Serial Ultrasonographic Appearance of Normal Uterus during Estrous Cycle in Miniature Schnauzer Dogs

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ABSTRACT

Serial ultrasonography was performed to measure the normal appearance of uterine during estrous cycle and to determine whether the unterine appearance was related to the sex hormone, progesterone and estrogen. The uterine appearances, shape, diameter and echogenicity were daily monitored with ultrasonography in 9 Miniature Schnauzer dogs undergoing 11 estrous cycles. During proestrus and estrous, the uterus became hypoechoic but developed hyperechoic luminal echo. In the longitudinal view, the shape of the uterus occasionally changed from rectangular to coiled or serpentine, compared to other stages of the cycle. The diameter of the uterus during proestrus and estrous was larger (range: 0.60~0.86 mm) than other stages (range: 0.48~0.62 mm) of the cycle. The rising estrogen concentrations (range: 14.51~16.86 pg/ml) in plasma during proestrus correlated with changes in the uterus ($p<0.05$). Progesterone concentrations were 0.08~0.15 ng/ml at the onset of proestrus, but rose 1.06~1.26 ng/ml at the end of proestrus. There was no relation to progesterone concentration from onset of estrus ($p>0.05$). There was dramatical changes in normal uterus and sex hormone during estrous cycle. Especially, the appearance, shape and diameter of uterus were related to plasma estrogen concentration during proestrus, correlated with other stages of the cycle.

(Key words : Miniature Schnauzer, uterine ultrasonograph, estrus cycle)

INTRODUCTION

Real-time ultrasonography has been used for several years in human medical gynaecology (England and Yeager, 1993). Subsequently, many veterinary investigators have utilized this imaging modality, most notably in the mare and cow (England and Yeager, 1993). In the bitch, the early use of ultrasound was confined to the diagnosis of pregnancy. However, more recently the full value of ultrasound imaging in dogs has been documented, and the technology is now finding wide applicability in mornitoring fetal development, in timing gestation and predicting parturition, in diagnosis and management of reproductive tract disease, and in supplementing breeding soundness examinations (England and Yeager, 1993). Other indications for ultrasonographic examination include infertility, persistent estrus, complications of the postpartum uterus, and complications following ovariohysterectomy (Bonagura and Kirk, 1995). A 5-MHz transducer is usually sufficient to visualize an enlarged fluid-filled uterus, fetal structures, or abdominal mass lesions; however, a 7.5- or 10-MHz transducer provides better detail in the examination of smaller structures and is recommended for most indications (Pennick and d'Anjou, 2008).

The ovaries and the uterus are the only female reproductive organs routinely visualized by means of ultrasonography (Pennick and d'Anjou, 2008). The normal nongravid uterus is inconspicuous, often difficult to identify in dogs, and usually not seen in cats (Pennick and d'Anjou, 2008). The uterus can be differentiated from small bowel by lack of peristalsis, lack of intraluminal gas, and absence of the layered appearance characteristic of small intestine (Nyland and Mattoon, 2002). The uterus is best identified in the caudal abdomen, where it appears as a tubular structure between the urinary bladder and the descending colon (Pennick and d'Anjou, 2008). The size and appearance of the uterus varies, depending on the size of the animal, number of previous pregnancies, disease states, stage of the estrus cycle, and whether the animal is pregnant (Pennick and d'Anjou, 2008; Nyland and Mattoon, 2002). Nulliparous bitches weighing 25 pounds are reported to have uterine horns in the range of 10 to 14 cm in length and 0.5 to 1.0 cm in diameter (Nyland and Mattoon, 2002).

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Several studies have reported the hormonal changes occurring during the estrous cycle in the bitch. In this species estrous is characterised by a sharp progesterone increase, because of preovulatory luteinization of mature follicles, and a decrease of estrogens (Rota *et al.*, 2007). The dramatically rising estrogen concentrations in venous plasma during proestrus correlate with dramatic changes in the uterus, the vaginal mucosa, and the vulva, as well as with follicular secretion and behavior patterns in the bitch (Feldman and Nelson, 2004). The bitch in proestrus is under the influence of estrogen. Progesterone is derived first from luteinized follicles and then corpora lutea. Both estrous and diestrus are phases dominated by progesterone (Feldman and Nelson, 2004).

England and Allen (1989) noted that the uterus enlarged and developed central radiating hyperechoic lines during estrous, and that these changes could be induced by the administration of estrogen. During metestrus the uterus was more difficult to image - a change the may have been associated with uterine glandular regression (England and Allen, 1989). However little attention has been given to imaging of the non-pregnant uterus in the bitch.

The objective of the present study was to measure the normal ultrasonographic appearance of uterine during estrous cycle and to determine whether the uterine appearance was related to the sex hormone, progesterone and estrogens, in Miniature Schnauzer dogs which is a common pet dog in Korea.

MATERIALS AND METHODS

1. Animals

The examination was performed on 9 Miniature Schnauzer dogs. They were aged 2～6 years and weighed between 5.0～8.9 kg. They were multiparous, having delivered twice or more. The dogs were fed a standard commercial dog food twice daily, and water was available *ad libitum*.

2. Definition of Estrous Cycle

1) Gross Examination

Each dog was examined twice daily for swelling of vulva and the presence of a vaginal discharge, which considered to signify the onset of proestrus. Each stage of estrous cycle was described according to following methods, as described by Holst (1986).

2) Proestrus

- Uterine and vaginal serosanguineous discharges are appeared through the vulva. Although the bitch attracts males during proestrus, she is not receptive to mating.

3) Estrous

- It is characterized by the bitch's acceptance of a male (Day 0).

4) Diestrus

- It is described that the first day after a period of estrous when a bitch refuses to allow a male to mate.

3. Vaginal Cytology

To estimate the stages of estrous cycle, serial vaginal smears were performed every other day from onset of proestrus to the onset of anestrus. Vaginal cytological evaluations were performed as described by Schutte (1967).

4. Plasma Progesterone (P_4) and Estrogen (E_2) Concentration Measurement

Blood samples were collected via cephalic veinpuncture placed immediately in chilled EDTA-coated tubes, and centrifuged for 10 min at 3,000 g. The plasma was stored at −25℃ until analysis. The plasma P_4 and E_2 concentrations were determined in duplicate using a each of commercial P_4 and E_2 kit (Progesterone-Coat-A-Count, Diagnostic Products Corporation, USA) by Gamma counter (EG & G Wallace, Finland), as described by Kim *et al.* (2000). Using a ^{125}I radioimmunoassay (RIA) that had been previously validated for a fertility breeding management (Okkens *et al.*, 2001), the plasma P_4 and E_2 concentrations were measured. The diestrus stage was described when plasma P_4 level was first greater than 1 to 2 ng/ml and the anestrus stage so did when plasma P_4 level reached less than 1 to 2 ng/ml.

5. Ultrasonographic Examination

The uterine appearances, shape, diameter, echogenicity during estrous cycle, 11 estrous cycles in 9 bitches. Serial ultrasonographic examinations were performed daily from onset of proestrus until diestrus. Before the ultrasonographic examination, the dogs were sheared and placed in lateral recumbency. Serial sonogram was performed using LOGIC 7 (GE Medical system Co, USA) with 10L MHz transducer on 9 Miniature Schnauzer dogs. All diameters were described in millimeters by electronic caliper built in the ultrasonography machine. Uterine shape, echogenicity and the uterine diameter were monitored. The

uterine diameter was defined as the serosa to serosa measurements in uterine horn. For the accuracy of the measurement, the diameter of the transverse section was measured when the shape was the closest to a circle and the diameter of the longitudinal section was measured when it was the largest.

6. Statistical Analysis

The statistical analysis was performed using the general linear-model procedure (SAS version 9.1, SAS Institute Inc., USA). SAS was used to determine the crude association between uterine appearances (shape, echogenicity and diameter) and sex hormones (P_4 and E_2). A p-value<0.05 was considered significant.

RESULTS

1. Appearances of the Uterus during Estrous Cycle

The ultrasonographic appearance of the uterus changed according to the stage of the estrous cycle (Table 1 and Fig. 1).

As shown in Table 1, the ultrasonographic appearance of the uterus changed according to the stage of the estrous cycle. During proestrus and estrus, the uterus became increasingly hypoechoic but developed hyperechoic luminal echo. In the longitudinal view, the shape of the uterus occasionally changed from rectangular to coiled of serpentine, compared to other stages of the cycle, which was tubular and elongated. The diameter of the uterus during proestrus and estrous was larger (range: 0.60~0.86 mm) than other stages (range: 0.48~0.62 mm) of the cycle.

2. The Average Concentrations of P_4 and E_2 for 11 Estrous Cycles

A total of 374 serum samples for 11 estrous cycles of 9 bitches was analyzed to determine the concentrations of P_4 and E_2 (Table 2 and Fig. 2).

Table 1. Characteristic appearances of uterus using ultrasonography in 9 miniature Schnauzer dogs

	Shape	Echogenicity	Diameter (mm)
Proestrus and estrus	Coiled or serpentine	Homogeneous Hypoechoic Hyperechoic luminal echo	0.60~0.86
Diestrus and anestrus	Tubular elongated	Uniformly hypoechoic	0.48~0.62

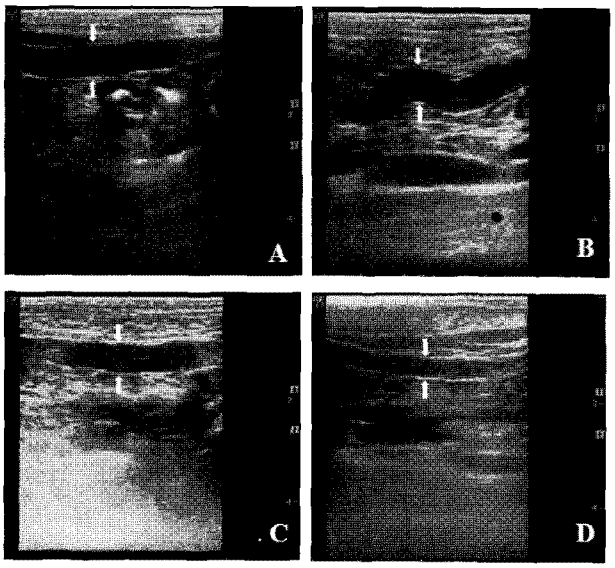

Fig. 1. Ultrasonograms made in the longitudinal plane showing Miniature Schnauzer uterus at various times of estrous cycle. The diameter of the uterus is marked (white arrow). (A): This proestrus uterus appears hypoechoic. (B): This estrous uterus appears central hyperechoic luminal linea and is coiled or serpentine. The uterus diameter is large compared to other stages. (C): The diestrus uterus changes tubular shape. (D): The anestrus uterus is decreased in diameter and elongated.

All hormonal data were normalized to the day of the onset of estrous (Day 0) for 11 cycles. P_4 concentrations were 0.08~0.15 ng/ml at the onset of proestrus, but rose 1.06~1.26 ng/ml at the end of proestrus. Serum P_4 concentrations continued to increase throughout estrus and then several weeks into diestrus. Serum E_2 concentrations peak 1 or 2 days before the onset of estrous and progressively decreased throughout estrous (Fig. 2).

The rising E_2 concentrations in serum between mid-proestrus and early estrous periods correlated with changes in the uterine diameter (UT diameter), compared to other stages of

Table 2. Mean (± SEM) serum P_4 and E_2 concentrations based on 11 estrous cycle periods in 9 miniature Schnauzer dogs

Stage	P_4 (ng/ml)	E_2 (pg/ml)
Proestrus	0.72± 0.5	7.85±2.6
Estrus	17.21±14.0	6.44±1.7
Diestrus	38.06± 1.7	5.04±0.4

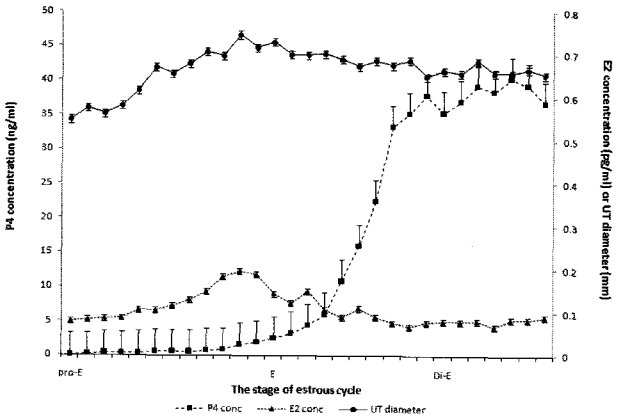

Fig. 2. Median P_4, E_2 concentrations and uterus diameter profiles in the 9 Miniature Schnauzer dogs during the observation period (Mean ± SEM).

the cycle ($p<0.05$). There was no relation to P_4 concentrations throughout periods of cycle ($p>0.05$).

DISCUSSION

Nylland and Matton (2002) noted that a normal, small, nongravid uterus can sometimes be imaged and identified as a solid, homogeneous, relatively hypoechoic structures. England and Yeager (1993) reported that the uterus was located dorsal or dorsolateral to the bladder and was a tubular hypoechoic structure. The uterus viewed in sonograms is sometimes composed of two distinct layers; a central homogeneous relatively hypoechoic region surrounded by a peripheral hyperechoic layer. The uterine horns have a similar appearance to the uterine body but are frequently smaller in diameter or not identified because of the presence of small intestine that interferes with ultrasound image (England and Yeager, 1993). However, empty small intestine characteristically has a central echo that is the lumen and its wall has multiple layers (the serosal interface, muscularis, submucosa, and mucosa). The small intestine is easy to correctly identify when there is peristalsis or gas in the lumen (Bonagura and Kirk, 1995). During the ultrasound examination of bitches in estrous after the collection of a vaginal cytology sample, there is frequently an echogenic line visible centrally within the uterine lumen (England and Yeager, 1993).

Throughout proestrus, estrous and early diestrus, a 1 mm hyperechoic luminal echo and a hypoechoic inner layer of the uterine wall are variably present. Detection of the uterus is easier at these times compared to anestrus because it is turgid and 1 to 3 mm larger in diameter (Bonagura and Kirk, 1995). It was enough to identify the uterus appearance for estrous cycle periods in ultrasonography with 10 MHz transducer in this study. It was able to identify an echogenic line within the uterine lumen. The uterus viewed in sonogram was composed of two distinct layers: a hypoechoic inner layer surrounded by a peripheral hyperechoic outer layer in longitudinal view. The shape of the uterus changed from linear tubular to coiled compared during proestrus and estrous. The uterine diameter was larger (range: 0.60～0.86 mm) than other stages (range: 0.48～0.62 mm). But, the edematous appearance of the uterus progressively decreased during mid-diestrus and anestrus. During anestrus, the imaging of the uterus was difficult.

The initial increase in uterine diameter and change of echogenicity during proestrus was similar to that previously reported (England and Allen, 1989). It has been suggested that this is associated with increasing E_2 during proestrus, as administration of exogenous E_2 produced a similar effect (England and Allen, 1989). Cruchten *et al.* (2003) noted that proliferation of endometrium is only noticed during proestrus and in the middle of estrus and the fact suggest that apoptosis is responsible for glandular regression in the cyclic canine endometrium. Apoptosis in the uterine surface epithelium of the hamster, mouse and rat is caused by E_2 withdrawal and can be prevented by treatment with E_2 and /or P_4 (Cruchten *et al.*, 2003). The lack of correlation between the apoptotic index and serum P_4 concentrations was also consistent with the distribution of E_2 and P_4 receptors in the cyclic canine endometrium (Vermeirsch *et al.*, 2000). The diameter of the uterus continues to increase several mm during early luteal phase, and therefore is easier to identify during estrous and the first week of two of the metestrous (diestrus) stage of the cycle, compared to other stages of the cycle. However, with the decline of plasma E_2 during estrous, the edematous appearance progressively decrease (England and Yeager, 1993). The uterus responds to increase in P_4 concentration by maintaining the glandular structure and vascularity required for pregnancy regardless of whether the bitch has mated. Maximum nonpregnant uterine size is seen 20 to 30 days following the onset of standing heat, a time coinciding with the highest P_4 concentrations (Feldman and Nelson, 2004). The uterus during anestrus undergoes self-repair. Complete repair of the endometrium to a basal state requires approximately 120 days after serum P_4 concentrations return to basal levels in the nonpregnant cycle and after 140 days in a fertile cycle (Feldman and Nelson, 2004).

In the study described here the changes of uterine diameter were specific enough to be related to serum E_2 concentration between mid-proestrus and early estrous periods ($p<0.05$). Throughout diestrus and anestrus, E_2 concentrations fluctuated, but there was no relation to uterine diameter.

Based on these findings, it appeared that dramatical changes in normal uterus and sex hormone (P_4 and E_2) during estrous cycle. Especially, the appearance, shape and diameter of uterus were related to plasma E_2 concentration during mid-proestrus and early estrus, correlated with other stages of the cycle in 9 Miniature Schnauzer dogs. However, additional study studies should be performed to clarify whether other sex hormones (FSH, LH) are related to uterine in the future.

CONCLUSIONS

Serial ultrasonographic examinations were performed on 9 Miniature Schnauzer dogs during estrous cycle periods to measure the normal appearance of uterus and determine whether the uterine appearance was related to the sex hormones (P_4 and E_2).

During proestrus and estrous, the uterus became hypoechoic but developed hyperechoic luminal echo. In the longitudinal view, the shape of the uterus occasionally changed from rectangular to coiled or serpentine, compared to other stages of the cycle. The diameter of the uterus during proestrus and estrus was larger (range: 0.60~0.86 mm) than other stages (range: 0.48~0.62 mm) of the cycle. The rising peak E_2 concentrations (range: 14.51~16.86 pg/ml) in plasma during proestrus correlated with changes in the uterus ($p<0.05$). P_4 concentrations were 0.08~0.15 ng/ml at the onset of proestrus, but rose 1.06~1.26 ng/ml at the end of proestrus. There was no relation to P_4 concentration from onset of estrus ($p>0.05$).

Based on these findings, there was dramatical changes in normal uterus and sex hormone during estrous cycle. Especially, the appearance, shape and diameter of uterus were related to plasma E_2 concentration during mid-proestrus and early estrus, correlated with other stages of the cycle.

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