Fine Needle Aspiration and Liver Cytology— A Simple Method for Diagnosis and Prognosis of Fatty Liver in Cattle

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Introduction

Liver lesions and abnormal liver function are relatively common in cattle, especially in association with metabolic diseases around calving time. More specifically, fatty liver occurs so frequently that it is described as "physiological fatty liver.[1] In the case of primary or secondary fatty liver disease, the "grade" of fatty liver change can be of prognostic value.

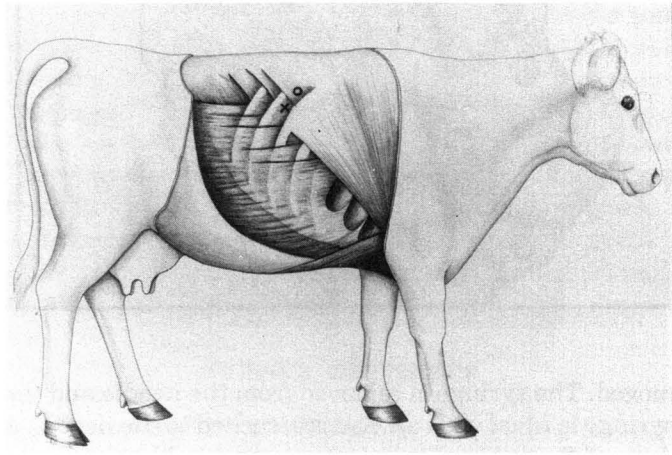
Diagnosis of hepatic disease is difficult, and evaluation of the degree of damage is even more difficult. While serum biochemical analyses, hepatic function tests and liver biopsies for histology and chemical analysis can be utilized, these are time consuming. Conversely, liver cytology from small aspirates from a fine needle aspiration (FNA), provides a simple, safe, rapid and inexpensive procedure, yielding accurate results.

FNA of bovine liver in large animal practice has not been commonly used. This procedure has been used by Dr. Holtenius, in Sweden since 1960.[2] This article describes the method in detail so that bovine practitioners can use this diagnostic aid in the diagnosis and prognosis of bovine hepatic disease.

Materials and Methods

FNA is a relatively simple procedure for which anesthesia or special restraint procedures are unnecessary. Cytologic specimens can be obtained from standing animals. The liver biopsy is performed through the second last (11th) intercostal space on the right side. The optimal location for needle entry is at or immediately above the boundary between the upper and middle thirds of the rib. With digital palpation from the top downward in the intercostal space, the operator will feel a "step" down at the correct biopsy site, just ventral to the Serratus Dorsalis Caudalis muscle (Figure 1). The hair is clipped and the skin is disinfected using standard procedure.

Figure 1. O: Serratus Dorsalis Caudalis muscle
X: Biopsy site



An 18 g disposable needle is passed through the skin and a long (i.e. 6 inch) 22 g needle is introduced through the larger needle. At the same time the direction of insertion is shifted somewhat cranially. A distinct sensation can be felt as the tip of the needle touches the visceral surface of the liver. The resistance felt when the needle has entered the liver is also quite characteristic. If the operator holds the hub of the biopsy needle between the thumb and index finger as it is passed through the disposable 18 g needle, it is unnecessary to use sterile gloves for this procedure. When the needle is well seated in the liver, a 10 ml. syringe is attached and suction is applied to the 2-4 ml mark and the tip of the needle is moved back and forth within the liver. The suction is stopped and the needle and syringe are re-

Figure 2. Grade O normal liver cells

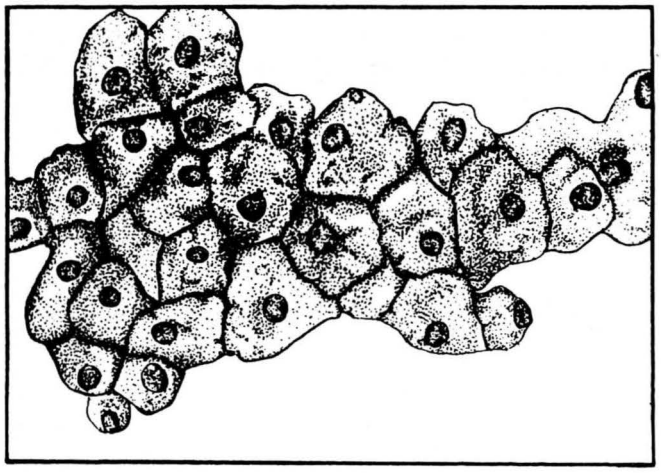
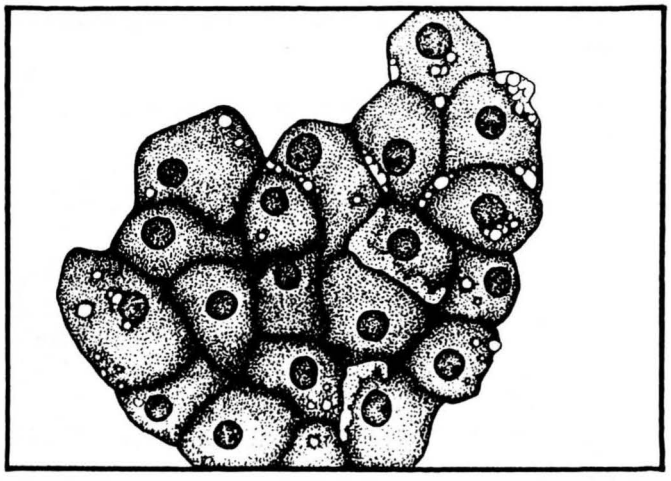


Figure 3. Grade 1 fatty liver change (Intracytoplasmic fat vacuoles)



moved. The syringe is removed from the needle and the syringe is filled with air and reattached to the needle. A drop of fluid (blood and hepatocytes) is blown on to a glass slide and a smear is made in the same manner as a blood smear. The smears should be made quickly, before any coagulation takes place and be air-dried as soon as possible.

If blood enters the syringe during the procedure, the suction should be immediately stopped. Too much blood is not desirable as this makes it more difficult to find the hepatocytes on the smear.

The slides are stained with Diff-Quick stain (a rapid Romanowsky type stain). With this method the slides are dipped 5 times in each of the solutions and rinsed with distilled water and dried. Before microscopy, a drop of oil and a coverslip should be added.

Examination of the slides should begin with 100X, to identify the hepatocytes. The examination should continue with 400X and 1000X to examine cellular detail.

Figure 4. Grade 2 fatty liver change

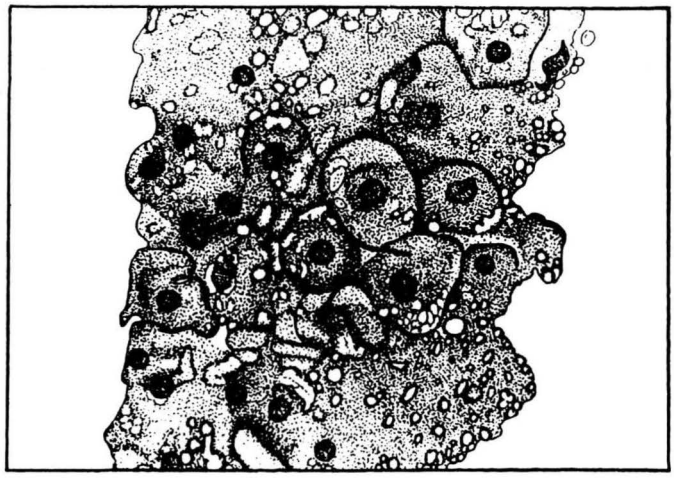
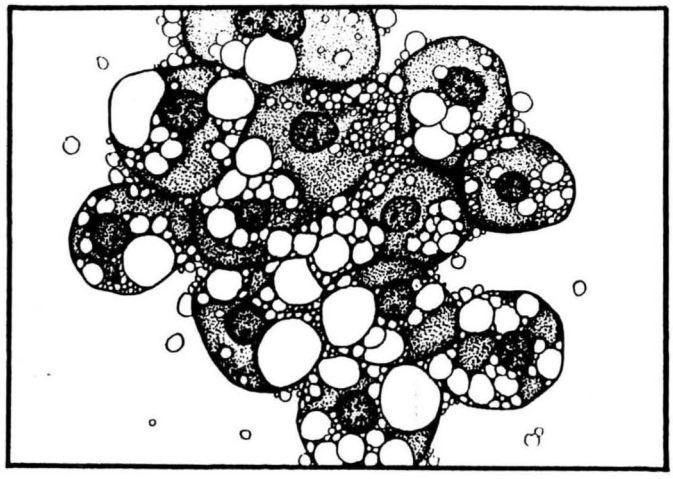


Figure 5. Grade 3 fatty liver change



Results and Discussion

With examination under low power (100X), cohesive clusters of hepatocytes can be found at the feathered edge of the smear. A normal specimen contains cohesive clusters of hepatic cells in regular sheets. Normal hepatocytes are large ovoid cells with abundant basophilic granular cytoplasm. It is important to have a thin preparation. The diameter of the hepatocyte nuclei should be approximately twice as large as a red blood cell (RBC) and the entire diameter of the hepatocytes should be 5-6 times that of RBC's. Variation in size is common, but the nuclear to cytoplasmic ratio is constant. The small nucleus is usually peripherally located and usually contains one small but distinct nucleolus.

Often a small amount of bile pigment can be found in the cytoplasm. A few leucocytes are usually present, probably a result of blood contamination. The background of normal specimens may contain debris and free nuclei.

Figure 6. Grade 4 fatty liver change (numerous pyknotic nuclei)

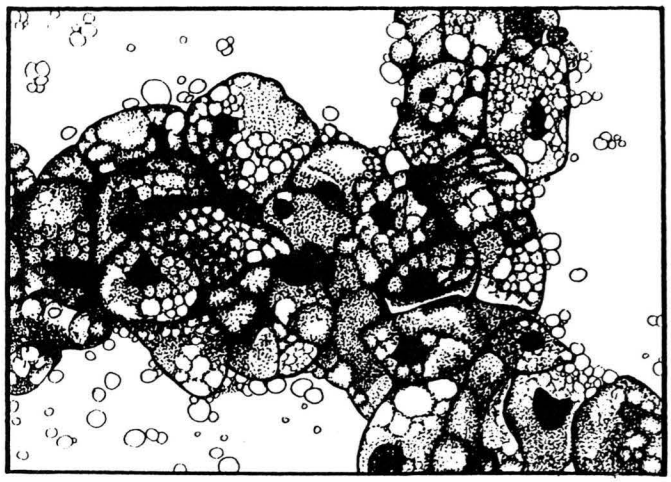
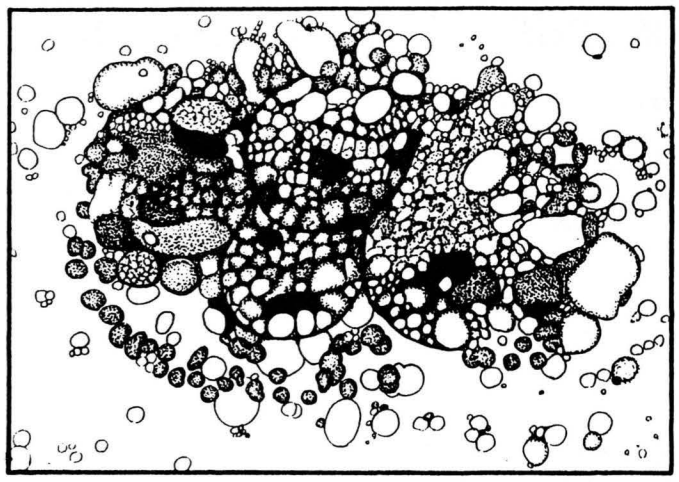


Figure 7. Grade 5 fatty liver change (extensive fat and pyknotic nuclei)



- A relatively common finding in clinically healthy animals is intranuclear inclusions, probably glycogen.[4,5] The nuclei are larger and surrounded by a thin nuclear membrane. In the nucleus, there appears to be a lack of the usual chromatin.

- Another quite common finding is accumulations of clusters of neutrophils. These may lie separate from the hepatocytes, but they are often associated with the hepatocytes. These clusters of neutrophils or microabscesses are often seen with traumatic reticulitis or enteritis.[6]

- The grade of fatty liver is evaluated after examination of several clusters of hepatocytes. The Swedish workers have used a scale of 0 (normal) to 5. (fig. 2-7).[7] Note that the important division line lies with grade 3, here we can notice nuclear changes.

- Fatty liver up to and including grade 3 gives a good prognosis. If there is primary underlying etiology causing lack of appetite we would expect a grade 4 and a poor prognosis. A liver lesion of such degree may take 5-6 weeks to improve. The condition must improve rapidly (1-3 days) if the cow is going to be able to reverse this fatty liver condition.

In practice FNA cytology of liver may be attempted on the second visit to an animal that has not responded to therapy. By taking the samples and with a few minutes staining and examination, we can obtain valuable information on possible further therapy and prognosis. The methodology is relatively simple and rapid. The detailed hepatocellular description in this article should allow the routine use of this diagnostic and prognostic tool in bovine practice.

Summary

Fatty liver in cattle is quite common around parturition. Diagnosis is difficult and often time and resource consuming. Hepatic FNA is a useful, fast and inexpensive diagnostic tool. This article describes in detail a method of fine-needle aspiration biopsy of liver. The fatty change/degeneration of liver is classified on a scale of 0 (normal) to 5 (fig. 2-7). Fatty change grade 3 or below gives a good prognosis. Fatty change grade 4 gives a guarded to poor prognosis, especially if primary illness is the cause of poor appetite.

Acknowledgements

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References

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