



# Histopathological changes in the pancreas of cattle with abdominal fat necrosis

Chikako TANI<sup>1</sup>#, Watanyoo PRATAKPIRIYA<sup>2</sup>#, Mineto TANI<sup>3</sup>,  
Takenori YAMAUCHI<sup>4</sup>, Takuya HIRAI<sup>2</sup>, Ryoji YAMAGUCHI<sup>2</sup>, Hitoshi ANO<sup>5</sup> and  
Hiromu KATAMOTO<sup>5</sup>\*

<sup>1</sup>Graduate School of Medicine and Veterinary Medicine, University of Miyazaki, 5200, Kihara, Kiyotake, Miyazaki 889-1692, Japan

<sup>2</sup>Laboratory of Veterinary Pathology, Faculty of Agriculture, University of Miyazaki, 1-1, Gakuen Kibanadai-nishi, Miyazaki 889-2192, Japan

<sup>3</sup>Laboratory of Large Animal Reproduction, School of Agriculture, Tokai University, Kawayo, Minami Aso-mura, Aso-gun, Kumamoto 869-1404, Japan

<sup>4</sup>Department of Hygiene and Preventive Medicine, Showa University, School of Medicine, 1-5-8, Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan

<sup>5</sup>Laboratory of Veterinary Internal Medicine, Faculty of Agriculture, University of Miyazaki, 1-1, Gakuen Kibanadai-nishi, Miyazaki 889-2192, Japan

**ABSTRACT.** The association between pancreatic disorder and abdominal fat necrosis in cattle remains unclear. The pancreases of 29 slaughtered cattle with or without fat necrosis were collected to investigate pathological changes. Japanese Black (JB) cattle were classified into the FN group (with abdominal fat necrosis; n=9) and N group (without fat necrosis; n=5). The pancreases were also collected from 15 Holstein Friesian (HF) cows. All JB cattle showed high body condition scores. Regarding the pathological findings, fatty pancreas which involves adipocyte infiltration into the pancreas and fat necrosis (saponification) were observed in 25 and 27 cases, respectively. Immunohistochemical staining with anti-Iba-1 antibody showed large numbers of macrophages surrounding the saponified fat in the pancreas. CD3-positive T cells were significantly more common in the pancreas of both the FN and N groups compared with the HF group ( $P<0.05$ ). Furthermore, fibrosis in the pancreas exhibited a correlative tendency with the formation of necrotic fat mass in the peritoneal cavity ( $P<0.1$ ). These results indicate that obesity leads to increased severity of pancreatic disorder, including fatty pancreas and pancreatitis. The pathological lesions in the pancreas may play a key role in abdominal fat necrosis through the inflammatory process.

**KEY WORDS:** abdominal fat necrosis, cattle, fibrosis, obesity, pancreatic lesion

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Fat necrosis frequently occurs in cattle and is characterized by the formation of necrotic fat masses in the abdominal cavity. These necrotic fat masses are multifocally found in various sites, including intestines, mesentery of the spiral colon, mesorectum and retroperitoneal area. This disease is very complex and causes intestinal obstruction and some clinical symptoms, such as sclerous feces, constipation and chronic anorexia. Lipodystrophy [2] and lipomatosis [3, 12, 28, 30, 38] are synonymous with this disease, which have been reported worldwide in the U.K. [3], Turkey [40], U.S.A. [28, 39], Egypt [7, 36] and Brazil [30]. Moreover, it has been documented in several species, including swines [6, 11, 38], goats [33], cats [2, 6] and humans [10, 37].

The incidence of fat necrosis has been reported in the cattle industry since the 1960s, especially in Japan [12, 14–16, 19, 22, 23, 31]. Obesity, heredity, lack of exercise and intake of a high-energy diet [14–16, 19, 31] have all been associated with the occurrence of fat necrosis. Moreover, fungal toxins, especially fescue toxicity, are considered to be a cause of abdominal fat necrosis in pygmy goats [33]. Ito *et al.* [12] have proposed the pathophysiology that excessive fattening causes hypertrophy of the adipose tissue followed by circulatory disturbance in the tissue, and subsequently, chemical changes of fat caused by an enzyme which has been oozed out of degenerated blood capillaries after the circulatory disturbance may produce the lesions. On the other hand, it has been well documented that acute pancreatitis, in which enzymatic juice leaks from the pancreas, is involved in the formation of necrotic fat lesions and systemic inflammatory response [2, 6, 37]. In swine, it has been suggested that this disease is also caused by a pancreatic disorder [11]. In addition, sodium taurocholate injection into pancreatic ducts in experimental rats led to acute pancreatitis followed by intra-abdominal fat necrosis [27, 34].

\*Correspondence to: Katamoto, H., Laboratory of Veterinary Internal Medicine, Faculty of Agriculture, University of Miyazaki, 1-1, Gakuen kibanadai-nishi, Miyazaki 889-2192, Japan. e-mail: katamoto@cc.miyazaki-u.ac.jp #Contributed equally to this work.

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In human medicine, obesity is widely recognized to be associated with a state of chronic, low-grade inflammation [35]. It has been noted that the amount of intrapancreatic fat increase with increasing BMI and fat in the pancreas during acute pancreatitis has a direct toxic effect on the pancreatic parenchyma [1]. Taken together, the inflammation of the pancreas attributed to obesity may be related to the etiology of bovine abdominal fat necrosis.

Therefore, it has been hypothesized that excess fattening induces fatty infiltration of the pancreas and causes acute or chronic pancreatitis followed by enzymatic juice leaks from the pancreas, and necrotic fat lesions occur in cattle. Little has been reported on the pathogenesis of bovine abdominal fat necrosis focused on the pancreatic lesions. The objective of this study was to investigate the possible association between severity of the pathological lesions in the pancreas and occurrence of abdominal fat necrosis in cattle.

## MATERIALS AND METHODS

### *Pancreas and necrotic fat mass samples*

The pancreases and necrotic fat masses from six Japanese Black (JB) steers and three JB heifers with abdominal fat necrosis (FN group) and the pancreases from five JB steers without fat necrosis as controls (N group) were collected during the slaughtering process at the Meat Center in Miyazaki Prefecture in June 2011. Pancreases were also collected from 15 Holstein Friesian (HF) cows (HF group) at the Meat Center in Kumamoto Prefecture in August 2011 to evaluate the relationship between degree of obesity and severity of pancreatic lesions. Chalky white firm mass in abdominal adipose tissue was diagnosed as necrotic fat mass by inspection and palpation. JB cattle were fattened in the barns of several private farms and were fed high-concentrate diets. HF cows were originally raised for milk production in the barns of several private farms. After the culling due to reproductive disorders, they were fed high-concentrate diets for at least 3 months before slaughter. All cattle were clinically healthy at slaughter. Body condition scores (BCSs) [8] were recorded for all cattle. The tissues were immersed in 10% neutral buffered formalin.

### *Histopathology*

Paraffin-embedded tissue samples were cut into 4- $\mu$ m-thick sections and stained with hematoxylin and eosin. The histological results were descriptively analyzed for microscopic findings in the lesions. Additionally, the histopathological severity of the pathological lesions in the pancreases was semi-quantitatively graded as no (-), minimal (+), moderate (++) or severe changes (+++). The severity of fatty infiltration was assessed according to the presence of fat vacuole in the low-power field (100 fold magnification): -, no vacuoles; +, 1-10; ++, 11-20; and +++, more than 20. The severity of saponification was also graded according to the presence of vacuole containing saponified fat in the low-power field: -, no vacuoles; +, 1-5; ++, 6-10; and +++, more than 10.

### *Immunohistochemistry*

Immunohistochemical studies were performed to identify the predominant inflammatory cells. The pancreases were prepared as 4- $\mu$ m sections on coated slides. Deparaffinization and rehydration were performed, and the samples were subjected to citrate buffer (pH 6) and heat-induced antigen retrieval. Subsequently, endogenous peroxidase blocking was performed by immersion in 3% diluted hydrogen peroxide solution. The primary antibodies were rabbit polyclonal anti-Iba-1 antibody (ionized calcium-binding adapter molecule 1; Wako Chemicals U.S.A., Inc., Richmond, VA, U.S.A.; diluted 1:250) as a macrophage marker and rabbit polyclonal anti-CD3 antibody (Dako Denmark A/S, Glostrup, Denmark; prediluted) to represent lymphocytes at 37°C in all serial section samples. EnVision polymer (Dako Denmark A/S) was utilized as the secondary antibody and visualized using 3,3'-diaminobenzidine (DAB; Sigma-Aldrich Co., St. Louis, MO, U.S.A.) in a horseradish peroxidase system. The tissues were counterstained with Mayer's hematoxylin. The bovine lymph node was used as a positive control in each tissue.

### *Data analysis*

The histological and immunological results were analyzed descriptively and semi-quantitatively, respectively. The immunopositive cells for each antibody were randomly counted in 10 high-power fields. Numbers of grade from 0 (-) to 3 (+++) were used in the statistical analysis. To compare groups, non-parametric methods were adopted since the numbers of cattle were not necessarily enough. Wilcoxon rank-sum test with or without Holm's correction and Kruskal-Wallis rank-sum test were used for comparison of numerical and ordinal variables. Correlations were estimated by Spearman's rank method. Logistic regression analysis was conducted to investigate the association between necrotic fat mass and suspected factors (*i.e.* four pancreatic lesions and BCS). However, multiple logistic regression was not because of sample size. The data were presented as the mean  $\pm$  SEM. All statistical analyses were conducted using R version 3.1.3 (The R Foundation, Vienna, Austria).  $P < 0.05$  was considered statistically significant in this study.

## RESULTS

### *Background of the FN, N and HF groups*

Three heifers and six steers of JB cattle were classified in the FN group (Table 1). Their mean age and BCS were  $29.7 \pm 0.4$  months old and  $5.0 \pm 0.0$ , respectively. The N group was composed of five steers of JB cattle, and their mean age and BCS were  $28.4 \pm 1.0$  months and  $5.0 \pm 0.0$ , respectively. The HF group consisted of 15 HF cows, and their mean age and BCS were  $74.9$

**Table 1.** Profiles of cattle and characteristic features of necrotic fat masses

No.	Age (months)	Sex	BCS	Necrotic fat mass	Location of mass (around or near)	Diameter of mass (cm)
FN1	28	Steer	5.0	+	Rectum	8
FN2	30	Steer	5.0	+	Pancreas	10
FN3	28	Heifer	5.0	+	Colon, rectum, pancreas	10, 8, 5
FN4	30	Heifer	5.0	+	Ileum, colon, rectum, kidney	150
FN5	31	Steer	5.0	+	Colon	50
FN6	29	Steer	5.0	+	Colon	30
FN7	30	Heifer	5.0	+	Colon	30
FN8	30	Steer	5.0	+	Colon	10
FN9	31	Steer	5.0	+	Colon	10
N1	25	Steer	5.0	–	–	–
N2	31	Steer	5.0	–	–	–
N3	28	Steer	5.0	–	–	–
N4	29	Steer	5.0	–	–	–
N5	29	Steer	5.0	–	–	–
HF1	95	Cow	3.3	–	–	–
HF2	61	Cow	3.5	–	–	–
HF3	39	Cow	2.5	–	–	–
HF4	99	Cow	2.0	–	–	–
HF5	78	Cow	4.5	–	–	–
HF6	110	Cow	2.25	–	–	–
HF7	35	Cow	2.75	–	–	–
HF8	50	Cow	3.5	–	–	–
HF9	86	Cow	1.75	–	–	–
HF10	92	Cow	2.0	–	–	–
HF11	89	Cow	3.5	–	–	–
HF12	79	Cow	3.25	–	–	–
HF13	90	Cow	3.5	–	–	–
HF14	45	Cow	1.75	–	–	–
HF15	77	Cow	2.25	–	–	–

BCS, body condition score; FN, fat necrosis; N, control; HF, Holstein Friesian.

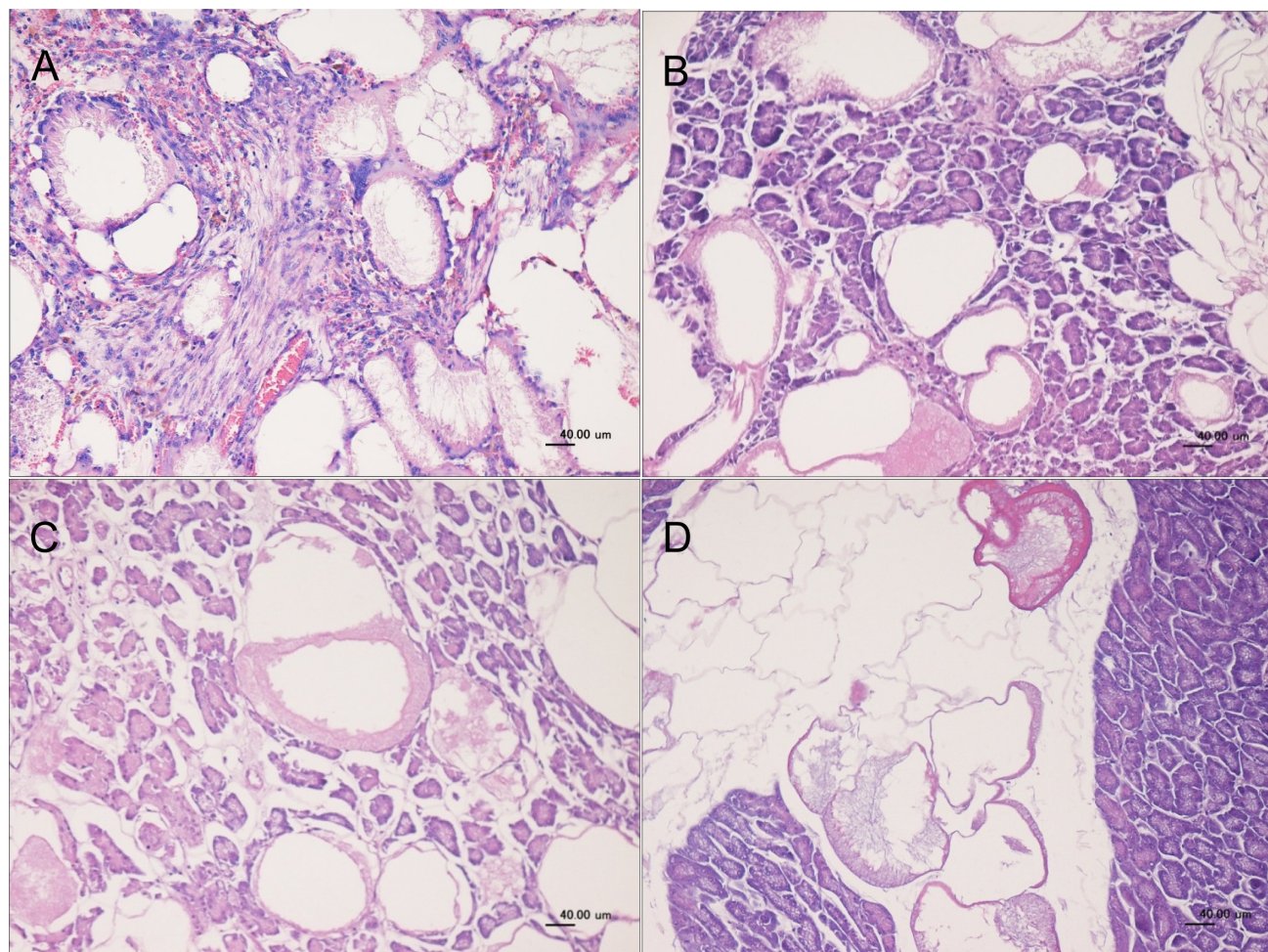
$\pm 6.0$  months and  $2.8 \pm 0.2$ , respectively. In the FN group, abdominal necrotic fat masses were observed in various locations, including the colon, rectum, retroperitoneum and pancreas.

#### *Pathological findings in necrotic fat masses and the pancreases*

Macroscopically, in the FN group, various organs including the pancreas, colon and rectum were encircled by hard adipose tissue with multifocal mineral deposits. The pancreases in the FN and N groups were enlarged and showed fatty light brown in color. Moreover, the cut surfaces of the pale and enlarged pancreases also showed excessive multifocal whitish strips of infiltrative adipose tissues accompanied by mineral deposits. The pancreases in HF group were macroscopically normal, and focal areas of the cut surface also exhibited infiltrative adipose tissue.

Histopathologically, the adipose masses surrounding the intestines in the FN group demonstrated severe fat necrosis with infiltration of inflammatory cells, especially macrophages and lymphocytes. Additionally, the formation of fat-laden foam cells, multinucleated giant cells and epithelioid cells was also noticeable (Fig. 1A). When the necrotic masses were located close to the intestine, aggressive infiltration of the fibrous tissues was observed, which invaded through the muscularis mucosal layer of the digestive tract. Scattered secondary calcium salt saponification was also observed in all lesions. Cholesterol clefts were occasionally found in the eosinophilic saponification adipocytes.

As shown in Fig. 1B and 1C, various grades of fatty infiltration (fatty pancreas), saponification (fat necrosis), acinar atrophy, infiltration of inflammatory cells and fibrosis were observed microscopically in the pancreases in the FN and N groups of JB cattle (Table 2). In addition, hemorrhage and calcification were observed in the samples from both groups. Plaques of saponification fat were observed in the pancreatic parenchyma and interlobular infiltrative adipose tissue, which were occasionally surrounded by inflammatory cells. Surprisingly, 73.3% (11 of 15) of the pancreases from the HF group also revealed minimal-to-moderate pancreatic fatty infiltration accompanied by either intrapancreatic or interlobular saponification (Fig. 1D) with less inflammatory and fibrous stromal infiltration (Table 3). Strikingly, 25 pancreatic samples (25 of 29; 86.2%) from both breeds showed various stages of fatty infiltration, and 27 pancreases (27 of 29; 93.1%) also showed fat necrosis in the pancreas, which was identified by evidence of saponification. Despite the macroscopically normal pancreases, fat necrosis in the pancreatic interlobular adipose tissue was obvious in the HF group. When comparing the two breeds, the lesions of fatty pancreas and pancreatic fat necrosis in the JB cattle were more severe than those in the HF cows.



**Fig. 1.** Histopathological findings of necrotic fat mass and the pancreases. (A) Necrotic fat mass from FN1. Severe fibrosis accompanied by macrophage accumulation and saponification was observed. (B) The pancreas from FN1. Severe fatty infiltration was observed in the pancreatic parenchyma, with eosinophilic saponification in the adipocytes. (C) The pancreas from N4. The lesions in the N group were similar to those in the FN group. (D) The pancreas from HF14. Fat necrosis was observed in the interlobular spaces of the pancreas. H&E. Bar=40 μm. FN, fat necrosis; N, control; HF, Holstein Friesian.

**Table 2.** Pathological changes in the pancreases of JB cattle

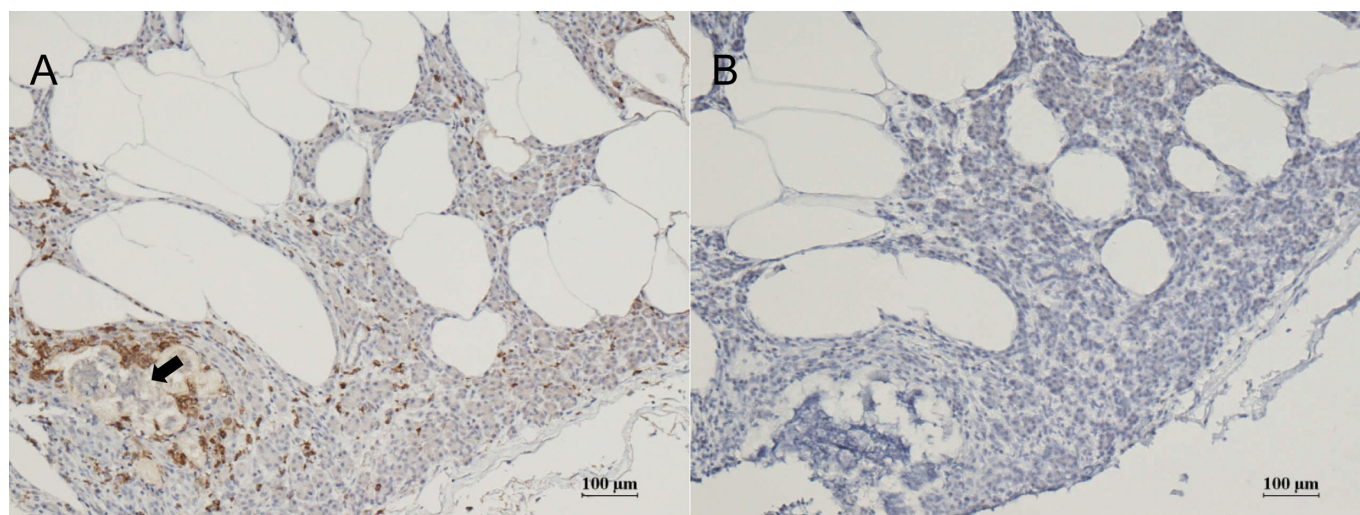
No.	Fatty pancreas	Fat necrosis (saponification)	Infiltration of inflammatory cells	Fibrosis
FN1	+++	+++	+	-
FN2	+++	+++	+++	+++
FN3	+++	+++	+++	++
FN4	+++	++	-	-
FN5	++	++	++	-
FN6	++	++	-	-
FN7	+++	+++	++	++
FN8	++	+	++	-
FN9	++	++	+++	++
N1	++	++	+	-
N2	+++	++	+++	-
N3	+++	++	++	-
N4	++	++	+++	-
N5	++	++	+	-

Pathological changes were semi-quantitatively graded as no (-), minimal (+), moderate (++) or severe changes (+++). JB, Japanese Black; FN, fat necrosis; N, control.

**Table 3.** Pathological changes in the pancreases of HF cows

No.	Fatty pancreas	Fat necrosis (saponification)	Infiltration of inflammatory cells	Fibrosis
HF1	++	+	-	-
HF2	+	+	-	-
HF3	+	+	-	-
HF4	+	+	-	-
HF5	+	+	-	-
HF6	+	+	-	-
HF7	+	+	+	-
HF8	+	++	-	-
HF9	-	-	-	-
HF10	-	-	-	-
HF11	++	+++	-	-
HF12	+	++*	-	-
HF13	+	++*	-	-
HF14	-	++*	-	-
HF15	-	++*	-	-

Pathological changes were semi-quantitatively graded as no (-), minimal (+), moderate (++) or severe changes (+++). HF, Holstein Friesian. \*Interlobular fat.



**Fig. 2.** Immunohistochemical sections of the pancreas from FN4. The serial sections were separately stained with anti-Iba-1 or CD3 antibody. (A) Large numbers of Iba-1-positive macrophages infiltrated into the pancreatic parenchyma and surrounded necrotic fat areas (arrow) and (B) there were few lymphocytic infiltrates (CD3-positive cells). HRP/DAB detection system. Bar=100  $\mu$ m. DAB, 3,3'-diaminobenzidine; FN, fat necrosis.

### *Predominant macrophages in the pancreas*

All of the pancreases exhibited a strong DAB immunoreaction against Iba-1 antibody, which is used as a macrophage marker (Fig. 2A). Iba-1 immunoreactivity was also strongly detected in macrophages surrounding the saponification fat. The scattered macrophages in the pancreatic parenchyma and fibrous stroma were also immunopositive. In contrast, positivity for CD3, which is known as a pan-T-lymphocyte marker, was occasionally seen (Fig. 2B). According to the semi-quantitative analysis, the numbers of CD3-positive T cells were significantly different among the FN, N and HF groups ( $P < 0.05$ ), whereas the numbers of Iba-1-positive cells were not. CD3-positive T cells were significantly more distributed in the pancreases from the FN and N groups compared with the HF group (FN vs HF;  $P < 0.05$  and N vs HF;  $P < 0.01$ , Fig. 3). Positive correlation between the numbers of CD3-positive T cells and those of macrophages was observed in FN and N groups as well as in FN, N and HF groups ( $\rho = 0.67$  and  $0.44$ , and  $P = 0.018$  and  $0.034$ , respectively).

### *JB cattle and HF cows*

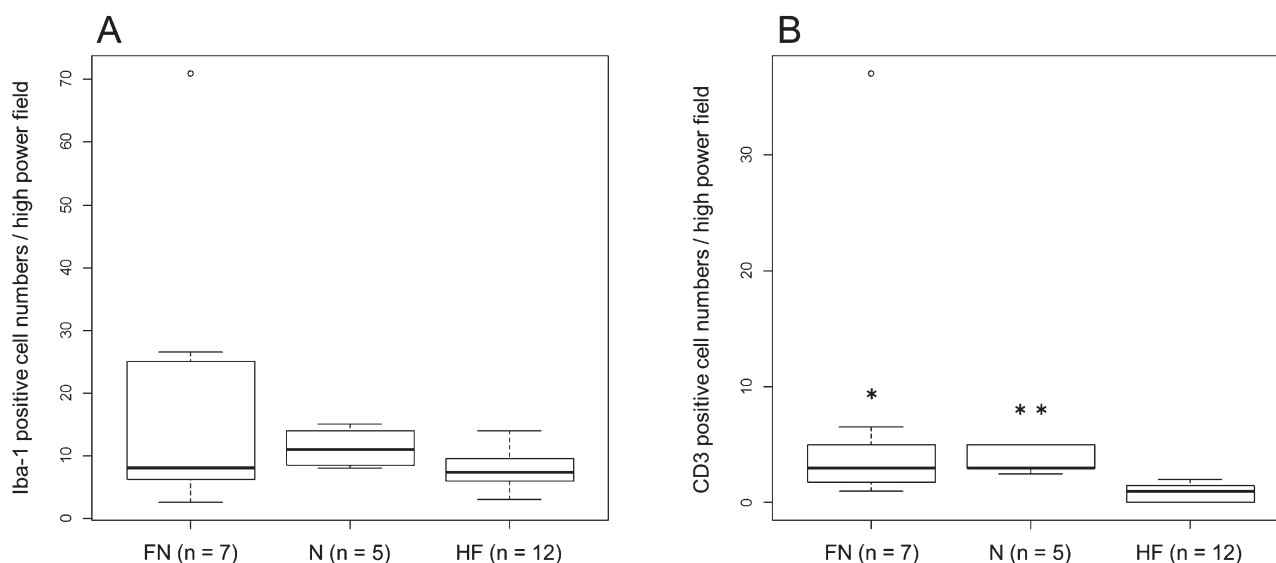
Association between the severity of four pancreatic lesions (*i.e.*, fatty pancreas, fat necrosis, infiltration of inflammatory cells and fibrosis) was estimated among all cattle and cows. Any of combination was significant. Fatty pancreas was associated with fat necrosis ( $\rho = 0.81$ ,  $P$  value  $< 0.001$ ), inflammatory cells ( $0.69$ ,  $< 0.001$ ) and fibrosis ( $0.45$ ,  $< 0.05$ ). Fat necrosis was associated with inflammatory cells ( $0.56$ ,  $< 0.01$ ) and fibrosis ( $0.51$ ,  $< 0.01$ ). Inflammatory cells was associated with fibrosis ( $0.57$ ,  $< 0.01$ ). BCS was associated with fatty pancreas ( $0.88$ ,  $< 0.001$ ), fat necrosis ( $0.74$ ,  $< 0.001$ ), inflammatory cells ( $0.73$ ,  $< 0.001$ ) and fibrosis ( $0.38$ ,  $< 0.05$ ). Furthermore, number of CD3-positive cells was also associated with fatty pancreas ( $0.74$ ,  $< 0.001$ ), fat necrosis ( $0.63$ ,  $< 0.001$ ), inflammatory cells ( $0.58$ ,  $< 0.01$ ) and the number of Iba-1-positive cells ( $0.44$ ,  $< 0.05$ ). Odds ratio and 95% confidence interval for fatty pancreas, fat necrosis and inflammatory cells were  $8.1$  ( $1.74$ – $37.5$ ,  $P < 0.01$ ),  $6.5$  ( $1.49$ – $28.5$ ,  $P < 0.05$ ) and  $2.5$  ( $1.18$ – $5.37$ ,  $P < 0.05$ ), respectively.

### *HF group*

BCS was associated with fatty pancreas ( $0.58$ ,  $< 0.01$ ) and fat necrosis ( $0.57$ ,  $< 0.05$ ). Fatty pancreas was also associated with fat necrosis ( $0.62$ ,  $< 0.05$ ).

### *FN group and N group*

Firstly, the FN and N groups were compared. Age and BCS were not significantly different between these groups. Additionally, no significant difference was observed between these groups with regard to the prevalence of the pancreatic lesions. Furthermore, no significant difference was observed regarding the severity of the four pancreatic lesions between the FN and N groups. Since the FN and N groups are considered to be homogeneous except for whether they had necrotic fat masses or not, correlations among the severity of the five factors (*i.e.*, necrotic fat mass, fatty pancreas, fat necrosis in the pancreas, infiltration of inflammatory cells and fibrosis) were estimated. Spearman's  $\rho$  and  $P$  values are shown in Table 4, and three positive correlations were observed. Fat infiltration in the pancreas was correlated with fat necrosis in the pancreas ( $\rho = 0.65$  and  $P = 0.012$ ). Fibrosis in the pancreas was correlated with both fat necrosis in the pancreas ( $0.64$  and  $0.013$ ) and the infiltration of inflammatory cells ( $0.56$  and  $0.038$ ). Furthermore, fibrosis in the pancreas and the formation of necrotic fat masses in the peritoneal cavity may be correlated ( $0.47$ ,  $0.093$ ). The number of CD3-positive cells was correlated with that of Iba-1-positive cells ( $0.67$ ,  $< 0.05$ ).



**Fig. 3.** Number of immunopositive cells in 10 high-power fields for anti-Iba-1 antibody (A) and anti-CD3 antibody (B). Iba-1-positive cells were not significantly different among the FN, N and HF groups. CD3-positive cells were significantly different among these three groups ( $P < 0.01$  by Kruskal-Wallis rank sum test). \*, \*\* Significant difference from the HF group with  $P < 0.05$  and  $P < 0.01$ , respectively. FN, fat necrosis; N, control; HF, Holstein Friesian.

**Table 4.** Correlation between necrotic fat mass and pancreatic lesions in the FN and N groups

	Necrotic fat mass	Fatty pancreas	Pancreatic fat necrosis	Infiltration of inflammatory cells	Fibrosis
Necrotic fat mass	–				
Fatty pancreas	0.15 ( $P=0.61$ )				
Pancreatic fat necrosis	0.33 ( $P=0.25$ )	0.65 ( $P=0.012$ )			
Infiltration of inflammatory cells	–0.077 ( $P=0.78$ )	0.15 ( $P=0.61$ )	0.19 ( $P=0.51$ )		
Fibrosis	0.47 ( $P=0.093$ )	0.33 ( $P=0.24$ )	0.64 ( $P=0.013$ )	0.56 ( $P=0.038$ )	–

Spearman's rank correlation rho and  $P$  value in the FN and N groups. FN, fat necrosis; N, control.

## DISCUSSION

Fat necrosis usually occurs in livestock, especially in JB cattle [31]. Its incidence is associated with economic loss in the Japanese cattle industry, because it causes intestinal obstruction, which can lead to death [3, 12, 29, 31, 36, 39, 40]. In general, it is mostly accidentally diagnosed by rectal palpation or during the slaughtering process. In the present study, necrotic fat masses were observed in various locations, including the colon, rectum, retroperitoneum and pancreas. These locations were consistent with those reported in previous studies [12, 31].

Shimada *et al.* [31] reported that the incidence of fat necrosis in JB cattle was higher than that in other breeds and that the lack of exercise in the pen and excessive feeding with concentrates combined with a shortage of roughage could induce abdominal fat necrosis in JB breeding cows. In addition, obesity was considered to be a predisposing factor for abdominal fat necrosis. BCS is a management tool that can be used to evaluate the nutritional status in cattle [8]. Normally, JB fattening cattle are given high-energy diets for producing marbled beef, while HF cows are fed suitable diets for milk production. Thus, BCSs in JB cattle are typically higher than those in HF cows. In this study, significant association was noted between BCS and pancreatic lesions.

The pathological findings showed that necrotic fat masses near the pancreas, colon and rectum were encircled by hard adipose tissue with multifocal mineral deposits. These findings were consistent with those of a previous report [12]. Histopathological analysis of the pancreases indicated that obese cattle tended to have fatty pancreases accompanied by saponification. Fatty infiltration of the pancreas occurs occasionally in cats, pig and cattle, usually as part of generalized obesity but sometimes restricted to the pancreas. Although there may be some pressure atrophy of the parenchyma, fatty infiltration is not functionally significant [13]. In association with abdominal fat necrosis, marked fat infiltration in the pancreas has been reported in a miniature Zebu bull affected by fat necrosis [28].

To confirm the inflammation in the pancreas, we investigated DAB immunoreaction of Iba-1 antibody, which is used as a macrophage marker [9], as well as positivity for CD3, which is known as a pan-T-lymphocyte marker [5]. Infiltration of CD3-positive T cells was more severe in the pancreases from the FN and N groups than in those from the HF group, whereas

macrophage distribution was not significantly different among the three groups. One of the main reasons for this is the fact that the numbers of cattle and cows were insufficient to compare the macrophage numbers among the groups. In fact, a positive correlation was found between the number of T cells and that of macrophages in the present study. Generally, infiltration of T cells is followed by that of macrophages. T cells have an essential role in the initiation and propagation of adipose inflammation [21]. Therefore, obesity was suggested to worsen the inflammatory status in the pancreas of cattle.

Regarding the correlation between necrotic fat mass and pancreatic lesions, fatty pancreas was related to pancreatic fat necrosis. However, infiltration of inflammatory cells was not related to pancreatic fat necrosis. These results may indicate different pathological stages, such as fatty pancreas accompanied by pancreatic fat necrosis and inflammation followed by fibrosis. Although it is unclear whether intrapancreatic fat evokes inflammation in the pancreas, nonesterified fatty acids generated from increased intrapancreatic fat in obese humans may exacerbate local pancreatic injury during acute pancreatitis [20].

Moreover, according to the results of our study, pancreatic fat necrosis and infiltration of inflammatory cells were related to fibrosis. Furthermore, fibrosis in the pancreas exhibited a correlative tendency with the formation of necrotic fat masses in the peritoneal cavity ( $P < 0.1$ ). Visceral fat necrosis has been known to occur with acute pancreatitis for over 100 years [10]. It has been recently suggested that a higher body mass index or obesity is associated with severe acute pancreatitis [18, 24, 26, 32]. Pancreatic fat increases with obesity [29], and increased fat deposition has been reported in the exacerbation of acute pancreatitis in humans [20]. Additionally, fibrosis of the pancreas results from chronic pancreatitis or duct obstruction, and the end-stage changes are associated with exocrine and endocrine insufficiency (diabetes mellitus) [17]. According to the results of our study, a similar pathological process in the pancreas to that in humans was noted in cattle. Namely, a higher BCS induces the infiltration of adipocytes into the pancreas followed by pancreatic fat necrosis or inflammation. As a result, the damaged regions are replaced by fibrous tissue. Chronic pancreatitis in a dog can lead to fat necrosis in the pancreas and mesentery which results from enzymatic digestion of lipids [4]. Visceral fat necrosis occurs as a result of pancreatic lipase leakage from the inflamed pancreas in obese mice with cerulein-induced pancreatitis [25]. In this study, necrotic fat masses were also observed in peripancreatic visceral adipose tissue (FN2 and 3). Therefore, although the leakage of pancreatic lipase was not examined in the present study, the inflammation of the pancreas caused by obesity might be associated with the etiology of bovine abdominal fat necrosis.

In conclusion, the results of this study indicated that obesity exacerbates pancreatic lesions in cattle, including fatty pancreas and pancreatitis. The pathological lesions in the pancreas may play a key role in abdominal fat necrosis through the inflammatory process. Further studies are essential to elucidate the cause and effect relationship between pancreatic disorder and abdominal fat necrosis.

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## REFERENCES

1. Acharya, C., Navina, S. and Singh, V. P. 2014. Role of pancreatic fat in the outcomes of pancreatitis. *Pancreatology* **14**: 403–408. [Medline] [CrossRef]
2. Aydin, Y., Temizsoyulu, D., Toplu, N. and Vural, S. A. 2002. Diffuse mesenteric lipodystrophy (massive fat necrosis) in a cat. *Aust. Vet. J.* **80**: 346–348. [Medline] [CrossRef]
3. Bridge, P. S. and Spratling, F. R. 1962. Bovine lipomatosis. *Vet. Rec.* **74**: 1357–1362.
4. Brown, D. L., Van Wettere, A. J. and Cullen, J. M. 2016. pp. 412–470. *In: Pathologic Basis of Veterinary Disease*, 6th ed. (Zachary, J. F. ed.), Elsevier, St. Louis.
5. Cantón, G. J., Katzer, F., Benavides-Silvan, J., Maley, S. W., Palarea-Albaladejo, J., Pang, Y., Smith, S., Bartley, P. M., Rocchi, M., Innes, E. A. and Chianini, F. 2013. Phenotypic characterisation of the cellular immune infiltrate in placentas of cattle following experimental inoculation with *Neospora caninum* in late gestation. *Vet. Res. (Faisalabad)* **44**: 60. [Medline] [CrossRef]
6. Charles, J. A. 2007. Pathology of domestic animals: volume 2. pp. 389–424. *In: Pancreas*, 5th ed. (Maxie, M. G. ed.), Saunders, Philadelphia.
7. el-Sebaie, A. and el-Amrousi, S. 1995. Bovine fat necrosis (Lipomatosis): an overview. *Berl. Munch. Tierarztl. Wochenschr.* **108**: 427–430. [Medline]
8. Ferguson, J. D., Galligan, D. T. and Thomsen, N. 1994. Principal descriptors of body condition score in Holstein cows. *J. Dairy Sci.* **77**: 2695–2703. [Medline] [CrossRef]
9. Golbar, H. M., Izawa, T., Juniantito, V., Ichikawa, C., Tanaka, M., Kuwamura, M. and Yamate, J. 2013. Immunohistochemical characterization of macrophages and myofibroblasts in fibrotic liver lesions due to *Fasciola* infection in cattle. *J. Vet. Med. Sci.* **75**: 857–865. [Medline] [CrossRef]
10. Hotchkiss, L. W. 1912. VIII. Acute pancreatitis with very extensive fat necrosis. *Ann. Surg.* **56**: 111–117. [Medline] [CrossRef]
11. Ito, T. 1973. A pathological study on fat necrosis in swine. *Jpn. J. Vet. Sci.* **35**: 299–310. [Medline] [CrossRef]
12. Ito, T., Miura, S., Ohshima, K. and Numakunai, S. 1968. Pathological studies on fat necrosis (lipomatosis) in cattle. *Jpn. J. Vet. Sci.* **30**: 141–150. [Medline] [CrossRef]
13. Jubb, K. V. F. and Stent, A. W. 2016. Pancreas. pp. 353–373. *In: Jubb, Kennedy, and Palmer's Pathology of Domestic Animals*, 6th ed. (Maxie, M. G. ed.), Elsevier, St. Louis.
14. Katamoto, H., Aoki, M., Shimada, Y. and Hakogi, E. 1996. Lipoprotein lipase activity of post-heparin plasma in Japanese black cattle affected with fat necrosis. *Br. Vet. J.* **152**: 339–345. [Medline] [CrossRef]
15. Katamoto, H., Kurihara, S. and Shimada, Y. 1990. Effects of isoprothiolane and phytosterol on lipogenesis and lipolysis in adipocytes from rats of dietary fat necrosis. *Jpn. J. Vet. Sci.* **52**: 1189–1197. [Medline] [CrossRef]
16. Katamoto, H., Yukawa, T. and Shimada, Y. 1996. Lipogenic and lipolytic activities in isolated adipocytes from cattle with fat necrosis. *Res. Vet. Sci.* **61**: 214–217. [Medline] [CrossRef]

17. Klöppel, G., Detlefsen, S. and Feyerabend, B. 2004. Fibrosis of the pancreas: the initial tissue damage and the resulting pattern. *Virchows Arch.* **445**: 1–8. [Medline]
18. Mentula, P., Kylänpää, M. L., Kemppainen, E., Repo, H. and Puolakkainen, P. 2007. Early inflammatory response in acute pancreatitis is little affected by body mass index. *Scand. J. Gastroenterol.* **42**: 1362–1368. [Medline] [CrossRef]
19. Motoi, Y., Kinno, S., Minamino, K., Shimbayashi, K. and Ushimi, C. 1984. Treatment and clinicobiochemical observations of cows affected with fat necrosis. *Jpn. J. Vet. Sci.* **46**: 281–289. [Medline] [CrossRef]
20. Navina, S., Acharya, C., DeLany, J. P., Orlichenko, L. S., Baty, C. J., Shiva, S. S., Durgampudi, C., Karlsson, J. M., Lee, K., Bae, K. T., Furlan, A., Behari, J., Liu, S., McHale, T., Nichols, L., Papachristou, G. I., Yadav, D. and Singh, V. P. 2011. Lipotoxicity causes multisystem organ failure and exacerbates acute pancreatitis in obesity. *Sci. Transl. Med.* **3**: 107–110. [Medline] [CrossRef]
21. Nishimura, S., Manabe, I., Nagasaki, M., Eto, K., Yamashita, H., Ohsugi, M., Otsu, M., Hara, K., Ueki, K., Sugiura, S., Yoshimura, K., Kadowaki, T. and Nagai, R. 2009. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat. Med.* **15**: 914–920. [Medline] [CrossRef]
22. Oka, A., Iwamoto, E. and Tatsuda, K. 2015. Effects of clay on fat necrosis and carcass characteristics in Japanese Black steers. *Anim. Sci. J.* **86**: 878–883. [Medline] [CrossRef]
23. Oka, A., Yamasaki, T., Shibatani, M., Suzuki, T. and Saito, T. 1988. Efficacy of isoprothiolane for the treatment of fat necrosis in cattle. *Br. Vet. J.* **144**: 507–514. [Medline] [CrossRef]
24. O’Leary, D. P., O’Neill, D., McLaughlin, P., O’Neill, S., Myers, E., Maher, M. M. and Redmond, H. P. 2012. Effects of abdominal fat distribution parameters on severity of acute pancreatitis. *World J. Surg.* **36**: 1679–1685. [Medline] [CrossRef]
25. Patel, K., Trivedi, R. N., Durgampudi, C., Noel, P., Cline, R. A., DeLany, J. P., Navina, S. and Singh, V. P. 2015. Lipolysis of visceral adipocyte triglyceride by pancreatic lipases converts mild acute pancreatitis to severe pancreatitis independent of necrosis and inflammation. *Am. J. Pathol.* **185**: 808–819. [Medline] [CrossRef]
26. Pavlidis, T. E., Pavlidis, E. T. and Sakantamis, A. K. 2010. Advances in prognostic factors in acute pancreatitis: a mini-review. *HBPD INT* **9**: 482–486. [Medline]
27. Pereda, J., Pérez, S., Escobar, J., Arduini, A., Asensi, M., Serviddio, G., Sabater, L., Aparisi, L. and Sastre, J. 2012. Obese rats exhibit high levels of fat necrosis and isoprostanes in taurocholate-induced acute pancreatitis. *PLOS ONE* **7**: e44383. [Medline] [CrossRef]
28. Reed, S. D. and Evans, D. E. 2010. Necrotizing infiltrative lipomatosis in a miniature Zebu bull (*Bos primigenius indicus*). *Vet. Med. Int.* **2010**: 810496. [Medline] [CrossRef]
29. Saisho, Y., Butler, A. E., Meier, J. J., Monchamp, T., Allen-Auerbach, M., Rizza, R. A. and Butler, P. C. 2007. Pancreas volumes in humans from birth to age one hundred taking into account sex, obesity, and presence of type-2 diabetes. *Clin. Anat.* **20**: 933–942. [Medline] [CrossRef]
30. Santos, A. D. S., Bandarra, P. M., Sonne, L., Pavarini, S. P. and Driemeier, D. 2008. Intestinal obstruction due to massive fat necrosis (lipomatosis) in a Jersey cow. *Cienc. Rural* **38**: 1483–1485. [CrossRef]
31. Shimada, Y., Katamoto, H., Ishida, S., Kobayashi, K. and Tohyoh, H. 1988. Therapeutic effect of isoprothiolane on bovine fat necrosis. *Jpn. J. Vet. Sci.* **50**: 1017–1024. [Medline] [CrossRef]
32. Shin, K. Y., Lee, W. S., Chung, D. W., Heo, J., Jung, M. K., Tak, W. Y., Kweon, Y. O. and Cho, C. M. 2011. Influence of obesity on the severity and clinical outcome of acute pancreatitis. *Gut Liver* **5**: 335–339. [Medline] [CrossRef]
33. Smith, G. W., Rotstein, D. S. and Brownie, C. F. 2004. Abdominal fat necrosis in a pygmy goat associated with fescue toxicosis. *J. Vet. Diagn. Invest.* **16**: 356–359. [Medline] [CrossRef]
34. Storck, G. and Björntorp, P. 1971. Chemical composition of fat necrosis in experimental pancreatitis in the rat. *Scand. J. Gastroenterol.* **6**: 225–230. [Medline] [CrossRef]
35. Suganami, T. and Ogawa, Y. 2010. Adipose tissue macrophages: their role in adipose tissue remodeling. *J. Leukoc. Biol.* **88**: 33–39. [Medline] [CrossRef]
36. Tharwat, M. and Buczinski, S. 2012. Diagnostic ultrasonography in cattle with abdominal fat necrosis. *Can. Vet. J.* **53**: 41–46. [Medline]
37. Van Minnen, L. P., Besselink, M. G., Bosscha, K., Van Leeuwen, M. S., Schipper, M. E. and Gooszen, H. G. 2004. Colonic involvement in acute pancreatitis. A retrospective study of 16 patients. *Dig. Surg.* **21**: 33–38, discussion 39–40. [Medline] [CrossRef]
38. Vítovec, J., Proks, C. and Valvoda, V. 1975. Lipomatosis (fat necrosis) in cattle and pigs. *J. Comp. Pathol.* **85**: 53–59. [Medline] [CrossRef]
39. Williams, D. J., Tyler, D. E. and Papp, E. 1969. Abdominal fat necrosis as a herd problem in Georgia cattle. *J. Am. Vet. Med. Assoc.* **154**: 1017–1021. [Medline]
40. Yilmaz, A. and Gülbahar, M. Y. 1995. Massive fat necrosis in a cow. *Ankara Univ. Vet. Fak. Derg.* **42**: 139–141.