大韓獸醫學會誌(1991) 第31卷 第1號
Korean J Vet Sci (1991) 31(1):109~121

Fine needle aspiration biopsy for the diagnosis of fatty liver in cattle

Bum-tae Hwang, Hong-ryul Han
College of Veterinary Medicine, Seoul National University
(Received Oct 20, 1990)

Fine needle aspiration biopsy에 의한 소의 脂肪肝診斷

黃 範 泰 · 韓 弘 栗
서울大學校 獸醫科大學
(1990. 10. 20 접수)

초록 : 소의 脂肪肝의 罹患率을 調査하고 fine needle aspiration biopsy에 의한 脂肪肝의 細胞學的 診斷을 試圖하였다.

肉眼的 또는 浮遊法에 의한 肝臟脂肪含量에 基礎한 소의 脂肪肝의 發生率은 韓牛암소에서 0.30%, 乳牛암소에서 4.70%, 乳牛숫소에서 0.15%였다. 脂肪肝은 肉眼的 所見으로 肝臟이 腫大되고 邊緣이 둔하며 가볍고 蒼白에서 朱黃色 色調를 보이나 肝臟의 色調와 脂肪肝의 重症度間에 恒常 相關關係가 認定되지는 않았다. 대부분의 正常肝臟과 重度의 脂肪肝을 除外하고는 肝臟의 脂肪浸潤은 中心靜脈周圍에서는 巨大脂肪球와 邊緣部에서는 小脂肪球 그리고 脈管部에 脂肪浸潤의 所見을 보였다. 浮遊法에 의한 肝臟脂肪含量과 比較한 細胞學上의 敏感度, 特異度, 正確度는 正常에서 94.4%, 95.2%, 94.9%, 輕度에서 64.3%, 100%, 87.2%, 中等度에서 100%, 83.3%, 82.2%, 그리고 重度에서 모두 100%였다. 細胞學的 所見은 組織學的 所見과 잘 一致하였다. Fine needle aspiraton biopsy에 의한 合併症은 臨床的으로 認知되지 않았다.

結論的으로 fine needle aspiration biopsy에 의한 細胞學的 診斷은 소의 脂肪肝 診斷에 있어서 組織學的 技法과 比較했을 때 單純하고 迅速하며 安全하며 經濟的인 方法으로 思料된다.

Key words: cattle, fatty liver, cytology, fine needle, aspiration biopsy.

Introduction

Fatty liver syndrome, fat cow syndrome, and hepatic lipidosis are terms that have been used to describe a disease syndrome in periparturient dairy cows. Fatty infiltration of the liver is part of a generalized fat mobilization syndrome which occurs in early lactation, particularly in high yielding dairy cows, as milk production outstrips appetite and body reserves are used to meet the energy deficiency.[1] Fatty liver was also induced by fasting in both nonlactating and lactating cows.[2,3] The mobilization of subcutaneous and internal body fat can result in the accumulation of fat in various organs such as liver, kidney, and skeletal and cardiac muscle.[4] The clinical fatty liver syndrome is associated with an increased incidence of postparturient disease,[5] suppression of immune response,[6] impaired reproductive function and reduced fertility.[7] Fifty per cent incidence of severe fatty liver was found in high yielding dairy cows.[8] Sixty six per cent of Friesian cows and thirty three per cent of Guernsey cows had a severe or moderate fatty liver in high yielding dairy cows.[9]

Diagnosis of clinical or subclinical fatty liver includes history taking, clinical pathology, and liver biopsy results. Fat content of biopsy samples can be estimated by one of many chemical or histological methods or buoyancy. Chemical methods generally involve estimation of either hepatic total lipid or triglyceride content.[10,11] In histological methods, hepatic lipid content may be estimated by point counting methods using plastic sections stained with toluidine blue[12~14] or frozen sections stained with Oil red O.[14] Hepatic lipid content can be estimated by buoyancy of needle biopsy samples in water or copper sulfate solutions[15] or glycerin solutions.[16] Fatty liver was diagnosed using an equation based on blood concentrations of nonesterified fatty acids, glucose, and AST at 7 to 13 days after calving.[17] In addition, a number of blood constituent change in the cows with fatty liver,[4,7,17~21] but serum chemistry values and liver function tests are generally not well correlated to the severity of fatty liver. When severe, it appears to result in clinical disease,[22] but clinical diagnosis is difficult because the signs are vague and nonspecific. Therefore, the reliable method to diagnose fatty liver remains the liver biopsy, but if rare, conventional liver biopsy has been accompanied by liver tear, hemorrhage, pneumothorax, peritonitis, subcutaneous emphysema, puncture of thoracic duct, increase of respiratory rate and pulse rate, apprehension, and death.[23~25]

In human medicine, the simple, less traumatic, and inexpensive fine needle aspiration biopsy has been performed on very wide indications such as biochemical analysis of aspirates,[26,27] nonneoplastic diseases,[28~34] and various tumors.[35~38] Fine needle aspiration biopsy has been increasingly accepted, especially in the cytodiagnosis of tumors in medical practice. Cytological puncture has been tested as a possible means of evaluating liver lesions in cattle.[39]

This study was performed (1) to investigate the morbidity of fatty liver in cattle, (2) to correlate gross findings of the liver and severity of fatty liver in cattle, (3) to apply the simple and less traumatic fine needle aspiration biopsy in the diagnosis of fatty liver in cattle, (4) to correlate cytological, histological findings, and hepatic lipid content by buoyancy, and (5) to evaluate the availability of cytology in the diagnosis of fatty liver in cattle.

Materials and Methods

Animals: Animals used in this study were 1998 cattle at the abbatoir or on the farm and this study was carried out from August to October of 1989. Fine needle aspiration biopsy was performed on 39 cattle. The rest were examined by floatation method or macroscopically. All the cows were in conditions of various reproductive cycle.

Fine needle aspiration biopsy technique: Fine needle aspiration biopsies were performed with 22-gauge, 6-inch Chiba needle with 25° bevel. Puncture site was 20~30cm to the right of midline of back in 10th intercostal space. Cattle were held in stanchion with a minimum restraint or confinement in a squeeze-chute. A 10cm square area at this site was shaved, prepared surgically with povidone iodine, and then 70% alcohol thoroughly. Two per cent lidocaine hydrochloride with or without epinephrine was infiltrated into the skin, the subcutaneous layer, and the area down to the peritoneum. The larger-bore short needle of 18 gauge or 19 gauge was penetrated the abdominal wall and musculature. The Chiba needle with stylet was introduced through the 18-gauge(19-gauge) pilot needle, and then introduced into liver parenchyma. A distinctive sensation can be felt as the needle tip nudges the capsular surface of the liver. The stylet was elminated from Chiba needle in the liver. The needle was then attached to a 10ml syringe. Negative pressure at needle bevel end was applied by withdrawing syringe plunger. The needle was then moved back and forth, once or twice to ensure adequate sampling of the liver. Once aspiration was complete, the syringe plunger was gently released to retain the sample within the needle lumen before the needle was withdrawn from liver(Fig 1).

Cytolological preparation and examination: The aspiration sample was expressed onto clean glass slides by first detaching the needle from the syringe and filling the barrel of the syringe with air. The needle was then reattached to the syringe. The specimen in the needle was expelled onto the glass

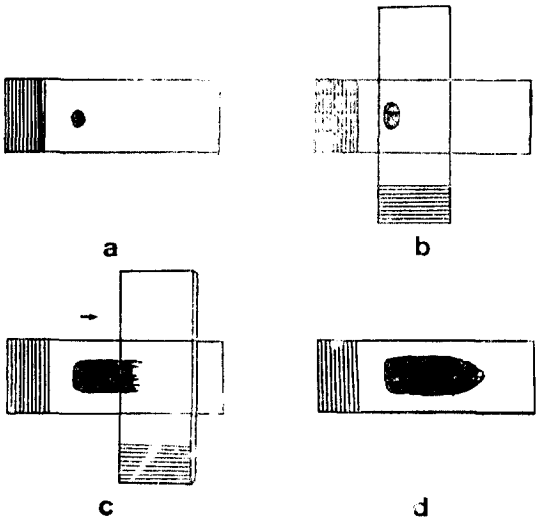


Fig 2. Squash preparation of aspirate.

slides in the periphery of the slides(Fig 2). When the aspirate contained a large amount of blood, the blood should be absorbed with gauze pads. A second glass slide was placed on top of the first and squashed the specimen on the slides. The two slides were pulled apart horizontally to spread the specimen along the slides. The slides were then air-dried. Some were fixed in 100% methanol for 5min. and then stained with Giemsa stain. The others were stained with Oil red O stain.[40] Cytological examination was performed under light microscope with the low power objective first and the high power objective for a closer examination.

Buoyancy: Liver samples for estimating hepatic lipid content were taken from cattle immediately after slaughtering. The buoyancy of the samples was performed according to the method of Herdt *et al*[15] and Scholz *et al*[16](Fig 3).

Histopathology: Liver specimens were taken from 39 cattle immediately after slaughtering. Sample was immediately fixed in 10% neutral buffered formalin. Five frozen sections(8μm in thickness) using Cryo-Cut II(American Optical) were performed at −25°C and stained with Oil red O stain[40] to demonstrate triglyceride. Ten fields were examined from each animal using the high power objective lens of a light microscope.

Classification of liver in cattle: Liver in cattle was subdivided into normal liver(<12%), mild fatty liver(<25%, >16%), moderate fatty liver(<39%, >25%), and severe fatty liver(>39%) according to the results based on hepatic lipid content by buoyancy from the method of Scholz *et al*[16]

Estimation of hepatic lipid content based on buoyancy*

Specific gravity			Hepatic lipid content(%)
1.000	1.025**	1.055**	
+	+	+	>39
−	+	+	<39, >25
−	−	+	<25, >16
−	−	−	<12

* : Data from Scholz *et al*[16]
**: Copper sulphate solution
+: Float
−: Sink

Calculation of diagnostic reliability of cytology: Diagnostic reliability of cytology compared with hepatic lipid content based on buoyancy was calculated according to the method of Phillips *et al.*[41] The equations are as follows:

$$\text{Sensitivity}(\%)=\frac{\text{true positive}}{\text{true positive}+\text{false positive}}\times 100$$

$$\text{Specificity}(\%)=\frac{\text{true negative}}{\text{true negative}+\text{false positive}}\times 100$$

$$\text{Accuracy}(\%)=\frac{\text{true positive}+\text{true negative}}{\text{total}}\times 100$$

Results

Incidence and severity of fatty liver in cattle: When the fatty liver was classified according to the amount of hepatic lipid content based on buoyancy or gross findings, 0.3 per cent of Korean native cows, 2.97 per cent of dairy cows, and 0.15 per cent of dairy bulls had a mild fatty liver. 0.99 per cent of dairy cows had a moderate fatty liver. 0.74 percent of dairy cows had a severe fatty liver. Incidence rate of fatty liver in dairy cows was 4.7 per cent, 15.7 times as high as that of Korean native cows, and 31.3 times as high as that of dairy bulls. Moderate and severe fatty liver in cattle occurred in only dairy cows(Table 1),

Table 1. Incidence of fatty liver in 1998 cattle detected macroscopically or by buoyancy

Grades	Korean native cattle		Dairy cattle	
	Cow(331)*	Bull(633)	Cow(404)	Bull(630)
Mild	1(0.30)**	—	12(2.97)	1(0.15)
Moderate	—	—	4(0.99)	—
Severe	—	—	3(0.74)	—
Total	1(0.30)		19(4.70)	1(0.15)

*: Number of cattle, **: Per cent

Gross findings of fatty liver: Cattle with fatty liver had large deposits of internal fat accumulated around heart, kidney, mediastinum, pelvic canal, and in omentum. Fatty liver showed gross evidence of fat deposition by its large size, round edges, greasy cut surface, light weight and a pale appearance in cases of mild fatty liver to a yellow-orange color in cases of severe fatty liver, but its color was not always correlated with the severity of fatty liver. The tendency of the liver was usually friable but varied to an extent from friable to cirrhotic.

Fine needle aspiration biopsy: The yield of the liver cells varied within wide limits such as single cells, small groups of cells, or coherent fragments of liver parenchyma. Cn rare occasions, only a few liver cells or a great number of liver cells were of little value for cytological interpretation. The latter would be crushed in the cytological smear. Most of aspirates contained a sufficient number of parenchymal cells for cytological evaluation. Fine needle aspiration biopsies were performed 2 or 3 times in each animal. The clinically significant complications of fine needle aspiration biopsies in the liver were not recognized. The shorter aspiration time, the smaller blood in aspirate.

Cytology and histopathology: Cn cytological examination, fat was usually observed in the hepatocytes as a single or multiple discrete droplets with clear and smooth borders, and various forms in the course of the coalescence of microvesicular droplets. In cells extensively filled with fat, the nuclei were eccentric and flattened against the cell membrane. Fat droplets were often spread extracellulary by the smear technique. Therefore, in this study, only intracytoplasmic fat droplets were estimated. For fine needle aspiration cytology the following classification was tried:

Normal liver: No intracytoplasmic fat droplet or fine fat droplets in several cells(Fig4, 5)

Mild fatty liver: Intracytoplasmic fat droplets of various sizes in most of the hepatic cells. Rarely eccentric and indented nucleus in the cytoplasmic periphery(Fig 6, 7)

Moderate fatty liver: Intracytoplasmic fat droplets of various sizes or large intracytoplasmic fat droplet in many cells. Eccentric and indented nucleus in the cytoplasmic periphery by large fat droplet and deformed several hepatic cells(Fig 8, 9).

Severe fatty liver: Large cells were filled with large fat droplets. Few nuclei were completely pyknotic. Eccentric and indented nucleus in the cytoplasmic periphery by large fat droplet(Fig 10, 11).

On histologic examination, fatty infiltration was characterized by the presence of large fat droplets in the central regions of the hepatic lobule, fine fat droplets in the periphery, and fatty infiltration in the pervascular region(Fig 12). Frozen sections stained with ORO method were examined with microscope. The following classification based on microscopic findings and hepatic lipid content by buoyancy was tried:

Normal liver: No fat droplet, or scattered single or multiple fine fat droplets in cytoplasms on sections(Fig 13)

Mild fatty liver: Multiple fat droplets of various sizes and sometimes large fat droplet in cytoplasms on sections(Fig 14).

Moderate fatty liver: Multiple fat droplets of various sizes in the peripheral areas and large fat

Table 2. Diagnostic reliability of cytology compared with hepatic lipid content based on buoyancy in 39 cattle (per cent)

Diagnostic reliability	Cytology			
	Normal liver	Mild fatty liver	Moderate fatty liver	Severe fatty liver
Sensitivity	94.4	64.3	100	100
Specificity	95.2	100	83.3	100
Accuracy	94.9	87.2	87.2	100

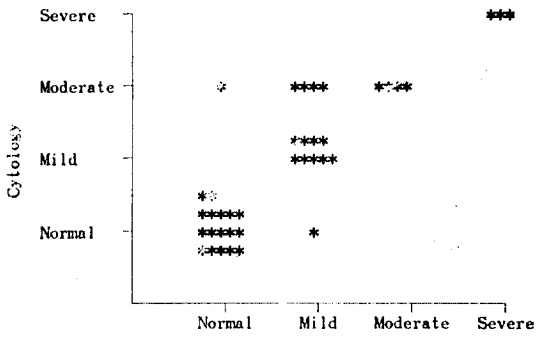


Fig 17. Correlation between grades of liver in the cytological smears and hepatic lipid content estimated by buoyancy.
Normal: Normal liver,
Mild: Mild fatty liver,
Moderate: Moderate fatty liver,
Severe: Severe fatty liver,
*: One cattle.

droplet in cytoplasms in the vicinity of central vein. Deformed nucleus and cell were often observed(Fig 15)

Severe fatty liver: Large fat droplets in most of the the hepatic lobules on sections. The nuclei of many hepatocytes were compressed against the cell membrane(Fig 16)

Correlation between the cytological grade and the grade based on buoyancy: The relation between the cytological grade of liver in smears and the grade based on buoyancy was in good agreement between the two methods as Fig 15 shows. Liver was diagnosed correctly as 17 of 18 normal livers, 9 of 14 mild fatty livers. all 4 moderate fatty livers, and all 3 severe fatty livers. Higher gradings in cytological smears compared with gradings based on buoyancy were more frequently encountered(5/39) than the reverse(1/39).

Analysis of the sensitivity, specificity, and accuracy of the cytological diagnosis compared with hepatic lipid content estimated by buoyancy is given in Table 2. The specificity and accuracy were more than 87.2% and 83.3% in the four grades of fatty liver, respectively. The sensitivity decreased to 64.3 % in the case of mild fatty liver.

Discussion

The fatty liver is a part of a generalized fat mobilization syndrome.[1,5] Factors stimulating mobilization of fat include negative energy balance,[42] hypoglycemia,[43] low serum insulin concentration,[44] and relatively high serum concentrations of lipolytic hormones such as growth hormone, placental lactogen, and prolactin.[45] All conditions occur in both late and early lactation. Fatty liver appears to be more related to the degree and rapidity of body weight loss[46,47] than to obesity *per se*. High serum concentrations of nonesterified fatty acid are closely associated with the development of fatty liver.[10,17] In a study of 20 clinically normal, high yielding dairy cows from an autumn calving herd carried out during the years of 1977~78, a 50 per cent incidence of severe fatty liver determined by histological analysis of needle biopsies taken one week after calving was found.[8] In a survey of the incidence and severity of post-parturient fatty liver in high yielding dairy cows of 151 cows and 46 heifers from four Friesian herds and one Guernsey herd, 66 per cent of Friesian cows and 33 per cent of Guernsey cows had a moderate or severe fatty liver at one week after parturition.[9] In a study of Holstein cattle at the slaughter house carried out in April to May of 1985, Lee and Choi[48] reported that 25.5 per cent of 94 Holstein cows and 21.3 per cent of 117 Holstein bulls had fatty liver

detected macroscopically and microscopically. In this study, 0.3 per cent of Korean cows, 2.97 per cent of dairy cows, and 0.15 per cent of dairy bulls had been suffering from mild to severe fatty liver. Low incidence rates of fatty liver can be explained as various reproductive cycle of the cows and improved herd management on the farms in Korea.

Cows which die either acutely or chronically have large deposits of internal fat located around heart, kidney, mediastinum, pelvic canal, and in omentum. The internal organ affected most severely is the liver which was enlarged and swollen with round edges, a pale color due to the intensive fatty metamorphosis,[5] and yellow color.[22] A piece of fatty liver placed in water will float due to the extensive fatty infiltration.[5] Discoloration varies from a pale appearances in cases of mild fatty liver to a yellow-orange color in cases of severe fatty liver and the tissue is friable and greasy.[49] In this study, gross findings are consistent with the observation above-mentioned but color of the liver was not always correlated to the severity of fatty liver, and the liver varied to an extent from friable to cirrhotic consistency. In animals with cirrhosis dying after the age of at least 2 years, the distribution of fatty change is very regular.[50]

This study indicates that fine needle aspiration biopsy can be valuable diagnostic aid in clinical bovine practice and its diagnostic values lie on the points that fine needle aspiration biopsies are much easier to perform than conventional coarse needle biopsy in the liver, and less traumatic to the liver. Many methods have been used for diagnosing and grading fatty liver of cattle. However, chemical methods, histological methods and buoyancy should be preceded liver biopsy and histological methods are suitable for laboratories with routine histological facilities. Liver biopsy has been accompanied by various complications. The risk from liver biopsy increases in proportion to the caliber of the cannula, the time during the biopsy needle remains in the liver parenchyma, and experience of the operater.[51] Blood chemistry and dye clearance tests are not well correlated with the severity of fatty liver and are difficult to differenciate fatty liver from other liver diseases. Using an multivariate equation based on blood concentrations of free fatty acids, glucose and GOT at 7~13 days after calving, it has been possible to correctly diagnose fatty liver in approximately 75 per cent of affected cows.[17] The validity of the equation needs to be tested on a larger scale before its routine use as a diagnostic aid. The lipid distribution of the liver is uniform.[52]

Fine needle aspiration biopsies have been used as the method to obtain liver specimens of various liver disorders in man and cattle. The technique applied in this study is similar to that of Holtenius[39] using needle 140mm long with an outer diameter of 0.6mm and Franzens' syringe(10ml Luer-Lock syringe). The basic equipments required to perform fine needle aspiration biopsy in this study are simple; it includes 22-gauge, 6-inch needle(Chiba needle), short-bore needle of 18 gauge or 19 gauge, 10ml disposable syringe, shaver, povidone iodine, alcohol skin preparation sponges, gauze pads, glass slides, and 100 per cent methyl alcohol for fixation. Most of these items are already available in most veterinary hospital and can be conveniently carried in a small package. In cytological preparation it is important to keep the needle touching the slide surface so that there is no air gap between needle and slide. Fragments of coherent liver tissue proved satisfactory for electron microscopy and under certain condition also for chemical analysis of liver tissue.[53] Tiny fragments or thin strands of tisue were processed histologically.[54] Complications were reported a unexpainable intrahepatic haematoma on the basis of available data in man.[53] In this study fine needle aspiration biopsy did not produce any complications in cattle.

Changes in liver of metabolic diseases in cattle are generally diffuse and involve cellular changes such as fatty change and nuclear abnormalities. If fatty changes are present, the fat vacuoles appear as negative images(H & E), an orange-color(Sudan III), and a negative image in the cytoplasm(gallocyanin-chrome-alum stain).[39] Cytological preparation showed hepatic cells with intracytoplasmic fat from patient with fatty liver caused by chronic alcoholism.[35] The MGG-stained cells contained small or large light, structureless areas in 10 cases of fatty liver of

human. In cells filled with fat, the nuclei were eccentric and flattened against the cell membrane.[29] Hepatocyte cytoplasm occasionally contained fat vacuoles. The fat was in one larger clear vacuole per cell or two to three smaller ones and the nucleus was usually pushed to the periphery and not indented by the vacuoles.[36] In fatty liver the most characteristic finding was the presence of numerous cytoplasmic vacuoles which were mostly Sudan III and Oil red O positive. Nuclei are pyknotic and the cytoplasm is filled with large vacuoles in the liver with marked fatty change.[32] Fat accumulation was graded as mild if less than 5% of hepatocytes contained lipid vacuoles, as moderate if 5% to 30%, and as severe if greater than 30%. More severe degrees of steatosis were usually associated with degenerative cytoplamic and nuclear changes.[33] Berge *et al*[55] reported a correlation between the histological and cytological evaluation of lipid content in 24 biopsies. Smears for cytology were obtained with a needle of 0.7mm in diameter and routinely stained with May-Grünwald-Giemsa. The fatty infiltration of the liver was estiamted in 4 degrees: No fatty infiltration −, Slight fatty infiltration(i.e. small droplets in a few liver cells) +, Moderate fatty infiltration(i.e. small and large droplets in several liver cells) ++, severe fatty infiltration(i.e. small and large droplets in most of the liver cells) +++. Fatty infiltration of the liver was diagnosed correctly as 15 of 24 psoriasis. The rest were not misclassified more exceedingly than one degree except for one case. These findings are similar to the present results but the liver was graded as normal liver, mild fatty liver, moderate fatty liver, and severe fatty liver in the cytological findings.

In the relation between the cytological examination and hepatic lipid content by buoyancy, the two methods are in good agreement. Higher gradings in cytological smears were often encountered severe fatty liver to normal liver in order. Herdt[52] describes the progression of liver morphometric changes in a cow approximately 1 week postpartum that has been treated for severe fatty liver manifested by clinical illness. When hospitalized, histologic examination of the liver was essentially abnormal. After 10 days of treatment, there was a slight histologic indication of improvement(not less than 25 per cent liver fat content by weight). Eight week after therapy, normal liver tissue interspersed with a few large fat droplets was observed on a histologic examination (13 per cent liver fat content by weight). Gerloff and associates[49] reported that liver fat concentration increased during the late dry period and peaked within the first three weeks of lactation. Fat is present in the liver in appreciable amounts even two week before calving, peaked at 1 week after calving, and falls back slowly to the normal level of almost zero by 26 weeks after calving.[20] Liver fat concentration increased before calving and peaked at 2 week after parturion, and decreased thereafter in cows with severe hepatic lipidosis.[56] Higher gradings in this study may be due to the fact that smears are taken from various chronological phases of liver fat accumulation above-mentioned and are not taken from the same specimen for hepatic lipid content by buoyancy and histology. The latter, however, may be partly resolved when biopsies are performed two or three times from each animal.

From these results, fine neelde aspiration biopsy as a diagnostic aid for fatty liver in cattle is considered as a simple, safe, and inexpensive method and can be performed in less reluctance of dairyman on the farm. In this study, however, since fine needle aspiration biopsy were performed in limited number of cattle, extensive study is needed under field conditions.

Summary

This study was carried out to investigate the morbidity of fatty liver in cattle at the abattoir and on the farm, and to cytodiagnose fatty liver in cattle by fine needle aspiration biopsy.

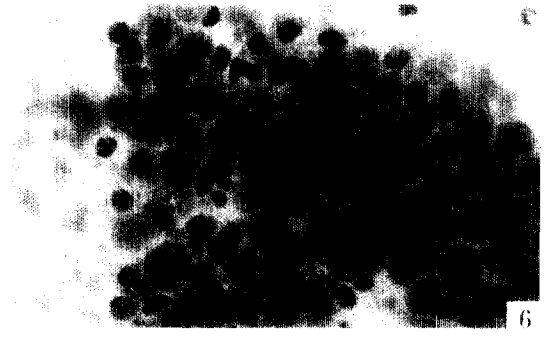
Incidence rates of fatty liver in cattle, detected macroscopically or based on hepatic lipid content by buoyancy, were 0.30% in Korean native cows, 4.70 % in dairy cows, and 0.15% in dairy bull. Fatty liver was enlarged, swollen with round edges, light weight, and pale to yellow-orange color, but its color was not always correlated to the severity of fatty liver. The findings of fat infiltration of the hepatic

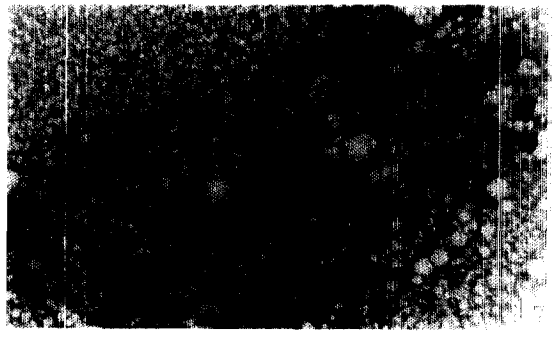
lobule were large droplets around central vein, fine droplets in the periphery, and fat infiltration in the perivascular region execpt for most of normal liver and severe fatty liver. The sensitivty, specificity, and accuracy of cytological finding compared with hepatic lipid content by buoyancy were 94.4%, 95.2 %, and 94.9% in normal cases, 64.3%, 100%, and 87.2% in mild cases, 100%, 83.3%, and 87.2% in moderate cases, and 100%, 100%, and 100% in sesvere cases, respectively. Cytological findings were well correlated with histological findings. Complications of fine needle aspiration biopsy were not recognized clinically.

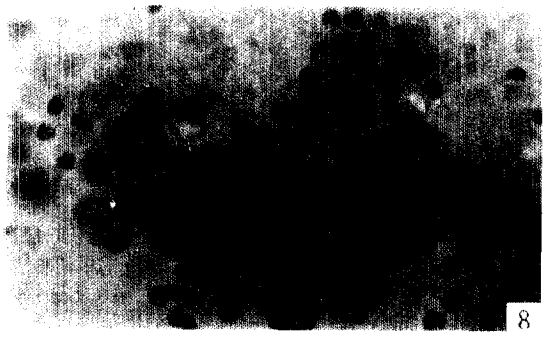
Consequently, the cytodiagnosis by fine needle aspiration biopsy is simple, rapid, safe, and economical method compared with histological techniques in the diagnosis of fatty liver in cattle.

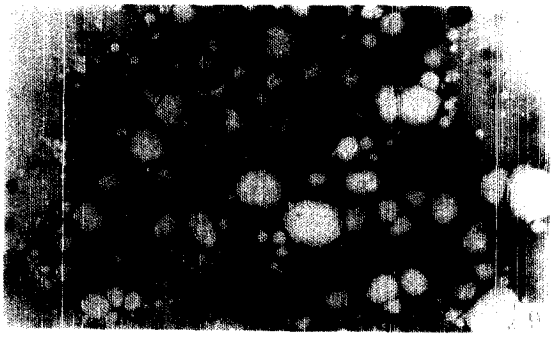
Legend for figures

Fig 1. Performance of fine needle aspiration biopsy in cattle.
Fig 3. Submergence of liver specimen into copper sulfate solution.
Fig 4. Normal liver on cytology. ORO stain, ×400.
Fig 5. Normal liver on cytology. Giemsa stain, ×400.
Fig 6. Mild fatty liver on cytology. ORO stain, ×400.
Fig 7. Mild fatty liver on cytology. Giemsa stain, ×400.
Fig 8. Moderate fatty liver on cytology. ORO stain, ×400.
Fig 9. Moderate fatty liver on cytology. Giemsa stain, ×400.
Fig 10. Severe fatty liver on cytology ORO stain, ×400.
Fig 11. Severe fatty liver on cytology. Giemsa stain, ×400.
Fig 12. Large fat droplets in the vicinity of central vein and small fat droplets in the peripheral area of the hepatic lobule. ORO stain, ×100.
Fig 13. Normal liver on histology. ORO stain, ×400.
Fig 14. Mild fatty liver on histology. ORO stain, ×400.
Fig 15. Moderate fatty liver on histology. ORO stain, ×400.
Fig 16. Severe fatty liver on histology. ORO stain, ×400.

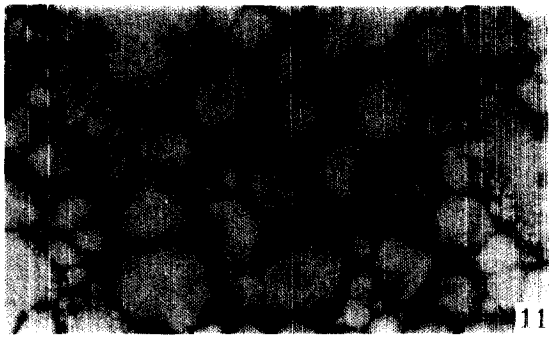


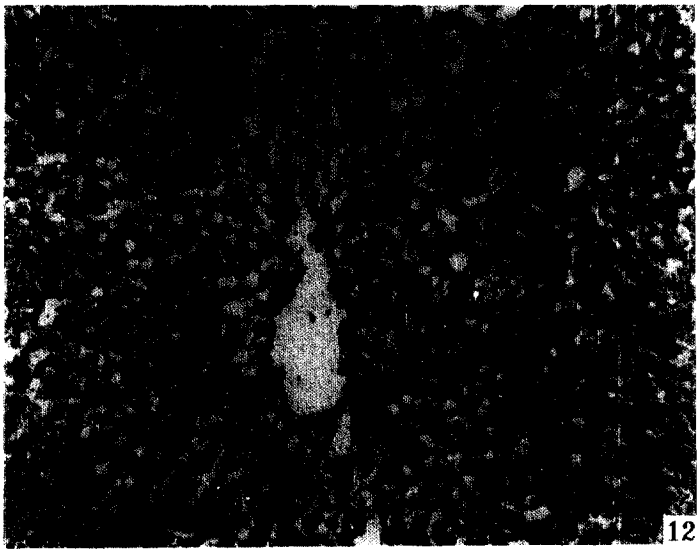


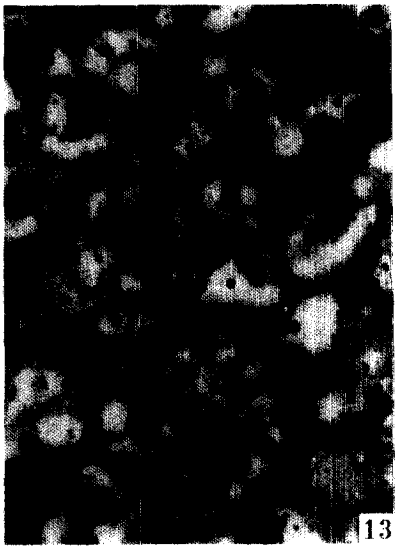


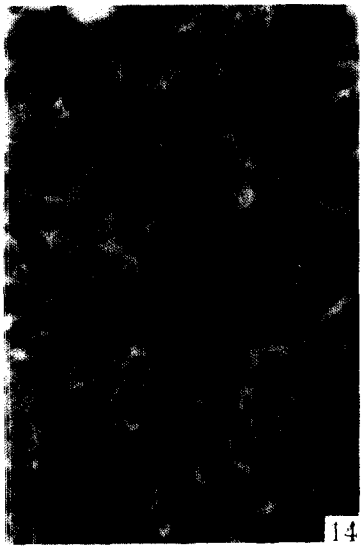


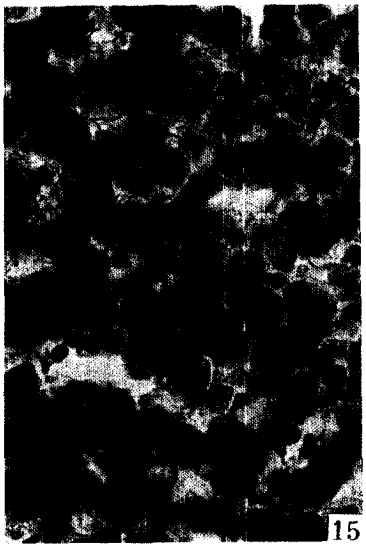

10


11


12


13


14


15


16

References

1. Roberts CJ, Reid IM, Rowlands GJ, Patterson A. A fat mobilisation syndrome in dairy cows in early lactation. *Vet Rec* 1981;108:7~9.
2. Brumby PE, Anderson M, Tuckly B, Storry JE. Lipid metabolism in the cow during stavation-induced ketosis *Biochemical J* 1975;146:609~615.
3. Reid IM, Baird GD, Heitzman RJ. Effects of fasting in non-lactating cows. A correlated biochemical and stereo-logical study of fasting-induced fatty liver. *J Agric Sci Camb* 1977;89:319~325.
4. Reid I, Roberts J. Faty liver in dairy cows. *In Practice* 1982;4:164~169.
5. Morrow DA. Fat cow syndrome. *J Dairy Sci* 1976;59:1625~1629.
6. McComack J. Fat-cow syndrome and its complications. *Vet Med/Small Animal Clinicians* 1978;73:1057~1060.
7. Reid IM. Reproductive performance and fatty liver in Guernsey cows. *Animal Reproduction Science* 1983;5:275~279.
8. Reid IM, Roberts CJ, Manston R. Fatty liver and infertility in high-yielding dairy cows. *Vet Rec* 1979;104:75~76.
9. Reid IM. Incidence and severity of fatty liver in dairy cows. *Vet Rec* 1980;107:281~284.
10. Gaal T, Roberts CJ, Reid IM, Dew AM, Copp CM. Blood composition and liver fat in post parturient dairy cows. *Vet Rec* 1983;113:53~54.
11. Herdt TH, Liesman JS, Gerloff BJ, Emery RS. Reduction of serum triacylglycerol-rich lipoprotein concentrations in cows with hepatic lipidosis. *Am J Vet Rec* 1983;44:293~296.
12. Collin RA, Reid IM. A correlated biochemical and stereo-logical study of periparturient fatty liver in the dairy cow. *Res Vet Sci* 1980;28:373~376.
13. Gaal T, Reid IM, Collin RA, Roberts CJ, Pike BV. Comparison of biochemical and histological methods of estimating fat content of liver of dairy cows. *Res Vet Sci* 1983;34:245~248.
14. Collins RA, Reid IM, William LA, Dennis PF. Histological measurement of fat content of liver of dairy cows. *J Comp Path* 1985;95:437~441.
15. Herdt Th, Goeders L, Liesman JS, Emery RS. Test for estimation of bovine hepatic lipid content. *JAVMA* 1983;182:953~955.
16. Scholz H, Zipori G, Höltershinken M. Erfahrungen mit einen neuen Instrumentarium für die Leberbiopsie beim Rind. *Collegium Veterinarium* 1988;1921~22.
17. Reid Im, Rewlands GJ, Dew AM, Collins RA, Roberts CJ, Manston R. The relationship between post-parturient fatty liver and blood composition in dairy cows. *J Agric Sci Camb* 1983;101:473~480.
18. Ubaldi A, Sabatini P, Corbella E. Electrophoresis of plasma LDH isoenzymes in the diagnosis of ketosis and steatosis in dairy cows. Paper No. 141. *International Symposium of Veterinary Laboratory Diagnosticians*. Lucerne, Switzeland, 1980;478~481.
19. Herdt TH, Gerloff BJ, Liesman JS, Emery RS. Hepatic lipidosis and liver function in 49 cows with displased abomasums. *XIIth World Congress on Diseases of Cattle*. 1982;522~526.
20. Reid IM, Roberts CJ. Subclinical fatty liver in dairy cows. *Ir Vet J* 1983;37:104~110.
21. Reid IM, Dew AM, Williams LA. Hematology of subclinical fatty liver in dairy cows. *Res Vet Sci* 1984;37:63~65.
22. Morrow DA, Hillman D, Dade AW, Kitchen H. Clinical investigation of a dairy herd with the cow syndrome. *JAVMA* 1979;174:161~167.
23. Schulz JA, Rossow N. Leberbiopsie unter Sichtkontrolle beim Rind. *Medizintechnik* 1965;5:104~106.
24. Udall RH, Warner RG, Smith SE. A liver biopsy technique for cattle. *Cornell Vet* 1952;42:25~27.
25. Schulz JA, Priboth W. Aspirationsbiopsie der Leber beim Rind. *Medizintechnik* 1965;5:148~149.
26. Wiebe T, Lundquist A, Belfrage P. Time-course of liver fat accumulation in man after a single load of ethanol. *Scan J Clin Lab Invest* 1971;27:33~36.
27. Laurell S, Lundquist A. Lipid composition of human liver biopsy specimens. *Acta Med Scand*

1971;189:65～68.
28. Lundin P, Lundquist A, Lunvall O. Evaluation of fine-needle aspiration biopsy smears in the diagnosis of liver iron overload. *Acta Med Scan* 1969;186:369～373.
29. Wasastjerna C, Thodén CJ, Ekelund P, Halta K. The bile canaliculi in cytological aspiration biopsies from patients with liver disorders. *Scan J Gastroent* 1970;5:327～331.
30. Johansen S, Myren J. Fine-nendle aspiration biopsy smears in the diagnosis of liver diseases. *Scan J Gastroent* 1971;6:583～588.
31. Stormay N, Å kerman M. Aspiration cytology in the diagnosis of granulomatous liver lesions. *Acta Cytol* 1973;17:200～203.
32. Dominis M, Čerlek S, Solter D. Cytology of diffuse liver disorders. *Acta Cytol* 1973;17:205～208.
33. Perry MD, Johnston WW. Needle biopsy of the liver for the diagnosis of nonneoplastic liver diseases. *Acta Cytol* 1985;29:385-390.
34. Shurbaji MS, Gupta PK, Newman MM. Hepatic actinomycosis diagnosed by fine needle aspiration. A case report. *Acta Cytol* 1987;31:751～755.
35. Sherlock P, Kim YS, Koss LG. Cytologic diagnosis of cancer aspirated material obtained at liver biopsy. *Am J Dig Dis* 1967;12:396～402.
36. Garney CN. Clinical cytology of the liver. *Acta Cytol* 1975;19:244～250.
37. Tao LC, Donat EE, Ho CS, McLoughlin MJ. Percutaneous fine needle aspiration biopsy of the liver: Cytodiagnosis of hepatic cancer. *Acta Cytol* 1979;23:287～291.
38. Whitlach S, Nuñez C, Pitlik D. Fine needle aspiration biopsy of the liver: A study of 102 consecutive cases. *Acta Cytol* 1984;28:719～725.
39. Holtenius P. Cytological puncture. A new method for the study of bovine hepatic disease. *Cornell Vet* 1961;51:56～63.
40. Haas E. Oil red O stain for lipids. *50 diagnostic special stains for surgical pathology*. Philadelphia: Lippincott Company, 1981;83～85.
41. Phillips WC, Scott JA, Blasczcynski G. Statistics for diagnostic procedures. *AJR* 1983;140:1265～1270.
42. Reid IM, Collins RA, Baird GD, Roberts CJ, Symonds HW. Lipid production rates and the pathogenesis of fatty liver in fasted cows. *J Agric Sci Camb* 1979;93:253～256.
43. Brockman RP. Effect of glycemic changes on lipolysis in sheep in vivo. *Metabolism* 1984;33:329～331.
44. Lomax MA, Baird GD, Mallinson CB, Symonds HW. Differences between lactating and non-lactating dairy cows in concentration and secretion rate of insulin. *Biochemical J* 1979;180:281～289.
45. Bauman DE, Currie WB. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorrhesis. *J Dairy Sci* 1980;63:1514～1529.
46. Gerloff BJ, Herdt TH, Emery RS. Relationship of hepatic lipidosis to health and performance in dairy cattle. *JAVMA* 1986;188:845～850.
47. Reid IM, Dew AM, Collin RA. The relationship between fatty liver and fertility in dairy cows: A farm investigation. *J Agric Sci Camb* 1983;101:499～502.
48. 李慶甲, 崔熙仁. 젖소에서의 간질환 진단을 위한 혈액화학치의 조사. 韓國臨床獸醫學會誌 1986;3:29～37.
49. Herdt TH. Fatty liver in dairy cows. In: *Vet Cli North Am. Food Animal Practice*. 1988;4(2):269～287.
50. Kelly WR. Cirrhosis. In Jubb KVF, Kennedy PC, Palmer N, ed. *Pathology of domestic animals*. 3rd ed. Orlando: Academic Press Inc, 1985;242～244.
51. Menghini G. One-second biopsy of the liver. Problems of its clinical application. *New Eng J Med* 1970;283:582～585.
52. Gaal T, Husveth F. Comparison of the liver biopsy sample and the "whole liver" in respect of lipid content and fatty acid composition of lipids. *Acta Vet Acad Sci Hung* 1983;31:51～56.
53. Lundquist A. Liver biopsy with a needle of 0.7 mm outer diameter. Safety and quantitative yield. *Acta Med Scan* 1970;188:471～474.
54. Berge G, Lundquist A, Rorsman H, Å kerman

M. Liver biopsy in psoriasis. *Br J Derm* 1970; 82:250~253.

55. Cochand-Priollet B, Chagnon S. Ferrand, J Blery M, Hoang C, Galian A. Comparison of cytologic examination of smears and histologic examination of tissue cores obtained by fine needle aspiration biopsy of the liver. *Acta Cytol* 1987;31:476~480.
56. Gerloff BJ, Herdt TH, Emery RS. Association of moderate and severe hepatic lipidosis in cattle with differing reproductive performance. *Can J Anim Sci* 1984;64(Suppl):250~251.