**Six types of Bovine Uterine Enlargement and Their Outcomes Monitored Using Mobile Ultrasonography**

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**ABSTRACT**

Bovine uterine enlargement can be attributed to single or multiple pregnancies in physiological cases, or pyometra, hydrometra, uterine tumors, myometritis or uterine perimetritis in pathological cases. However, clinical differential diagnosis has been complicated, thus proper diagnosis and treatment have been considerably difficult. Six types of uterine enlargement from 26 clinical cases were sequentially monitored using transrectal mobile ultrasonography and their outcomes, after appropriate treatments were investigated. They were 12 cases of pyometra, 4 cases of myometritis, 4 cases of hydrometra, 3 cases of perimetritis and 3 cases of uterine tumors. In the cases involving uterine tumors, all of 3 cases were lymphosarcoma on postmortem examinations. The prognosis of chronic myometritis was usually poor; however, prompt and proper diagnostic treatment based on ultrasonographic findings such as combination treatment with prostaglandin F₂α (PGF₂α), estradiol benzoate and povidone iodine infusion increased the possibility of pregnancy. This suggests that a mobile ultrasonography may be beneficial for conducting a differential diagnosis of uterine enlargement.


**Keywords**: cows, mobile ultrasonography, myometritis, pyometra, uterine enlargement

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**INTRODUCTION**

Bovine uterine enlargement is usually classified into one of six types: single or multiple pregnancies in physiological cases, or pyometra (Földi et al. 2006; El-Tahawy and Fahmy 2011), hydrometra (Hatipoglu et al. 2002), uterine tumor (Honda et al. 2009; Burton et al. 2010), myometritis (Bruun et al. 2002; Sheldon et al. 2006), or uterine perimetritis (Hatipoglu et al. 2002; Földi et al. 2006) in pathological cases.

In clinical examinations, uterine enlargement is often discovered by rectal palpation during the estrus cycle. Diagnosis of an enlarged uterus has usually been determined only by transrectal palpation and thus information about the enlarged uterus has been limited. Therefore, most cows with uterine diseases have been culled due to failure of conception (Sheldon et al. 2006). It is too difficult to distinguish pyometra and pregnancy with only rectal examination in the enlarged uterus.

Recently, ultrasound techniques and devices have been developed, and among them mobile ultrasound machines have become a routine component of diagnostic investigation in reproductive management (Melendez et al. 2004; Silvestre et al. 2009; Medan and El-Aty 2010). In addition, accurate diagnosis of ovarian disease has become possible through ultrasound imaging (Hansen et al. 2000; McDougall 2010) and hormonal analysis (Amrozi et al. 2004; Iwakuma et al. 2008a, 2008b). Although ultrasound techniques have been commonly used for diagnosis and treatment of reproductive disorders in the field, differential diagnosis of uterine diseases by ultrasonography has not yet been fully developed (Földi et al. 2006; LeBlanc 2007).

Uterine pregnancy is classified into three types in cows, which include single and multiple pregnancies plus the carrying of twin fetuses, either in the ipsilateral or bilateral uterine horns. There are several reports indicating that postpartum bacterial contamination of uterine lumen causes uterine infection and the administration of antimicrobial agents...
is effective in preventing uterine infection (Galvão et al. 2009; Ali et al. 2010; Kaufmann et al. 2010). In this study, a total of 26 clinical cases of bovine uterine enlargement during the estrus cycle were sequentially monitored using transrectal mobile ultrasonography and their outcomes after treatment were investigated. Although postmortem examination of the uterus with myometritis in culled cows has been reported (Hatipoglu et al. 2002), there are few reports in the literature regarding the treatment of chronic myometritis. The aim of the present paper is to provide clear clinical definitions of uterine diseases, especially uterine enlargement observed using ultrasonography and to evaluate the effectiveness of treatment based on ultrasonographic diagnosis during the estrus cycle. In addition, a differential diagnosis of myometritis and uterine tumors will be discussed.

MATERIALS AND METHODS

Subjects
A total of 26 cases, 15 Holstein (H) and 11 Japanese Black (JB) cows, were examined during the period 2006 to 2008. They included 4 heifers, 10 primiparous and 12 multiparous cows (42.9 ± 27.9 months: means ± SD), and were raised on 15 commercial farms in Miyazaki and Kumamoto prefectures, Japan. The system of housing for the cows was either a tie stall barn, a free stall barn, or a loose barn.

Examination of the reproductive tract
As for the examination of the reproductive tract, every case was palpated per rectum. The left and right ovaries, cervix and uterine horns were scanned per rectum using mobile ultrasonography with a 7.5 MHz transducer (SonoSite 180 Plus, USA) covered by a plastic glove for rectal palpation. Moreover, the pathological characteristics of the uterine cavity, fluid, endometrium, myometrium and perimetrium were thoroughly determined using an ultrasound probe. The information generated through ultrasonic imaging has clarified the shape of the uterus, uterine thickness, and accumulation of fluids in the uterine lumen. Furthermore, images of the uterus were saved in the device for the further computer aided medical image analysis.

Diagnosis of uterine diseases
In this study, bovine uterine disorders with enlargement were classified into six types by ultrasonographic images. Pyometra and myometritis, hydrometra, uterine tumor, and perimetritis were diagnosed according to the definitions by Parkinson (2009) and Risco et al. (2007), Smith (2009) and Matsas (2007), Parkinson (2009) and Steenholdt (2007), and Risco et al. (2007), respectively.

Treatment of the enlarged uterus
Twenty-five mg of PGF2α (Dinoprost Tromethamine: Pronalgon F®, Pfizer Animal Health, Tokyo, Japan), 1 mg of estradiol benzoate (Ginandol®, Sanko-Yell Pharmaceuticals, Tokyo, Japan) and 50 ml of 2% povidone iodine were used for treatment of the enlarged uterus as needed. A standardized dosage of these drugs is typically applied in clinical practice. Twenty-five mg of PGF2α and 1 mg of estradiol benzoate were frequently injected in three to four days intervals, from one to five times on each occasion. In addition, intrauterine infusion with 50 ml of 2% povidone iodine was performed one to three times at 7-day intervals in each case until the uterine fluid was excluded and the enlarged myometrium returned to normal. In the cases of perimetritis which was characterized by adhesion of the uterus to abdominal organs, antibiotic treatment was not done because any acute infections had already disappeared.

Artificial insemination
Once the cows exhibited estrus after treatment, artificial insemination (AI) was conducted using AM/PM methods by the veterinarian. The assessment of pregnancy was performed 27 days after artificial insemination using mobile ultrasonography and if an echogenic embryo or fetus was observed inside the uterine cavity with functional corpus luteum (CL) in the ovary, the cows were diagnosed pregnant.

Histopathological examination
In the cases of myometritis and uterine tumor, the uteri were collected after slaughter and examined macro- and microscopically. The uterine tissues were soon fixed with 10% buffered formalin and were then paraffin-embedded, sectioned in slices 5 to 6 micrometers thick, and stained with hematoxylin and eosin (H&E).

Reproduction performance
Age (months), number of parity, days postpartum to treatment, days to AI after treatment, number of AI attempts, and days to conception after treatment were all analyzed according to the disease type.

Statistical analysis
Days postpartum to treatment, days to AI after treatment, number of AI attempts and days to
conception after treatment were analyzed with Mann-Whitney U-test between the cases of pyometra and myometritis.

**RESULTS**

In this study, bovine uterine enlargement from 26 clinical cases were classified into six types such as pyometra, myometritis, hydrometra, uterine tumors, and uterine perimetritis in pathological cases or single or multiple pregnancies in physiological cases. 700 cattle were bred over three years with a 1.24% (26 cases/2,100 heads) incidence of pathological diseases. The age (in months), number of parity, days postpartum to treatment, days to AI after treatment, number of AI attempts, and days to conception after treatment in cows with six types of uterine enlargement were listed in Table 1. The ultrasonic findings in six types of uterine enlargement are shown in Figure 1, and each characteristic of ultrasound diagnosis is as follows:

**Pyometra:** Using rectal palpation a loose substance was noted in the enlarged uterus. In the ultrasound diagnosis, milky and patchy substances in the uterine horns were diagnosed as hypoechogenic particles in a low echo structure. Thickening of the uterine muscle layer was not observed (Figure 1a, Japanese Black cow, No.5) Identification of the endometrium and muscle layer was possible. Twelve cases, one Holstein and 11 Japanese Black cows: age (average ± SD), 50.2 ± 33.6 months; parity, 2.5 ± 2.5; days postpartum to treatment, 90.8 ± 61.0; days to AI after treatment, 40.1 ± 9.4; Number of AI attempts, 1.6 ± 0.9; days to conception after treatment, 63.4 ± 48.8, were diagnosed as pyometra based upon ultrasound diagnosis. Ten cases were cured by 25 mg PGF₂α injection once or twice and intrauterine infusion of 2% povidone iodine at 7-day intervals zero or one time and managed to become pregnant. Two cases were culled.

**Myometritis:** The hard and enlarged uterus was noted by rectal palpation. Ultrasound image of the myometritis was shown in Figure 1b. Thicker muscle layer was noted. Thicker muscle layer was noted. 4 cases, two Holstein and two Japanese Black cows: age (average ± SD), 32.5 ± 6.8 months; parity, 1.3 ± 0.5; days postpartum to treatment, 86.8 ± 39.1; days to AI after treatment, 81.0 ± 34.3; Number of AI attempts, 2.0 ± 2.0; days to conception after treatment, 149.3 ± 156.5, were diagnosed as myometritis based upon ultrasound diagnosis. Two cases were cured by 25 mg PGF₂α injection once and intrauterine infusion of 2% povidone iodine at 7-day intervals zero or one time and managed to become pregnant. One case was culled.

**Hydrometra:** The uterine horns were filled with a large amount of fluid. The uterine muscle layer was not observed in the ultrasound diagnosis. No single or multiple pregnancies were noted in physiological cases. 4 cases, two Holstein and two Japanese Black cows: age (average ± SD), 34.5 ± 31.6 months; parity, 1.5 ± 2.4; days postpartum to treatment, 131 ± 60.8; days to AI after treatment, 26.5 ± 4.9; Number of AI attempts, 1.5 ± 0.7, were diagnosed as hydrometra based upon ultrasound diagnosis. Two cases were cured by 25 mg PGF₂α injection once and intrauterine infusion of 2% povidone iodine at 7-day intervals zero or one time and managed to become pregnant. One case was culled.

**Tumor:** Single or multiple pregnancies were noted in physiological cases. The uterine muscle layer was not observed in ultrasound diagnosis. One case, a Japanese Black cow: age (average ± SD), 52.3 ± 29.9 months; parity, 5.5 ± 2.1; days postpartum to treatment, 141.3 ± 80.8, was diagnosed as a uterine tumor based upon ultrasound diagnosis. One case was culled.

**Perimetritis:** Single or multiple pregnancies were noted in physiological cases. The uterine muscle layer was not observed in ultrasound diagnosis. Two cases, one Holstein and one Japanese Black cows: age (average ± SD), 31.3 ± 1.3 months; parity, 32.1 ± 1.3; days postpartum to treatment, 72.3 ± 32.4; days to AI after treatment, 78.3 ± 41.5; Number of AI attempts, 2.3 ± 2.3; days to conception after treatment, 179.5 ± 208.6, were diagnosed as perimetritis based upon ultrasound diagnosis. Two cases were culled.

**Pregnant:** Single or multiple pregnancies were noted in physiological cases. No single or multiple pregnancies were noted in physiological cases. One case, a Japanese Black cow: age (average ± SD), 29.3 ± 2.9 months; parity, –; days postpartum to treatment, 72.3 ± 32.4; days to AI after treatment, 78.3 ± 41.5; Number of AI attempts, 2.3 ± 2.3; days to conception after treatment, 179.5 ± 208.6, was diagnosed as pregnant based upon ultrasound diagnosis.

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**Table 1** Bovine uterine enlargement classified into six types using mobile ultrasonography and their outcomes

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<th>Type</th>
<th>(n)</th>
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<th>Age (months)</th>
<th>Parity</th>
<th>Days postpartum to treatment</th>
<th>Days to AI after treatment</th>
<th>No. of AI attempts</th>
<th>Days to conception after treatment</th>
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<td>90.8 ± 61.0</td>
<td>40.1 ± 19.4</td>
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<td>Ave ± SD</td>
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<td>1.3 ± 0.5</td>
<td>86.8 ± 39.1</td>
<td>81.0 ± 34.3</td>
<td>2.0 ± 2.0</td>
<td>149.3 ± 156.5</td>
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<td>Ave ± SD</td>
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<td>34.5 ± 31.6</td>
<td>1.5 ± 2.4</td>
<td>131 ± 60.8</td>
<td>26.5 ± 4.9</td>
<td>1.5 ± 0.7</td>
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<tr>
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<td>H(No.8)</td>
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<td></td>
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<td>52.3 ± 29.9</td>
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<td>1.3 ± 0.6</td>
<td>72.3 ± 32.4</td>
<td>78.3 ± 41.5</td>
<td>2.3 ± 2.3</td>
<td>179.5 ± 208.6</td>
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<td>Pregnant</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>pregnant</td>
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</table>

JB: Japanese Black cow, H: Holstein cow
–: Data was not derived
tritis is shown in Figure 1b (Holstein cow, No.3). The uterine wall is partly or entirely hard and thickened, and the lumen is distended due to the accumulation of purulent or mucopurulent material. Identification of the endometrium and muscle layer is difficult. Four cases, 3 Holstein and one Japanese Black cows; age (average ± SD), 32.5 ± 6.8 months; parity, 1.3 ± 0.5; days postpartum to treatment, 86.8 ± 39.1; days to AI after treatment, 81.0 ± 34.3; Number of AI attempts, 2.0 ± 2.0; days to conception after treatment, 149.3 ± 156.5, were diagnosed as myometritis. Three cases were cured and one case was culled. Twenty-five mg of PGF2α and 1 mg of estradiol benzoate were frequently injected three to five times at three or four day-intervals and intrauterine injection of 2% povidone iodine was done twice at seven-day intervals leading to pregnancy.

**Hydrometra:** An ultrasound image of hydrometra is shown in Figure 1c (Japanese Black cow, No.13). By ultrasound diagnosis, watery substance within the distended uterus was diagnosed as an echo free structure. Also, the sediments in watery substance could be observed floating within the uterine lumen by vibration. The endometrium is extremely thin, and echo images of the endometrium and muscle layers are indistinct. Four cases, 2 Holstein and 2 Japanese Black cows; age (average ± SD), 34.5 ± 31.6 months;

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**Figure 1** Ultrasonic findings in six types of uterine enlargement.

a: Ultrasound image of pyometra in a cow (JB, No. 5). Note the nonuniform echogenic appearance of the uterine contents, which included several hyperechogenic particles. No thickening of the uterine muscle layer was seen.

b: Ultrasound image of a myometritis in a cow (H, No. 3). An oblique longitudinal uterine section. Note the variable echogenic liquid and the presence of many hyperechogenic particles in the uterine cavity as well as the thick uterine wall (double-headed arrow).

c: Ultrasound image of a transverse uterine section of hydrometra in a cow (JB, No. 13). Note accumulation of the anechoic liquid in the uterine cavity and very thin uterine wall.

d: Ultrasound image of a longitudinal uterine section of tumor in a cow (JB, No. 15). The thick uterine wall (double-headed arrow) and a slight accumulation of intrauterine liquid could be seen.

e: Ultrasound image of perimetritis in a cow (H, No. 9). Note the connective tissue (double-headed arrow) in the adhesive region.

f: Ultrasound image of a gravid uterus. Embryo, aged 35 days (1.5 cm long).

g: Ultrasound image of a twin pregnancy on day 45 in a 4-year-old Holstein cow. Two embryos are easily visible and separated by a chorioallantoic membrane commonly known as the “twin line”. Bar = 10 mm
parities, 1.5 ± 2.4; days postpartum to treatment, 131 ± 60.8; days to AI after treatment, 26.5 ± 4.9, were diagnosed as hydrometra. Injection with 25 mg of PG-F2α and intrauterine infusion of 2% povidone iodine was done once or twice at seven-day intervals on each occasion. AI was conducted in two cases, but no cases achieved pregnancy and thus all were culled.

**Uterine tumor:** The hardened and enlarged uterus was noted by rectal palpation much like myometritis. The ultrasound image of the uterine tumor is shown in Figure 1d (Japanese Black cow, No.15). According to the ultrasound diagnosis, the uterine wall was completely hardened and thickened. The uterine lumen was distended with small amounts of mucus present. Three cases, 2 Holstein and 1 Japanese Black cows; age (average ± SD) 52.3 ± 29.9 months; parities, 2.7 ± 2.1; days postpartum to treatment, 141.3 ± 80.8, were diagnosed as having uterine tumors. By rectal palpation and ultrasound diagnosis, the uterine wall was noted as being entirely hardened and thickened in all 3 cases. These were treated in the same manner as the myometritis cases. However, the symptoms in the 3 cases did not improve and the increase in the uterus size indicated a poor prognosis. All 3 cows failed to achieve pregnancy and were culled. The histopathological inspections revealed the large-sized lymphocytes with atypia and nuclear division infil-

**Figure 2** Pathological finding of myometritis and tumor

a: An enlarged uterus with myometritis in a 2-year-old Holstein cow (H, No. 4).

b: The mass in the right uterine horn was caused by thickening of the endometrium. Severe infiltration of inflammatory cells, mainly lymphocytes, neutrophils and macrophages as well as proliferation of fibroblast were noticed in the endometrium. HE stain (x 200 magnification).

c: Perivascular infiltration of similar inflammatory cells was recognized in the muscle layer of right uterine horn. HE stain (x 200 magnification).

d: Uterine tumor (JB, No. 15). This female was 2 years old and enlargement of left uterine horn was palpated by rectal examination 3 months postpartum, while right uterine horn was normally palpated. Bar (d) = 20 mm.

e: In the left uterine horn lesion, mucosal tissue under epithelium or muscle layer was in part replaced with diffusely proliferated tumor cells. Few uterine glands (arrow) can be seen. HE stain. Bar (e) = 200 μm.

f: Histopathological examination of the lesion revealed the infiltration of large-sized lymphocytes (arrowheads) with atypia and nuclear division (arrow) into the endometrium and myometrium. The lesion was diagnosed as lymphosarcoma. Two types of tumor cells were recognized, one consisted of densely stained small circular cells and the other of round cells which showed moderate inequality in size, atypia and rarely binuclear. In the latter cells, nuclear division was recognized in scattered manner. No tumor cells were recognized in the right uterus horn. HE stain. Bar (f) = 25 μm.
trated into the endometrium and myometrium, and the uterine lesions were diagnosed as lymphosarcoma (Figure 2).

**Perimetritis:** In 3 (Holstein cow, No.2~4) of 4 cases diagnosed as myometritis, myometrial inflammation extended to the perimetrium and the adhesions of the uterus to abdominal organs were observed (Figure 1e). These 3 cases, 3 Holstein cows (Holstein cow, No.9 ~11); age (average ± SD), 29.3 ± 2.9 months; parity, 1.3 ± 0.6; days postpartum to treatment, 72.3 ± 32.4; days to AI after treatment, 78.3 ± 41.5; days to conceive after treatment, 179.5 ± 208.6; were diagnosed as perimetritis. Perimetritis is characterized by adhesion of the uterus and the adhesion appears as an echogenic structure. In these cases, inflammatory signs such as pain and fever were not noted. The inflammation in adhesive organs caused by perimetritis had disappeared and no general symptoms were observed. Thus they were not treated with antibiotics. The treatment of myometritis by intrauterine infusion of 2% povidone iodine and PGF$_{2\alpha}$ injection reduced the thickening of the uterine muscle layer. In spite of these adhesions, two of the three cases became pregnant.

In days postpartum to treatment and number of AI after treatment, there was no significant difference between the cases of pyometra and myometritis. Days to AI after treatment and days to conception after treatment in the cases of myometritis tended to be longer than pyometra, but there was no significant difference between them.

In the cases of pregnancy, there were two types of twin pregnancy, i.e., (1) two embryos observed in the bilateral uterine horns and (2) two embryos observed in the unilateral uterine horn with a connect- ed chorioallantoic membrane known as the twin line.

**DISCUSSION**

In this study, bovine uterine enlargement during the estrus cycle was divided into six types by ultrasonography. Mee et al. (2009) evaluated the pre-breeding ovario-uterine status according to the six-grade system using ultrasound reproductive tract scoring and concluded that these scores were significantly associated with subsequent fertility. The puerperal fever and endometritis due to compromised uterus often occur within 1 month postpartum, and thus a great deal of research on postpartum uterine diseases has been published (LeBlanc 2007; Iwakuma et al. 2008a; Kaufmann et al. 2010). However, the diagnosis and treatment of enlarged uterine disease in the breeding period after the voluntary waiting period had not been made clear. In the present study, treatments using 2% povidone iodine intrauterine infusion and injection with PGF$_{2\alpha}$ and estradiol benzoate were efficient for treating cows with chronic myometritis after the voluntary waiting period. In addition, three of the four cases became pregnant using this treatment. Ali et al. (2010) reported that a regimen consisting of intrauterine infusion with 100 ml of 0.1% acriflavine and PGF$_{2\alpha}$ injection was efficient for treating metritis in female camels. Days to AI after treatment and days to conception after treatment in the cases of myometritis tended to be longer than pyometra. This difference might indicate that in pyometra return to estrus occurs after mainly removal of the pus from the uterus, while myometritis is accompanied by myometrial chronic inflammation and takes longer periods to recover. Until now, bovine myometritis had not been considered treatable; however, cows with myometritis in this study could become pregnant without a withdrawal period for milk, so it was suggested that this treatment for curing the condition might have a great clinical significance.

Further, in this study, ten of twelve cases with pyometra became pregnant using the treatment. However, the cows (n=4) with hydrometra using this treatment did not result in pregnancy, though 2 cows received AI. Thus, all of them were culled. In pyometra cases, endometrial tissue with a fluctuated surface was often observed. On the other hand there appeared to be a thin endometrial tissue in the hydrometra cases, but this was not confirmed by the ultrasound image. The uterus gland which secretes nutritional fluid inside the uterus exists in the endometrium. In hydrometra cases, it was considered that the endometrium suffered irreversible damage and the treatment was ineffective.

Ultrasonography can examine deep areas of the uterus in real time, which is not possible by rectal palpation. An ultrasound diagnosis can reveal more information than a rectal palpitation, making prompt and proper treatment in uterine disease possible (Melendez et al. 2004; Mee et al. 2009; Medan and Abd El-Aty 2010). Ultrasound imaging of high or low echo intensity and the movement of intrauterine substances can determine whether treatment by PGF$_{2\alpha}$ is warranted. In the cases of thickened uterine wall, ultrasound imaging after intrauterine treatments with 50 ml of 2% povidone iodine can ensure a diagnostic differentiation between myometritis and uterine tumors. In myometritis, the involution of uterine
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muscles by this treatment was observed; however, in uterine tumors the uterine muscles became thicker.

Furthermore, in this study a diagnosis of myometritis by ultrasound imaging was confirmed by histopathological examination (Sheldon et al. 2006; Honda et al. 2009). Bovine leukemia virus infection is widespread in Japan (Usui et al. 2003; Honda et al. 2009; Murakami et al. 2013). An ultrasound-guided biopsy of a mass might be necessary to perform a diagnosis (Chapwannya et al. 2010).

In conclusion, bovine uterine enlargement can be attributed to physiological cases and pathological cases. Moreover uterine diseases differential diagnosis and tentative treatments were previously limited by rectal palpation; however, these can be improved by using ultrasonography. Therefore, the spread of ultrasound diagnosis could provide prompt treatments of uterine diseases.

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REFERENCES


要約

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