

## Determination of the Genital Structures using Ultrasound in Canine Prenatal Fetuses

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### ABSTRACT

The objective of this study was to evaluate the initial detection time and development of the fetal genital structures using ultrasound in twelve pregnant small bitches. The initial detection time of the fetal genital structures was as follows: genital tubercle at days 32.6; os penis at days 45.2; labia at days 45.7; scrotum at days 47.5. Ultrasonograms of fetal genital structure according to gestational stage were as follows: Undifferentiated stage (before day 35), the genital tubercle was observed to have a small elevation and just a hyper-echogenic structure in the midline between the umbilical cord and the tail in male and female fetus. Migration stage (between day 35~45), the genital tubercle was observed as a hyper-echogenic, bilobular, oval shaped and the genital tubercle began to migrate from the initial position toward the umbilical cord in males, and toward the tail in females. Differentiated stage (after day 46), the penis and os penis were observed to stand out in the abdominal wall and the scrotum was observed toward the perineal region in male fetuses. The labia was detected at the base of the tail in female fetuses. These results indicate that ultrasound of fetal genital structures could be useful for fetal gender determination and a completely prepartum evaluation of the canine fetus.

(Key words : canine fetus, external genitalia, genital tubercle)

### INTRODUCTION

Real-time ultrasound fetal monitoring techniques have documented fetal progress and organ development at various stages of gestation in great detail (Kähn, 1990). Ultrasound has been used to determine the fetal gender by means of identifying genital structures in bovine (Ali, 2004), equine (Mari *et al.*, 2002), and ovine species (Santos *et al.*, 2007). The genital tubercle is the embryonic structure that differentiates into the penis and prepuce in males and the vulva and clitoris in females (Noden and De Lahunta, 1985; Kähn, 2004). During differentiation, the tubercle moves from the initial position between the hind limbs toward the umbilical cord in males, or towards the tail in females (Kamimura *et al.*, 1994).

In large animals, the fetal gender was determined by ultrasonographic appearance and relative location of genital tubercle, and also visualization of the scrotum for males and mammary glands for females (Curran *et al.*, 1989; Kamimura *et al.*, 1994).

As seen above, many studies reported that fetal gender could be determined by ultrasonographic evaluation in large animals. However, in multiparous species (canine and feline),

only one study of fetal gender determination by evaluating the external genitalia has been reported (Zambelli *et al.*, 2004).

The purpose of this study was to determine the initial detection time of the genital structures throughout pregnancy with ultrasonographic examination and to provide the basic data for fetal gender determination and a complete prepartum evaluation of the fetus in dog.

### MATERIALS AND METHODS

#### 1. Animals

A total of twelve healthy small bitches (7 Pekinese, 3 Cocker Spaniel, 1 Miniature Schnauzer, 1 Chihuahua) were aged 2 to 6 years old, weighing 4.0 to 8.0 kg. They were housed individually, fed a standard commercial food twice daily, and water was available *ad libitum*.

#### 2. The Estimation of Optimal Mating Time and Mating

To estimate optimal mating time, vaginal cytological evaluations were performed daily from the initial day of proestrus to the initial day of diestrus as described by Schutte (1967)

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and mating were performed when the cornification index is  $\geq 90\%$ , as described by Kim *et al.* (2000).

### 3. The Estimation of Ovulation Time

The ovulation day (Day 0) was estimated as the first day that the plasma progesterone increased above 4.0 ng/ml, as described by Kim *et al.* (2000). Gestation lengths were calculated as the interval from the ovulation day to the parturition day.

### 4. Ultrasonographic Examination

Serial ultrasonographic examinations were performed daily using LOGIQ 7 (GE Medical system Co, USA) with 10 MHz linear transducer on 47 litters from twelve small bitches to determine the initial detection time of the fetal structures from 15 days of gestation to parturition in dorsal recumbency. Fetuses were examined made to view the entire fetus sequential through cross sectional, frontal, and sagittal planes.

Gestational age at the time of initial detection and the appearance of the fetal genital structures (genital tubercle, penis, os penis, scrotum, and labia) were recorded.

## RESULTS

The average gestational length of twelve small bitches was  $63.4 \pm 1.6$  (mean  $\pm$  SD, range: 61 ~ 65) days and the average litter sizes was 3.9 (total litter size, n=47). The accuracy of prepartum litter size at 30 days of gestation by ultrasound were 100%, compared with actual litter size.

### 1. The Time of Initial Detection of the Fetal Genital Structures

As shown in Table 1, the genital tubercle was first detected on day 32.6 (range: 29 ~ 35). In males, the os penis and scrotum

Table 1. Mean and range of initial detection time of fetal genital structures in canine 47 fetuses by ultrasound

Pregnancy features	Day after ovulation	
	Mean	Range
Genital tubercle	32.6	29 ~ 35
Os penis	45.2	43 ~ 48
Labia	45.7	43 ~ 48
Scrotum	47.5	46 ~ 48

were first detected on day 45.2 (43 ~ 48) and 47.5 (46 ~ 48), respectively. Around the same time, in females, the labia was first detected on day 45.7 (43 ~ 48).

### 2. The Ultrasonographic Appearances of the Fetal Genital Structures

The ultrasonographic appearance of the fetal genital structures according to gestational age were shown in Fig. 1 ~ Fig. 5.

Before day 35, the ultrasonographic appearance of the genital tubercle was similar in males and females (Fig. 1). It was observed as a hyper-echogenic structure, and was identified between the umbilical cord and tail in the sagittal views. In this stage frontal and cross sectional views, the genital tubercle was located between the hind limbs.

Between day 36 ~ 40, the genital tubercle was a hyper-echogenic, bilobular structure, and oval shaped. In male fetuses, the genital tubercle was moved from the initial position toward the umbilical cord, and was located over or cranial to the fetal urinary bladder (Fig. 2. A ~ C). Contrarily, in female fetuses, the genital tubercle was located just behind the hind limbs and caudal to the fetal urinary bladder (Fig. 2. D ~ F).

Between day 41 ~ 45, In male fetuses, the genital tubercle was close to the umbilical cord and in some of the fetuses the penis was observed (Fig. 3. B, C). In female fetuses, the genital tubercle was located towards the base of the tail (Fig. 3. E, F).

Between day 46 ~ 50, the penis seemed to be just a round bump that stood out in the abdominal wall and the os penis was observed with a hyper-echogenic structure inside the penis (Fig. 4. A). The scrotum was observed between the hind limbs toward the perineal region in male fetuses (Fig. 4. B). The labia was detected at the base of the tail in female fetuses (Fig. 4. C).

Between day 51-parturition, the external genitalia of the male and female fetuses were observed as the complete shape and the urethra was identified inside the os penis in the male fetus (Fig. 5. A, B).

## DISCUSSION

The assessment of fetal gender by ultrasonography and related studies have not been reported to date in canine. The results of the present study showed that the fetal genital tubercle was first observed as a hyper-echoic structure on days 32.6 between the hind limbs. And the male external genitalias

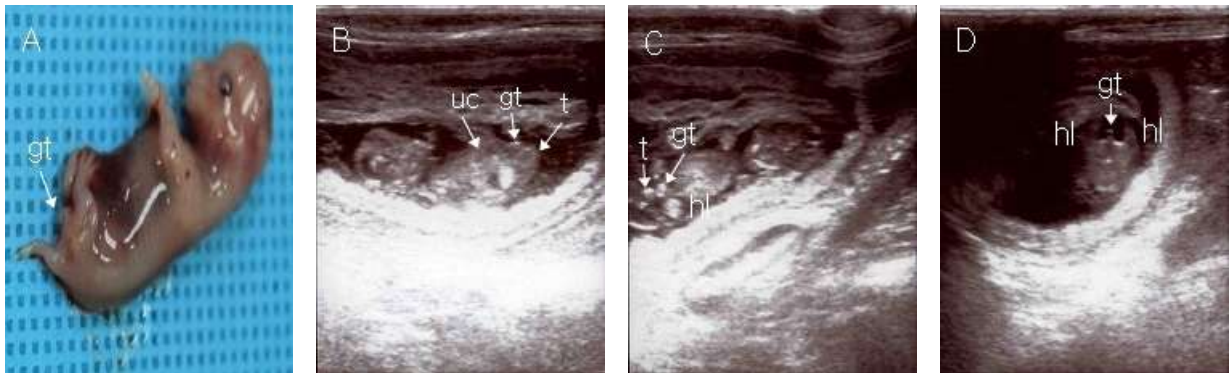


Fig. 1. Ultrasonographic images of genital tubercle in 30~35 days old fetuses in dogs. A: Genital tubercle (gt) of a 32 days old fetus, gross anatomical view. B: Genital tubercle (gt) located between the umbilical cord (uc) and the tail (t), sagittal view. C: Genital tubercle (gt) located between the hind limbs (hl) and the tail (t), frontal view. D: Genital tubercle (gt) located between the hind limbs (hl), cross-sectional view.

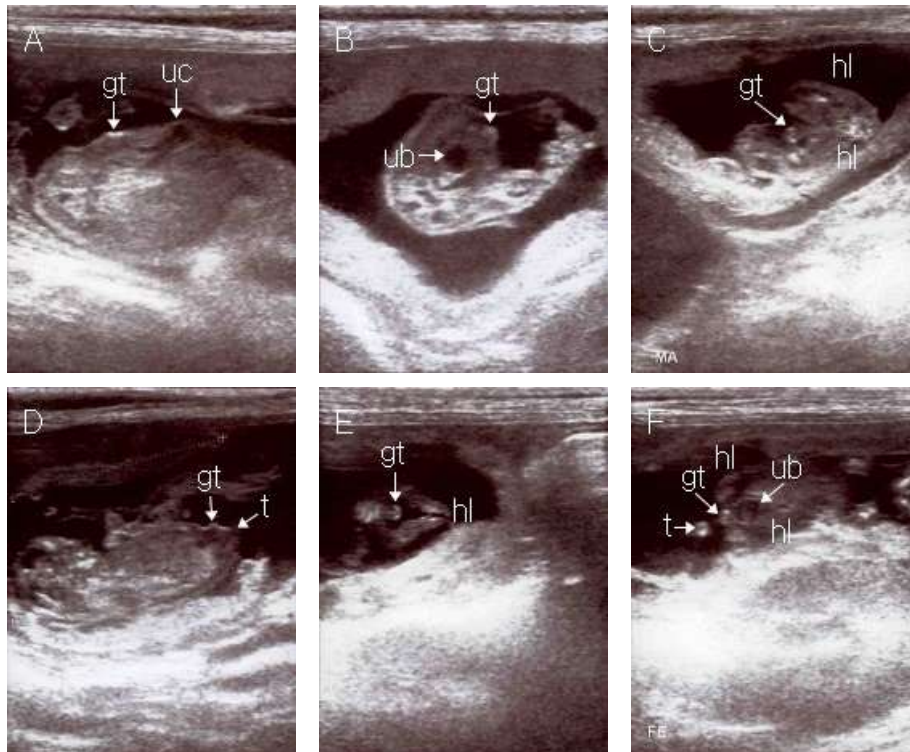


Fig. 2. Ultrasonographic images of genital tubercle in 36~40 days old fetuses in dogs. A: Male fetus (38 days), genital tubercle (gt) located toward the umbilical cord (uc), sagittal view. B: Male fetus (38 days), genital tubercle (gt) located ventral abdominal wall, urinary bladder (ub), cross-sectional view. C: Male fetus (40 days), genital tubercle (gt) located between the hind limbs (hl), frontal view. D: Female fetus (38 days), genital tubercle (gt) located toward the tail (t), sagittal view. E: Female fetus (38 days), genital tubercle (gt) located just caudal to the hind limb (hl), cross-sectional view. F: Female fetus (38 days), genital tubercle (gt) located between the hind limbs (hl) and the tail (t) and more caudal to the urinary bladder (ub), frontal view.

(os penis and scrotum) were first detected on days 45.2 and 47.5, respectively. Around the same time, the female external genitalia (labia) was first detected on days 45.7. Evans (1993)

reported that the genital tubercle was remarkably observed at days 30, the genital swellings become enlarged to the scrotum in the male fetus and developed to the labia in the female

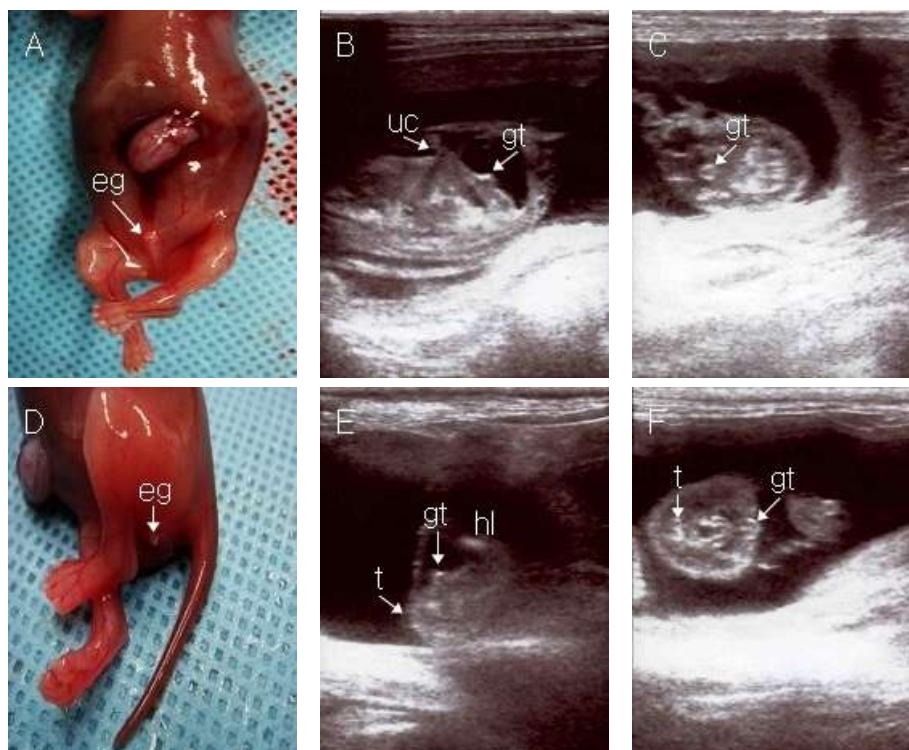


Fig. 3. Ultrasonographic images of genital tubercle in 41~45 days old fetuses in dogs. A: Male fetus (43 days), external genitalia (eg), gross anatomical view. B: Male fetus (43 days), genital tubercle (gt) located toward the umbilical cord (uc), sagittal view. C: Male fetus (43 days), genital tubercle (gt) located ventral abdominal wall, cross-sectional view. D: Female fetus (43 days), external genitalia (eg), gross anatomical view. E: Female fetus (43 days), genital tubercle (gt) located toward the tail (t), sagittal view. F: Female fetus (43 days), genital tubercle (gt) located at base of the tail (t), cross-sectional view.

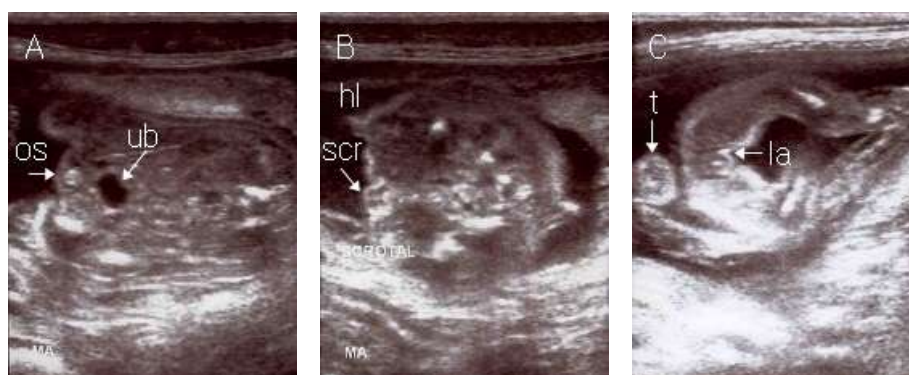


Fig. 4. Ultrasonographic images of external genitalia in 46~50 days old fetuses in dogs. A: Male fetus (48 days), os penis (os) located ventral abdominal wall, urinary bladder (ub), cross-sectional view. B: Male fetus (48 days), scrotum (scr) located between the hind limbs (hl), cross-sectional view. C: Female fetus (48 days), labia (la) located under position of tail (t), cross-sectional view.

fetus by gross anatomical view at days 45.

Undifferentiated stage of the genital tubercle (before day 35), the ultrasonographic appearance of the genital tubercle was observed to have a small elevation and just a hyper-echogenic structure in the midline between the umbilical cord and the

tail. In this stage, the genital tubercle was observed to have similar appearance in male and female fetus, as described by Evans (1993). And the genital tubercle was identified between the umbilical cord and tail in the sagittal views, and was located between hind limbs in the frontal and cross sectional

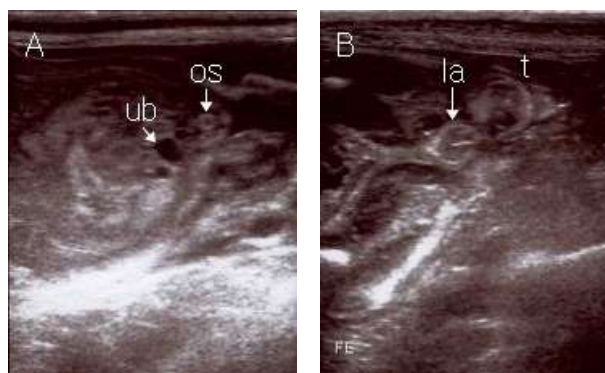


Fig. 5. Ultrasonographic images of external genitalia at day 51-parturition of fetuses in dogs. A: Male fetus (55 days), os penis (os) located ventral abdominal wall, urinary bladder (ub), cross-sectional view. B: Female fetus (58 days), labia (la) located under position of tail (t), cross-sectional view.

views, in agreement with the results of Kähn (2004).

Migration stage of the genital tubercle (between day 35 ~ 45), the genital tubercle was observed as a hyper-echogenic, bilobular structure, and oval shaped in ventral abdominal area. Kamimura *et al.* (1994) reported that the bilobular characteristics of the genital tubercle make it easy to distinguish between other echogenic structures by ultrasound in ventral area of fetus. In this stage, identification of the genital tubercle was relatively easy with ultrasonographic planes. And also, the genital tubercle began to migrate from the initial position toward the umbilical cord in males, and toward the tail in females, as reported by Curran *et al.* (1989). And the migration of the genital tubercle was estimated nearly completed in male and female fetuses around day 45. In this stage, the genital tubercle reached close to the umbilical cord in male fetuses and located towards the base of the tail in female fetuses. In this way, the fetal gender determination was possible by assessment of the relative location of the genital tubercle in this stage, as described by Kamimura *et al.* (1994).

Differentiated stage of the genital tubercle (after day 46), the penis and os penis were observed to stand out in the abdominal wall and the scrotum was observed toward the perineal region in male fetuses. The labia was detected at the base of the tail in female fetuses. In this stage, determination of fetal gender was very easy by confirmation of the external genitalia in male and female fetuses. However sometimes, the labia was unobserved associated to the presence of the coccygeal vertebrae and determination of the fetal gender was more difficult in the

female fetuses. Quintela *et al.* (2011) reported that identification of the clitoris at the base of the tail in bovine female fetuses was relatively more difficult because many hyperechogenic structures were closely observed to the tail area. In this case, the fetal gender determination was possible through the check of the external genitalia of both fetal areas (umbilical cord and tail).

In conclusion, the genital tubercle and the external genitalia was detected by ultrasound in canine fetus. The fetal gender determination could be possible by assessment of the relative location of the genital tubercle between day 35 ~ 45 and the confirmation of the external genitalia after day 46.

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