Studies on freezing of bovine embryos: use of ethylene glycol as a cryoprotectant and direct transfer of frozen-thawed embryos to recipient animals

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Solid-phase extraction of estrogen from bovine blood plasma for their peripheral detection by radioimmunoassay

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A study of liver and corticosteroid-induced alkaline phosphatase isoenzymes activities associated with glucocorticoid hepatopathy in the dog.

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An excess of endogenous and exogenous glucocorticoids causes a reversible vacuolar hepatopathy accompanied by increased serum alkaline phosphatase (ALP). Increased serum ALP activity has primarily been attributed to the presence of a specific corticosteroid-induced isoenzyme of ALP (CALP). However, increased CALP is a less consistent finding in all treated dogs. Despite vacuolar hepatopathy and increased ALP isoenzyme activity being well recognized as the sequelae to glucocorticoid excess, the relationship between the degree of increased ALP isoenzyme activity and the magnitude of vacuolar hepatopathy is uncertain. This

study thus investigated the relationship between serum liver ALP (LALP) and CALPs and the degree of vacuolar hepatopathy. This was to be achieved by (i) devising a method that adequately quantifies all the ALP isoenzymes in serum, (ii) quantifying the magnitude of vacuolar hepatopathy, and (iii) localizing the 2 isoenzymes at the subcellular level and determine their relationship to the vacuoles.

In the first chapter of this study the ALP isoenzymes in serum were quantified by combining wheat germ lectin (WGL) precipitation and levamisole inhibition the ALP isoenzymes. Levamisole at 4.2 mM inhibits both bone ALP (BALP) and LALP leaving CALP while 50 mg/l WGL precipitates BALP in serum. The LALP isoenzyme was then calculated from the equation: TALP=BALP+LALP+CALP. BALP, LALP and CALP standards from serum of puppies, bile-duct ligated dogs and dogs given 4.4 mg/kg/day prednisolone for 30 days, respectively, were used. The suitability of standard sera was tested by affinity electrophoresis. The assay was then applied to determine normal serum age dependent ALP levels and also in abnormal conditions that result in high serum ALP levels. It was concluded that combining WGL and levamisole inhibition provided adequate quantification of canine ALP isoenzymes and that the method has great potential for routine use.

In the second and third chapters of this study, the relationship between serum ALP isoenzymes and the degree of glucocorticoid-induced hepatopathy was investigated. The degree of hepatopathy was determined using vacuolization scores and digital analysis of histograms on the hepatic parenchyma as treatment with prednisolone progressed. Ten dogs were used i.e., 2 as controls, another 2 were injected with 1.1 mg/kg prednisolone and the last 4 dogs were injected 4.4 mg/kg prednisolone. The changes in TALP, CALP and LALP were compared to changes in vacuolization scores and digital analysis values. It was observed that although increased TALP subsequently resulted in a concomitant increase in both vacuolization scores and digital analysis values, only in cases where increased TALP was predominantly due to CALP was there a significantly higher degree of vacuolar hepatopathy. CALP was found to directly correlate with the severity of vacuolization while LALP that increased in all treated dogs and thus was nonspecific to the degree of vacuolization. It was thus concluded that CALP may have a direct bearing on the degree of vacuolization in glucocorticoid-induced hepatopathy.

In final chapter of this study, the subcellular location and distribution of LALP and CALP relative to the vacuoles was investigated histochemically, in order to verify the observations made in second and third chapters. Liver biopsies were taken in normal and in prednisolone treated dogs at day 20. The biopsies were cryopreserved until examination at –80°C. The ALP isoenzyme distribution and subcellular location was determined using an ALP stain with or without 4.2 mM levamisole in order to stain for CALP activity alone. In controls, LALP was confined to the periportal zone whereas CALP was not observed. At day 20 post treatment, LALP became homogenous throughout the hepatic zones but CALP was mostly confined to the periportal zone around severely vacuolized hepatocytes. The association between CALP and the vacuoles was interpreted to be the reason why the isoenzyme only increased significantly after the degree of vacuolization significantly increased.

It was finally concluded that although both LALP and CALP may increase during glucocorticoid induced hepatopathy, only CALP seems to correlate well with the degree of vacuolization and that the reason why CALP correlates directly with degree of vacuolization is due to the isoenzyme's subcellular location on the margins of

these vacuoles. Clinically, CALP may be used as a serum indicator of the severity of steroid hepatopathy.

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Studies on clinical application of endoscopic ultrasonography in the dog.

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Endoscopic ultrasonography (EUS) is widely used as a technique for diagnostic imaging of the pancreas in human medicine. The author performed EUS on the normal pancreas and for experimentally induced pancreatic disorders in dogs to investigate the efficacy of this modality in diagnosis of pancreatic disorders in the dog. The EUS device used in the present study was a Hitachi/Pentax FG-32UA echoendoscope with a curved-array ultrasonic transducer, mounted in front of the objective lens. The EUS device was connected to a Hitachi EUB-565A ultrasound scanner. Dogs were placed in dorsal recumbency under general anesthesia, the tip of the ultrasonic endoscope was inserted into the stomach, and all examinations of the pancreas were performed from within the stomach.

The first chapter describes EUS performed on 12 normal adult dogs to establish the procedure for imaging the pancreas using anatomical landmarks, and to collect EUS images of the canine normal pancreas. EUS provided good images of most of the pancreas except for the edges of each pancreatic lobe. Useful information on the pancreatic parenchyma, including the pancreatic lobular structure, pancreatic duct and vessels of the pancreas was obtained by EUS. Blood flow in the vessels was detected by color Doppler and pulsed-wave Doppler examination. These results suggested that EUS is available as an effective diagnostic modality for the canine pancreas.

The second chapter describes EUS examination, gray-scale histogram analysis of EUS images and transcutaneous ultrasonography (TUS) done in seven dogs with caerulein-induced pancreatitis. One dog was subjected to laparotomy and biopsy specimen collection for histopathology. By EUS, the pancreatic lesions were first detected at 60 minutes after the start of caerulein infusion. This was earlier than when they were first detected using TUS. EUS findings included swelling, subcapsular hypoechoic areas and anechoic stripes through the pancreatic parenchyma. No marked change of histogram analysis was seen until 30 minutes. From 30 to 60 minutes, a decrease of the mean brightness (mean data of brightness : MD) of the pancreatic parenchyma was first observed. These changes of the MD reflected histopathological findings, including vacuolization of acinar cells and interstitial edema of the pancreas. These findings indicated that EUS can detect delicate and diffuse changes of the pancreatic parenchyma. Furthermore, gray-scale histogram analysis reflected histopathological changes more sensitively than