Liver Function in Cows with Retained Placenta

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Abstract: The aim of the present study was to compare the liver function of cows with retained placenta (n = 21) with that of control post-partum cows (n = 12). Liver biopsies of the cows with retained placenta showed that the mean percentage of fat in the liver parenchyma was 32.5%. The serum glucose, albumin, cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), total calcium and inorganic phosphorus levels of cows with retained placenta were significantly lower than those of control cows whereas the aspartate aminotransferase (AST) and gamma glutamyltransferase (GGT) serum concentrations of cows with retained placenta were greater than those of control cows. In conclusion, this study indicated that retained placenta might be associated with fatty liver.

Key Words: Cow, retained placenta, liver function.

Retensiyo Sekundinarum'lu İneklerde Karaciğer Fonksiyonu

Özet: Sunulan çalışmanın amacı, postpartum dönemdeki normal ineklerle (n = 12) retensiyo sekundinarum şekillenmiş ineklerin (n = 21) karaciğer fonksiyonlarını karşılaştırmaktır. Retensiyo sekundinarumlu ineklerden alınan karaciğer biyopsi örneklerinin incelenmesi sonucu karaciğer paranşimindeki ortalama yağ oranı % 32,5 olarak belirlendi. Retensiyo sekundinarumlu ineklerde serum glukoz, albumin, kolesterol, trigliserid, yüksek densiteli protein (HDL), düşük densiteli protein (LDL), total kalsiyum ve inorganik fosfor seviyeleri kontrol grubuna göre önemli oranda düşük olmakla birlikte, serum aspartat aminotransferaz (AST) ve gamma glutamiltransferaz (GGT) seviyeleri kontrol grubundan yüksekti. Bu çalışmada, retensiyo sekundinarumla karaciğer yağlanması arasında bir ilişki olabileceği sonucuna varıldı.

Anahtar Sözcükler: İnek, retensiyo sekundinarum, karaciğer fonksiyonu.

Introduction

Extending the interval between successive calvings causes important economic losses. Retained placenta is an important disorder that a variable degree of metritis commonly accompanies. When retained placenta is accompanied by metritis, a decreased pregnancy rate, increased calving-conception intervals and culling rate and a decrease of milk production are observed (1-4).

Another disorder of high producing dairy cows in early lactation is fatty liver (5-8). The development of fatty liver in cows may adversely affect immune defence mechanisms and increase the susceptibility to infectious diseases. Fatty liver has been shown to result from problems in postparturient dairy cows, including metabolic diseases, displaced abomasum, retained placenta, metritis, mastitis and susceptibility to infertility (2,3,6,7,9-11).

The aim of the present study was to evaluate the liver function in cows with retained placenta.

Materials and methods

The present study was conducted at the Livestock Research Institute in Konya, Turkey. A total of 21 Brown-Swiss cows with retained placenta were used. Twelve healthy Brown-Swiss post-partum cows were selected as control cows. The ages of both healthy and diseased cows ranged from 5 to 10 years. All the cows were in the third or later lactation.

Blood and biopsy samples were collected about 7 days after calving. Blood samples were taken from the jugular vein just before the liver biopsies took place, i.e. between 10.00 and 11.00 h. Precautions were taken to avoid unnecessary stress at bleeding. Sera were obtained and immediately centrifuged for 20 minutes at 3000 rpm. Collected sera were stored at - 20 °C until assayed for analysis. Sera were analysed for glucose, urea, creatinine, total protein, albumin, globulin, total bilirubin (T-bil), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), creatine phosphokinase (CPK), cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), total calcium and inorganic phosphorus levels. All the analyses were performed on an automated analyser (Olympus AU 5200) using commercial test kits.

Liver biopsy was performed in the right side of the eleventh intercostal space by percutaneous needle biopsy from each animal. Liver samples were put in 10% formaldehyde-Ca solution and fixed for 16 hours. Thin sections (12 μ m) were cut from each sample, stained with Sudan Black B and Oil Red O and examined under light microscopy. The percentage volume of visible fat in hepatic parenchyma cells was estimated by sterogical point counting method (5). Five fields from each sample were examined at x 1100 though the oil immersion lens

and a 100 point eyepiece graticule. The average volume fraction of liver cell parenchyma occupied by Oil Red O positive droplets was recorded. Fat infiltration less than 10 μ m²/100 μ m² was considered mild, and 10-20 μ m²/100 μ m² moderate and over 20 μ m²/100 μ m² severe (6).

A two-sample t-test (Minitab statistical package) was performed to compare the serum concentrations of biochemical marker between cows with retained placenta and control cows.

Results

Stereological analysis of liver biopsies samples of the 21 cows with retained placenta revealed a mild, a moderate and a severe fatty liver in 3, 5 and 13 cows, respectively. The mean percentage of fat in the liver parenchyma of cows with retained placenta was $32.5 \pm 2.52\%$ (mean \pm SEM). The cows with severe fatty liver exhibited the typical signs of anorexia and depression, and a marked decrease milk production and progressive debilitation. No fat infiltration was observed in the liver biopsy samples of the control cows.

Biochemical parameters obtained from cows with retained placenta and controls are shown in the Table.

Parameters	Retained Placenta (n: 21)	Control (n: 12)	Р
Glucose (mg/100 ml)	61.6 ± 6	88.9 ± 7.3	0.0076**
Urea (mg/100 ml)	23.95 ± 2.2	19.58 ± 6.27	0.13
Creatinine (mg/100 ml)	1.33 ± 0.24	1.25 ± 0.08	0.75
T-Protein (g/100 ml)	7.37 ± 0.22	7.77 ± 0.16	0.15
Albumin (g/100 ml)	2.93 ± 0.07	3.32 ± 0.07	0.0011**
Globulin (g/100 ml)	4.43 ± 0.19	4.45 ± 0.13	0.96
T-Bilirubin (mg/100 ml)	0.50 ± 0.09	0.32 ± 0.051	0.10
AST (U/I)	120.6 ± 19	78.5 ± 9	0.045*
GGT (U/I)	42.4 ± 7.8	22.33 ± 2.1	0.021*
CPK (U/I)	239 ± 53	154.5 ± 21	0.15
Cholesterol (mg/100 ml)	96.1 ± 6.8	141.3 ± 9.8	0.0009***
Triglyceride (mg/100 ml)	15.57 ± 1.4	21.5 ± 1.6	0.0092**
HDL (mg/100 ml)	74 ± 5.5	97.9 ± 4.3	0.0018**
LDL (mg/100 ml)	19.4 ± 2.7	39.9 ± 7	0.017*
T-Ca++ (mg/100 ml)	7.89 ± 0.1	8.75 ± 0.21	0.0017**
I- Phosphorus (mg/100 ml)	4.43 ± 0.27	5.78 ± 0.43	0.016*

Table. Biochemical parameters in cows with retained placenta and control groups and their statistical significance (mean ± SEM).

* P < 0.05 ** P < 0.01 *** P < 0.001

The mean serum levels of AST and GGT levels of cows with retained placenta were significantly greater than those of control cows while the mean serum levels of glucose, albumin, cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), total calcium and inorganic phosphorus of cows with retained placenta were significantly lower when compared to those of control cows.

Discussion

Our results suggest that a fatty liver is associated with the retention of placenta in cows. Indeed, we have shown that the mean percentage of fat in the liver of cows with retained placenta is $32.5 \pm 2.52\%$ (mean \pm SEM). The fat infiltration of the liver was associated with an increase in hepatic enzymes serum concentrations (AST, GGT) and a decrease in serum levels of glucose, albumin, cholesterol, triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL), total calcium and inorganic phosphorus.

In dairy cows, fatty liver has been shown to result in a variety of problems during the peripartum period, including metabolic diseases and increased susceptibility to infections (2,6,11-14). Morrow et al. (15) and West (16) found a high incidence of retained placenta accompanied by metritis. Aslan et al. (6) and Nizamlioğlu et al. (17) observed a moderate fatty liver and a mean percentage of fat infiltration of 25% in cows with retained placenta.

Mild and moderate fatty liver, but not severe fatty liver, may result in a liver dysfunction without hepatocyte destruction and a subsequent increase in liver specific serum enzyme activities (8,11,18-20). Endotoxin dependent infectious diseases such as metritis cause destruction and necrosis of the liver and this phenomenon is associated with various degrees of hepatic dysfunction (21). Hepatic function can be severely impaired by fatty infiltration of the liver. One of the numerous consequences of fatty infiltration of the liver is a drop in serum albumin levels (22) since albumin serum level is a marker of the liver's synthetic function (16,23). In the present study, the albumin and serum levels of cows with retained placenta were lower than those of control cows, suggesting that the hepatic function is altered. The high serum glucose levels of the control cows do not allow us to conclude that the serum glucose levels are decreased in these cows.

The measurement of serum activities of hepatic enzymes can be useful, but it has limitations (8,18). Indeed, AST activity in serum is fairly well correlated to fatty liver, but this enzyme is non-specific of hepatic tissue (2,24). AST activity should be interpreted in conjunction with that of a liver-specific enzyme, such as GGT, or muscle-specific enzyme, such as CPK, to determine the source of the tissue insult (8,25). Lotthammer (26) has shown that serum activity of AST is a very sensitive indicator of liver disorders for clinical and subclinical diagnosis. Bogin et al. (18) and Sevinc et al. (8) have shown that cows with fatty liver had higher levels of GGT and AST. In the present study, the increase in AST and GGT activities in cows with retained placenta when compared to control cows may be due to the accumulation of lipids in the hepatocytes.

Abnormal lipid and lipoprotein concentrations are often associated with liver disorders (27-29). Several authors (8,17,28,30-32) reported that triglyceride, cholesterol and HDL-cholesterol concentrations are decreased in cows with fatty liver. In the present study we have shown that the serum levels of cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol of cows with retained placenta are lower than those of control COWS. The decrease of LDL-cholesterol serum concentration in cows with retained placenta could result from several mechanisms including a decrease in the conversion of VLDL to LDL. Another explanation for the LDL decrease could be an increase in LDL catabolism. The decrease in serum HDL-cholesterol level may be related to the lower cholesterol serum levels of cows with retained placenta because HDL-cholesterol consists of about 60% cholesterol (28,33).

The decrease in calcium serum levels can be attributed to the fatty liver, which induces a decrease in calcium mobilisation (6,9). Such hypocalcaemia might play an important role in the incidence of retained placenta and uterine infections (2,9,12,34). Decreases in calcium and phosphorus serum levels in cows with retained placenta were previously described (6,11,14,35).

In conclusion, our results indicate that notable changes in serum lipid and lipoprotein levels and of some biochemical parameters altered in cows with retained placenta might be useful for the assessment of liver function.

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