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NOTE

Theriogenology

Blood lactate concentration as diagnostic predictors of uterine necrosis and its outcome in dairy cows with uterine torsion

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ABSTRACT. In order to determine blood lactate concentrations (bLac) and their validity as a diagnostic marker in bovine uterine torsion, blood samples were taken from 54 Holstein cows with uterine torsion before the correction of torsion. bLac in a group of cows with and without uterine necrosis were 15.0 and 3.0 mmol/*l*, respectively ($P<0.01$). Moreover, bLac in a group of dead or culled dams and in that of survived dams were 10.2 and 3.1 mmol/*l*, respectively ($P<0.01$). Furthermore, the proposed diagnostic cutoffs for bLac based on ROC analysis for detection of uterine necrosis and poor prognosis in dams were set at >5.0 and >6.5 mmol/*l*, respectively. These findings suggest that in dairy cows with uterine torsion, an increase in bLac is a diagnostic predictor of uterine necrosis as well as poor prognosis in dams.

KEY WORDS: dairy cow, lactate, prognosis, uterine necrosis, uterine torsion

In dairy cows, uterine torsion accounts for approximately 20% of dystocia cases that attended veterinary services [3, 14, 19, 20]. The duration and severity of uterine torsion and the extent of circulation insufficiency in the uterine tissue affect the prognosis [2, 12, 14, 17, 23]. In severe cases, circulation insufficiency results in edemas of the broad ligament of the uterus, bleeding, bloody ascites, thromboses of uterine vessels and uterine necrosis [14, 19–21]. Also, prognosis is poor when torsion is accompanied by a large uterine rupture, bleeding and necrosis [7, 12, 14]. The number of cases requiring correction of uterine torsion and/or cesarean delivery increases with severity of torsion [14], indicating that determining the severity of torsion is essential in treatment planning. However, no accurate diagnostic criteria are available, so uterine necrosis and/or a rupture are often found only after laparotomy [14, 20]. Diagnostic methods enabling accurate determination of torsion severity and prediction of its prognosis are needed.

Lactate forms via anaerobic metabolism (e.g., during intense muscle exercise), and the blood lactate concentrations (bLac) also increase in pathological conditions, such as low tissue perfusion and peripheral circulatory impairment [4, 5, 16, 24]. It has been reported that bLac correlate with colic in horses, and with poor prognosis of displaced abomasum in dairy cows and gastric dilation-volvulus in dogs [10, 11, 13, 15, 18, 24, 25]. Pathology indicative of congestion and necrosis can also be seen in some dairy cows with uterine torsion, suggesting that bLac might be useful in diagnosis of disease severity and prognosis. Portable devices measuring bLac are commercially available, and their diagnostic reliability has been reported in dairy cow [6, 8]. Furthermore, activities of aspartate aminotransferase (AST) and creatine phosphokinase (CK) in blood, which increase with cell disruption, were reported as prognostic indices in buffaloes with uterine torsion [1, 2], but not in Holsteins.

Against this background, the aims of this study were 1) to compare bLac and AST and CK activities among different correction difficulties in dairy cows and among groups with different outcomes and 2) to investigate possible use of bLac as a diagnostic marker of uterine necrosis in dairy cows with uterine torsion and to determine diagnostic thresholds.

Subjects were 54 Holstein dairy cows that developed uterine torsion between April 2014 and September 2015 in Nemuro, Hokkaido, Japan. Uterine torsion developed during delivery in 53 cows on the 240th or later day of gestation and on the 150th day of gestation in 1 case. The uterus rotated toward the left in 36 cases, with a rotation of ≤180 degrees in 18, 181–360 degrees in 14

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Table 1. Relationship among G1-4 and the direction and degree of torsion [n (left: right)]

	Grade 1	Grade 2	Grade 3	Grade 4	Total
<180	16 (10 : 6)	5 (4 : 1)	5 (4 : 1)	1 (0 : 1)	27 (18 : 9)
181−360	7 (5 : 2)	6 (6 : 0)	5 (3 : 2)	3 (0 : 3)	21 (14 : 7)
>360	0 (0 : 0)	1 (1 : 0)	1 (0 : 1)	4 (3 : 1)	6 (4 : 2)
Total	23 (15 : 8)	12 (11 : 1)	11 (7 : 4)	8 (3 : 5)	54 (36 : 18)

Table 2. Mortality rates and median (range) bLac levels and AST and CK activities by corection difficulties of uterine torsion

	Normal	(n)	Grade 1	(n)	Grade 2	(n)	Grade 3	(n)	Grade 4	(n)
Mortality (%)										
Dam	-		0	(0/23)	0	(0/12)	45.5	(5/11)	75.0	(6/8)
Fetus	-		13.0	(3/23)	50.0	(6/12)	81.8	(9/11)	100.0	(8/8)
bLac (mmol/*l*)	1.3 (0.8−2.6)	(7)	3.1 (1.4−6.0)	(23)	3.4 (1.1−5.5)	(12)	3.4 (1.4−10.2)	(11)	15.0 (5.0−25.0)**	(8)
AST (U/*l*)	-	-	82 (65−118)	(13)	85 (68−120)	(8)	95 (88−113)	(9)	104 (84−178)	(5)
CK (U/*l*)	-	-	144 (81−373)	(13)	214 (133−371)	(8)	220 (141−370)	(9)	269 (173−327)	(5)

**P<0.01, compared with the values of Normal, Grade1, 2 and 3.

and ≥360 degrees in 4. The uterus rotated toward the right in 18 cases, with a rotation of ≤180 degrees in 9, 181–360 degrees in 7 and ≥360 degrees in 2. Cows culled for reasons other than uterine torsion were excluded from the study.

Blood specimens were collected from the coccygeal vein before correction of the torsion. bLac were measured in 54 subjects, and AST and CK activities were measured in 35 subjects. Blood specimens were collected and stored in tubes containing heparin. bLac were measured using a portable lactate measuring device (Lactate Pro2, Array KDK, Kyoto, Japan). Blood specimens, collected and kept in plain tubes, were centrifuged at 1,500 *g* for 10 min, and serum samples were kept at or below −20°C before measurement of AST and CK activities using an automated biochemical analyzer (Bio Majesty JCA-BM8060, JEOL, Tokyo, Japan).

Difficulties in correcting torsion were classified into grade (G) 1–4 depending on the course of treatment. Kruskal-Wallis test and then Turkey-Kramer honestly significant difference test were performed for comparisons among the four grades. G1 was defined as the mildest torsion, which was successfully corrected by rotating the fetus per vaginam, rolling of the cow or elevating the dam's hind legs, leading to successful vaginal delivery. G2 was defined as torsion that was successfully corrected during labor, but required a cesarean section due to insufficient cervical dilatation. G3 was defined the torsion without necrosis that was corrected only after a cesarean section. G4 was defined as torsion with necrosis that was corrected only after cesarean section. The diagnosis of uterine necrosis was made when the visual inspection of dark purple uterine wall was accompanied by one or more of findings, such as the accumulation of bloody ascites, thickening of uterine myometrium and thrombosis. Table 1 shows the relationships among G1–4 and the direction and degree of torsion. In addition, blood was collected in the second stage of labor in 7 cows who were going through a normal vaginal delivery of a live fetus without intervention. A cesarean section with a right flank incision was performed using the conventional procedure in the dam placed in a recumbent position.

Subjects were divided into two groups according to the outcome of the dam: survival longer than 30 days after delivery (surviving dam group) or not (deceased dam group, including dams that died naturally or were culled). Similarly, subjects were divided into two groups, according to whether the fetus was alive (the live birth group) or stillborn (the stillbirth group). Mean values were compared among the groups by Mann-Whitney U test. Receiver operating characteristic (ROC) analysis was performed to calculate threshold values of bLacfor uterine necrosis and prognosis. JMP ver. 11 (SAS Institute Inc., Cary, NC, U. S. A.) was used for statistical analysis. A P value of <0.05 was considered statistically significant.

Table 2 shows the results of comparisons among the groups with different correction difficulties. The mortality rates in both dams and fetuses increased as the degrees of torsion severity increased from G1 to G4. Compared with the normal delivery and G1–3 groups, the bLac was significantly higher (15.0 mmol/*l*, P<0.01) in the G4 group. AST and CK activities in serum showed an increasing trend with torsion severity, although the differences were not statistically significant.

The results of comparisons between different outcomegroups are shown in Table 3 (A and B). Median bLac in the deceased dam group and the stillbirth group were 10.2 mmol/*l* and 4.5 mmol/*l*, respectively, which were significantly higher than those in the corresponding surviving groups (both P<0.01). Also, median serum AST activity was significantly higher in the deceased dam group than in the surviving dam group (P<0.05), while there were no differences in serum CK activity among the groups. Figure 1 shows the results of ROC analysis of bLac. The threshold bLac for distinguishing G4 (torsion with uterine necrosis) from G1-G3 (torsion without uterine necrosis) was 5.0 mmol/*l* (100% sensitivity and 89.1% specificity, P<0.01), while that for unfavorable prognosis in dams was 6.5 mmol/*l* (72.7% sensitivity, 100% specificity, P<0.01).

The severity of uterine torsion is thought to depend on the degree of rotation and disease duration. However, because it is difficult to accurately keep track of time in actual clinical practice, we investigated whether it is possible to predict disease

Table 3. Median (range) bLac levels and AST and CK activities by outcomes of uterine torsion

A : Dam

	Total	Survived	(n)	Dead or Culled	(n)
bLac (mmol/*l*)	54	3.1 (1.1−6.0)	(43)	10.2 (3.4−25.0)**	(11)
AST (U/*l*)	35	85 (65−125)	(28)	104 (90−178)*	(7)
CK (U/*l*)	35	172.5 (81−373)	(28)	269 (209−327)	(7)

B : Fetus

	Total	Alive	(n)	Stillborn	(n)
bLac (mmol/*l*)	54	2.95 (1.4−6.0)	(28)	4.5 (1.1−25.0)**	(26)
AST (U/*l*)	35	84 (65−118)	(17)	91.5 (68−178)	(18)
CK (U/*l*)	35	167 (81−373)	(17)	231 (133−370)	(18)

* $P<0.05$ and ** $P<0.01$, compared with the surviving animals.

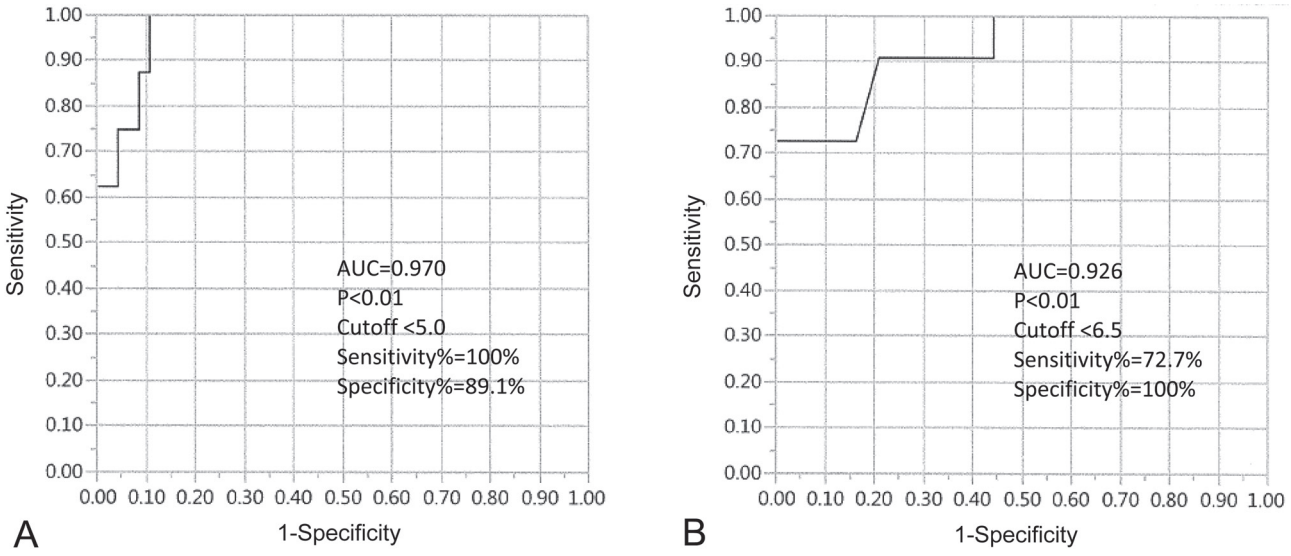

Fig. 1. A, a ROC curve of G4 with uterine necrosis vs G1-3; B, a ROC curve of mother cows with poor prognosis vs. survival.

severity from hematological findings in this study. All the groups with different correction difficulties, but not the G1 group, had laparotomy, and therefore, it is unclear whether some cows in the G1 group had uterine necrosis. However, we think this is unlikely because of an extremely low mortality rate in both dams and calves and because of sufficient cervical dilatation and vaginal delivery after the correction of uterine torsion. AST and CK activities in serum are known to be elevated by cell disruption and also by necrosis and disruption of uterine cells, and the utility of AST and CK activities as prognostic predictors of uterine torsion has been reported in buffalo [1, 2], but not in Holstein dairy cows. Also, no study has reported bLac measurements in dairy cows with uterine torsion. In this study, elevated serum AST activity was observed in the deceased dam group; otherwise, there were no significant differences in serum AST and CK activities. Slipping and falling when standing up are thought to be possible causes of uterine torsion [3], and elevated AST and CK activities in blood may be due to skeletal muscle damage caused by slipping and/or falling. Compared with AST, the activity of CK lasted shorter, which might have contributed to the difference in results between AST and CK.

Significant increases in bLac were observed in cases of severe torsion with uterine necrosis, in the deceased dam group and in the stillbirth group. It has been reported that impaired perfusion and ischemia of the small intestine induce anaerobic glycolysis, thereby increasing bLac in horses with colic [10]. In dairy cows that undergo surgery for displaced abomasum, increases in bLac were associated with findings indicative of poor prognosis, such as a discolored (deep purple) abomasum, venous thromboembolism and elevated ascitic fluid volume [9, 13]. We found elevated bLac in cows with uterine torsion of G4 severity, in which a discolored (deep purple) uterus, venous thromboembolism and elevated bloody ascitic fluid volume were observed upon cesarean section. This may be due to a pathology similar to that in horses with colic and dairy cows with displaced abomasum.

We usually try to correct uterine torsion by rotating the fetus per vaginam, rolling of the cow and/or elevating the dam's hind legs, and perform a cesarean section when correction is unsuccessful. Without an accurate diagnosis of the severity, more procedures tend to be attempted in cows with severer torsion, and thus, dams are in the extremely weakened condition when a

cesarean section is finally performed. In this study, despite having severe torsion, treatment was successful in two of the cows in the G4 group. bLac in these cows were <6.5 mmol/*l* (that is, 5.0 and 5.3 mmol/*l*). In addition, one of the cows had a partial uterus resection, whereas the area of uterine necrosis was localized. These findings suggest that a bLac of ≥5.0 to <6.5 mmol/*l* is a predictor of uterine necrosis that is difficult to correct non-invasively and therefore a cesarean section should be selected in the early stage of the disease. Furthermore, there is a risk of post-correction mortality due to ischemia-reperfusion injury in cows with torsion accompanied by uterine necrosis [14]. Thus, it is necessary that procedures including possible hysterectomy [22] be considered for those animals. In addition, a bLac of ≥6.5 mmol/*l* may be a sign of poor prognosis.

In conclusion, the results of this study revealed that an elevated bLac indicates, and thus can serve as a predictor of, uterine necrosis and poor prognosis in dairy cows with uterine torsion.

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