

A STUDY OF ULTRASONOGRAPHIC CHARACTERISTICS OF THE CERVIX OF BITCHES DURING ANOESTRUS, OESTRUS, AND PREGNANCY

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SUMMARY: The main objective of the present study was to investigate the ultrasonographic characteristics of the cervix of bitches during anoestrus, oestrus, and pregnancy by using two-dimensional transabdominal ultrasonography. Ultrasonographic examinations were performed on 40 bitches using an ultrasound scanner (MyLab30vet, Esaote, Genoa, Italy) with a linear-array transducer (Esaote LA-522, Esaote, Genoa, Italy). According to the results, the cervix was a mild to moderate hyperechoic cylindrical structure lined by hyperechoic serosal margin in anoestrous bitches. The mean diameter of the cervix in anoestrus was 0.93 ± 0.37 cm (range, 0.59 - 1.81 cm), while the cervical canal could not be clearly demarcated by ultrasonography. However, in bitches in oestrus, the mean diameter of the cervix was 1.32 ± 0.11 cm (range, 1.2 - 1.45 cm). The cervical canal was visible as a hypoechoic irregular passage with the mean diameter of 1.5 ± 0.7 mm (range, 1.2 - 2.7 mm). In pregnant bitches, the mean diameter of the cervix was 1.57 ± 0.47 cm (range, 0.82 - 2.18 cm). The cervical canal of pregnant bitches was occluded with the cervical mucus plug which was heterogeneous echogenicity. The diameter of the cervix of pregnant bitches was significantly higher than that of bitches in anoestrus ($P=0.001$). Further, it was found that the diameter of the cervix increased when the pregnancy advanced. In conclusion, this study evaluated the morphological characteristics of the cervix in bitches during anoestrus, oestrus, and pregnancy. The findings of this study can be used as useful information to evaluate the cervix of bitches in clinical reproductive practice.

Key words: Bitch, cervix, ultrasonography.

INTRODUCTION

In many species, including dogs, the cervix is a sphincter between the uterus and vagina, and a filter to physiological and pathological processes related to fertilization and reproduction (Silva *et al.*, 1995). Anatomically, the cervix is a long connective tissue structure which is longitudinally occluded by epithelial folds, and the mucosa is lined with columnar epithelium cranially and stratified squamous epithelium caudally (England *et al.*, 1989; Silva *et al.*, 1995). The cervical canal extends from the internal ostium to the external ostium in a dorsoventral direction, with the external ostium being in close apposition to the ventral area of the vaginal fornix (Silva *et al.*, 1995). The cervix is dynamic in nature and changes take place in

different physiological states of the bitch, with relaxation of the cervical folds observed during oestrus under the influence of oestrogen (Concannon *et al.*, 1975; Olson *et al.*, 1984). On the other hand, closure of the cervical canal occurs as a result of occlusion by thick mucus which is stimulated by progesterone and the crystallization of the anterior vaginal fluid during pregnancy (Smith *et al.*, 1974; Silva *et al.*, 1995).

Investigation about changes of the cervix is important for the assessment of cervical incompetence and preterm labour of the pregnancy (Silva *et al.*, 1995; Nott *et al.*, 2016). It would also be useful to identify abnormalities of

the cervix in certain pathological conditions. The importance of post-uterine endoscopic and radiographic appearances of the cervix of live bitches has been described by Lindsay (1983). Meanwhile, two-dimensional transabdominal ultrasonography is considered as one of the best methods for determining the staging of pregnancy in bitches (England *et al.*, 2006; Aissi *et al.*, 2008; Blanco *et al.*, 2008). In particular, the characteristics and measurement of ultrasonographic features of the uterus of the dam can be used to predict impending abnormalities in pregnancy and the process of parturition which are useful to manage canine pregnancy safely and effectively (Blanco *et al.*, 2008). However, to our knowledge, there is dearth of information related to ultrasonographic findings of the cervix of the bitch. The main objective of the present study was to investigate the ultrasonographic characteristics of the cervix of clinically normal bitches during anoestrus, oestrus, and pregnancy by using two-dimensional transabdominal ultrasonography.

MATERIALS AND METHODS

Animals

Ultrasonographic examinations were performed on 40 bitches (15 in anoestrus, 20 pregnant, and 5 in oestrus) between 1 and 5 years of age, with bodyweights ranging from 8 to 30 kg. These bitches were presented to the Veterinary Teaching Hospital, University of Peradeniya, Sri Lanka for general checkups, pregnancy diagnosis, or elective surgical procedures, such as ovariohysterectomy or caesarean section. The bitches were subjected to thorough clinical examination to assess the clinical condition. Bitches which showed signs of oestrus were confirmed with vaginal cytological evaluation. In pregnant bitches, the pregnancy was confirmed with abdominal ultrasonography. The biparietal diameter and body diameter of the fetus were measured during ultrasonographic examination and were used to assess the gestational age according to the method adopted by Luvoni *et al.* (2006). A written consent from the owner was obtained before each

ultrasonographic examination.

Ultrasonography procedure

Two-dimensional transabdominal sonograms were obtained using an ultrasound scanner (MyLab30vet, Esaote, Genoa, Italy) with a linear-array transducer (Esaote LA-522, Esaote, Genoa, Italy) with frequency of 7.5 MHz. All the necessary dimensions were obtained from the sonographic images using electronic calipers. In each required parameter, at least 3 measurements were taken, and the means of measurements were calculated as the final value. Real-time images were recorded on videotape, and static images were sent to a computer equipped with specialized software for storage and off-line evaluation.

Vaginal cytological evaluation

Bitches which were in oestrus were confirmed with vaginal smears evaluation. As described by Antonov (2017), the cotton swab was passed into the vagina and then the swab was rotated through a complete revolution in each direction and withdrawn. Then, the cotton tip was lightly rolled from one end of a glass microscope slide to the other. The smears were stained using a Wright's Giemsa stain and examined by light microscopy (x 100 oil immersion). Bitches that showed 80-100% cornified cells with a few red blood cells in the vaginal smears were considered to be in oestrus (Simmons, 1970; Srinivas *et al.*, 2004; Antonov, 2017).

Evaluation of ultrasonographic characteristics of the cervix

Transabdominal ultrasonographic examinations were performed with the bitches in dorsal recumbency. The linear-array transducer was placed on the mid-ventral abdomen between the umbilicus and the pubis to obtain sagittal images and then the transducer was slightly angled towards the pelvic cavity and moved gently to visualize the cervix. The cervix was located by observing the urinary bladder, uterine lumen, descending colon, and subiliac lymph nodes

which serve as the anatomical landmarks. The maximum diameter of the cervix was measured at the internal ostium, middle, and towards the external ostium, and the mean was calculated as the final value. The echogenicity of the cervix and the patency of the cervical canal were recorded.

Analysis of the data

All measurements were recorded on a Microsoft Excel spreadsheet. Statistical analysis was done using the software Minitab®. Descriptive statistics, such as mean, range (minimum to maximum), and standard deviations of the mean were calculated for relevant parameters. The mean cervix diameters of pregnant bitches and the bitches in anoestrus were compared using the two samples t-test. Further, the diameter of the cervix was compared in pregnant bitches after categorizing them into 3 groups according to their gestational age: 4 to 5 weeks, 6 to 7 weeks, and 8 or more weeks. Pregnancy categories were

compared with each other using the two samples t-test. The P value < 0.05 was considered as statistically significant.

RESULTS

Ultrasonographic characteristics of the cervix of bitches in anoestrus

Fifteen clinically normal bitches which were in anoestrus without evidence of oestrous signs were examined. The breeds were Rottweiler (n=2), crossed bred (n=4), German Shepherd (n=5), Doberman (n=2), and Labrador (n=2). Figure 1A represents the sonograph of the cervix of a 3 year-old German Shepherd. The cervix was observed dorsal to the neck of the urinary bladder and it extends from the uterus towards the pelvic cavity between the neck of the urinary bladder and the descending colon. The cervix was more or less cylindrical in shape. It was a mild to moderate hyperechoic structure lined by

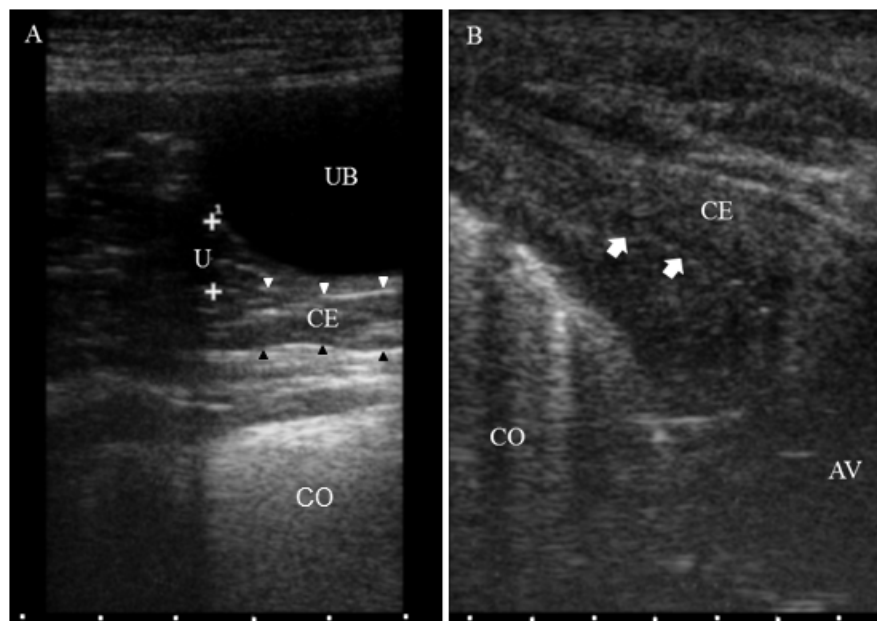


Figure 1. Trans-abdominal sonograms of the cervix (CE) of (A) a 3 year-old German Shepherd in anoestrus. The cervix (among arrow heads) is visible dorsal to the urinary bladder (UB) and it extends from the uterus (U) towards the pelvic cavity between the UB and ventral to the descending colon (CO). The cervix is more or less cylindrical in shape. (B) A 2 year-old German Shepherd 3 days after the onset of oestrous signs showing heterogeneous cervix. The cervical canal can be seen as hypoechoic capillary passage with irregular margin (arrows) which opens into the anterior vagina (AV). Top is ventral, bottom is dorsal, and right is caudal. The distance between two consecutive dots on the scale at the bottom of the images is equal to 1 cm.

hyperechoic serosal margin. The mean diameter of the cervix of bitches in anoestrus was 0.93 ± 0.37 cm (range, 0.59 - 1.81 cm). The cervical canal of bitches in anoestrus could not be clearly

demarcated ultrasonographically. The external ostium of the cervix could not be imaged in 5 bitches in the group.

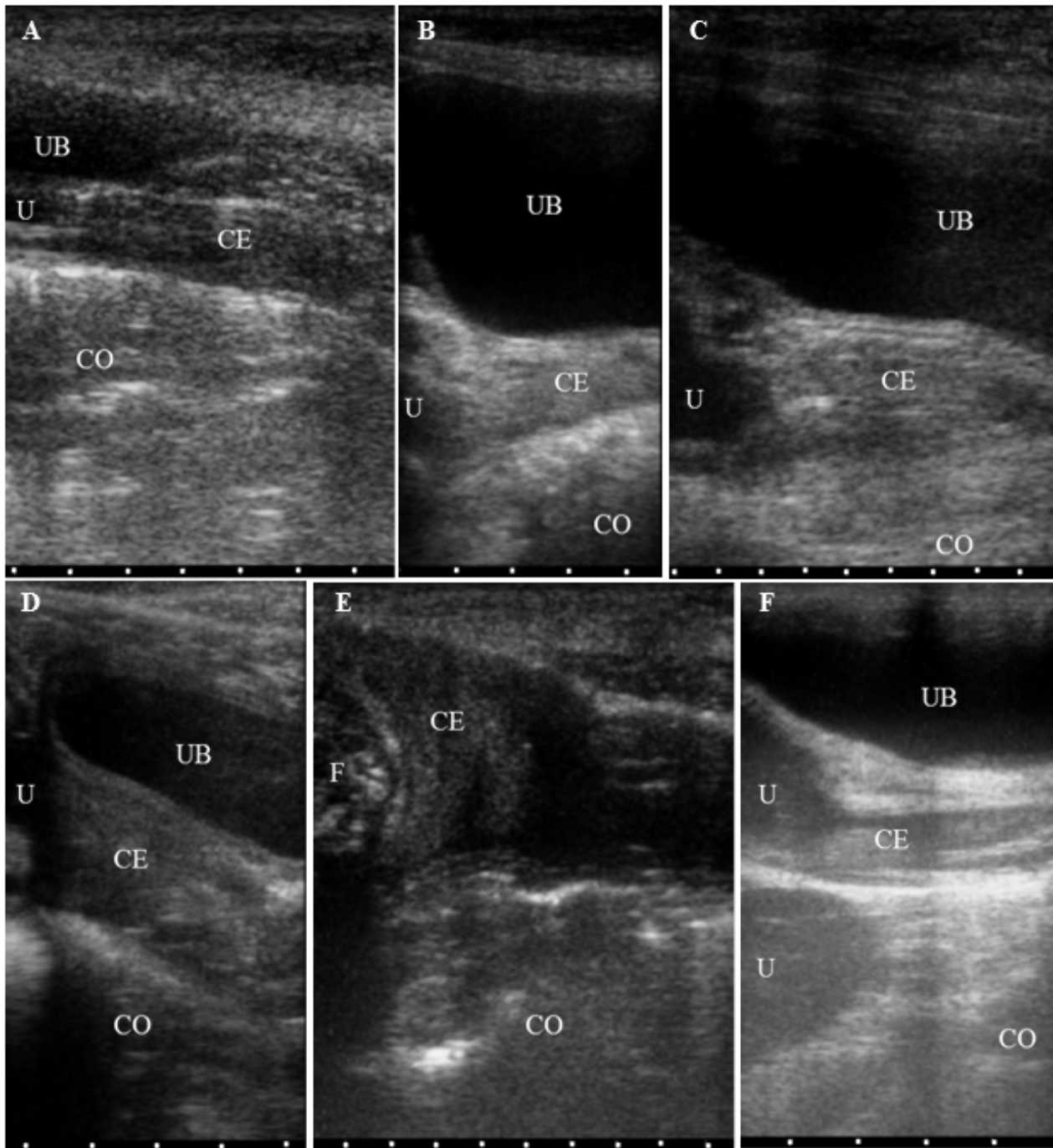


Figure 2. Trans-abdominal sonograms of cervix (CE) with mucus plug of (A) 2 year-old Labrador at 3 weeks of pregnancy, (B) A 2 year-old German Shepherd at 5 weeks of pregnancy. A well-developed mucus plug with enlarged cervix can be seen dorsal to the urinary bladder (UB) and ventral to the descending colon (CO). (C) A 5 year-old German Shepherd at 7 weeks of pregnancy, (D) A 4 year-old Pomeranian at 9 weeks of pregnancy, (E) A 3 year-old Dachshund a few hours before whelping showing partially dissolved mucus plug with hypoechoic area towards the external ostium. (F) A 3 year-old crossed bred after whelping the first puppy showing the opened cervix which is filled with hypoechoic materials. Top is ventral, bottom is dorsal, and right is caudal. The distance between two consecutive dots on the scale at the bottom of the images is equal to 1 cm.

Ultrasonographic characteristics of the cervix of bitches in oestrus

Five bitches in oestrus which were confirmed both with oestrous signs and vaginal smear evaluation were included in the study. The breeds were Rottweiler (n=1), German Shepherd (n=1), Labrador (n=2), and crossed bred (n=1). Figure 1B shows the ultrasonographic appearance of the cervix of a 2 year-old German Shepherd 3 days after the onset of signs of oestrus. The cervix appeared as a well demarcated heterogeneous structure. The mean diameter of the cervix of bitches was 1.32 ± 0.11 cm (range, 1.2 - 1.45 cm). The cervical canal was visible as a hypoechoic capillary passage with irregular margin which opens to the anterior vagina. The mean diameter of the cervical canal was 1.5 ± 0.7 mm (range, 1.2 - 2.7 mm).

Ultrasonographic characteristics of the cervix in pregnant bitches

Twenty pregnant bitches were included in this study. The breeds were German Shepherd (n=5), Rottweiler (n=5), Pomeranian (n=3), Boxer (n=2), Terrier (n=2), Crossed bred (n=1), Shih Tzu (n=1), and Labrador (n=1). They were observed under 3 major categories according to the gestational age: 4-5 weeks (n=7), 6 to 7 weeks (n=5), and 8 weeks or more (n=8). Some cervical changes which were observed during the different stages of pregnancy are shown in the figure 2. The cervical canal of pregnant bitches was occluded with the cervical mucus plug (operculum) which was heterogeneous echogenicity (Fig. 2). The cervix of pregnant bitches gave the appearance of a bottle neck between the uterus and the vagina. The mean cervical diameter of the pregnant bitches was 1.57 ± 0.47 cm (range, 0.82 to 2.18 cm), which was significantly higher than the diameter of the cervix of the bitches in anoestrus ($P < 0.0001$). The mean diameter of the cervix of bitches at 4 to 5 weeks and 6 to 7 weeks of pregnancy were 1.14 ± 0.39 cm (range, 0.82 to 1.77 cm) and 1.65 ± 0.12 cm (range, 1.46 to 1.77 cm), respectively, and were significantly different with the P value of 0.03. The mean cervix diameter of the bitches at 8 weeks or more in pregnancy was 2.00 ± 0.21

cm (range, 1.56 ± 2.18 cm), which was significantly different from those between 6 to 7 weeks of pregnancy ($P = 0.003$). There was a significant enlargement of the cervix with the advancement of pregnancy in bitches. Ultrasonographic changes of the cervix, such as dissolving the mucus plug and opened cervix few hours before and after whelping the first puppy are shown in the figures 2E and 2F, respectively.

DISCUSSION

In the present study, the transabdominal ultrasonographic characteristics of the cervix of bitches were investigated during anoestrus, oestrus, and pregnancy. The findings provide reference values for the cervix of bitches, which can be used during ultrasonographic examinations. Further, the ultrasonographic findings would be useful to distinguish certain pathological conditions of the cervix in bitches.

In the present study, it was possible to observe a heterogeneous cervix with hypoechoic cervical canal of bitches which were in oestrus. These findings together with other methods, such as vaginal smear evaluation and hormonal investigation assays would increase the accuracy of oestrus detection in bitches. Meanwhile, poor techniques of artificial insemination are considered as the most common cause of subfertility after artificial insemination in bitches (Mason *et al.*, 2018). This is mainly because the cervix acts as a mechanical barrier during the artificial insemination process. Thus, real-time visualization of cervix as described in the present study can be used as an additional aid in transcervical insemination which involves instillation of semen into the uterus through the cervix (Mason *et al.*, 2018). This would enhance the success rate in artificial insemination technique in canine reproduction.

In addition, in human studies, it is reported that the structure of the cervix is integral to the maintenance of pregnancy, keeping the developing fetuses in utero and forming a barrier to the ascent of microorganisms from the vagina

(Nott *et al.*, 2016). Weakness of the cervix may lead to deficiency of this barrier and is associated with subsequent preterm birth (Nott *et al.*, 2016). In the present study, there was a remarkable enlargement of the cervix in pregnant bitches which could be detected with transabdominal ultrasonography. Furthermore, the diameter of the cervix of the pregnant bitches increased significantly as the pregnancy advanced. This enlargement of the cervix could be due to the formation of the mucus plug inside the cervix. The mucus plug acts as a protective barrier by deterring the passage of microorganisms into the uterus, and contains a variety of antimicrobial agents, including immunoglobulins (England *et al.*, 1989). Thus, ultrasonographic monitoring of the mucus plug would indicate the possible threats associated with the pregnancy. Furthermore, in the present study, it was possible to detect certain changes of the mucus plug in the cervix during the initiating process of parturition (Fig. 2E). Therefore, the clinical translation of this knowledge will enable the early and appropriate identification of bitches that need veterinary intervention during the delivering process.

However, there are some limitations in our study. Bitches of several breeds were used when comparing the diameter of the cervix in the present study. If bitches were categorized into the small, medium, and large group according to their body weight, the results would provide values for each category of dogs. However, the number of bitches used in different categories of this preliminary study was limited. Thus, bitches of different breeds were studied together giving more emphasis on their physiological status.

In conclusion, to the best of our knowledge, this is the first study to evaluate ultrasonographic characteristics of the cervix during anoestrus, oestrus, and pregnancy in bitches. Therefore, the findings of the present study provide useful information to evaluate the cervix of bitches in veterinary clinical reproductive practice.

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