New Approaches to Preparing for Cytologic Examination

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As a general procedure, always make direct smears. Large quantity specimens should be preserved with anticoagulants to prevent clotting. Even transudates, which are not turbid, should be preserved with anticoagulants. Small amounts of fibrin forming in these fluids are sufficient to entrap cells. The Wright's smears prepared from such fluids contain clusters of cells held together for no visible reason since fibrin is not demonstrated with this stain.

Blood is the most common fluid examined cytologically. The principles followed in making blood smears may be applied to all types of fluids. The conventional glass slide blood smear should have a feathered edge on a portion of the glass slide which may be examined with a microscope. The cells that flow to the feathered edge will also marginate to the sides of smears. The cells marginated to the edge of smears will usually be intact while similar cells carried to the feathered edge may be damaged. The length of the smear and the distribution of cells in the smear will depend on: 1) the viscosity of the fluid, 2) the size of the drop, and 3) the speed of the spreader slide. The angle of the spreader slide is usually 45°. Lower angles of the spread slide will result in thinner smears when other factors are constant. Fluids with viscosity lower than blood will have nucleated cells and particulates carried to the feathered end. Large cells, cell clusters, cells with abundant cytoplasm, platelets and bacteria will also concentrate at the feathered end and margins of smears when the fluid is of low viscosity. Cells carried to the feathered end are subject to sheer forces and the scrambled egg effect is readily apparent. Smears made by this method are valuable in that a gradient of cells is established and an ultra-thin portion of the smear is assured.

Alternate methods of direct smear preparation of low viscosity fluids are necessary to concentrate cells and reduce cell damage at the feathered end. The smear may be made as for a feathered end except that the spreader slide is stopped short of feathering the fluid. The spreader slide is removed and the straight end examined for cells. The slide may be tilted at a steep angle to allow a short back flow of fluid for a distance of a few millimeters. Several attempts may have to be made to achieve this result. The smears of low viscosity fluids made by this method will have cells concentrated in the back flow area. Smear damage is avoided and elegant smears with cells spread thin, the fried egg effect, are possible by this method.

Direct smears of fluffs with high viscosity generally have uniform cell distribution. Smears made of normal synovial fluid have uniform cell distribution even when the spreader slide is advanced very slowly to insure a thin smear.

Concentration Methods

Concentration methods facilitate examination of low cellularity fluids. Concentration techniques affect cell distribution and create additional artifacts which may be absent on direct smears. The longer cells remain in fluids, the greater the opportunity for degenerate changes to take place.

Centrifugation is the most common concentration method. Slow speeds (1500 rmp) should be used. The supernatant fluid is removed. The sediment is suspended in a small quantity of fluid left on the specimen. Difficulty is encountered in developing a uniform suspension of cells. Damaged with ruptured cells with exposed nucleoprotein tend to stick together in clusters. Alternate concentration methods may be required to assure thinly-spread cells. Cerebral spinal fluids and transudates of low cellularity yield small amounts of sediments which may be lost in the process of smear preparation.

Procedures based on the principle used in cytology centrifuges have been developed as simple concentrating methods. The quantity of fluid concentrated may range from a few drops to 0.5 to 1.0 ml. A simple method is based on sedimentation. A filter paper with an 8-10 mm hole is folded around a slide with the hole centered on the slide. A receptacle with a larger inside diameter is fixed over the hole. A simple unit consists of a rubber washer fixed to the slide with paper clips. A small quantity of fluid is placed in the center of the washer and the fluid is absorbed on the filter paper. Care must be taken to ensure that absorption of the fluid is at a slow rate to ensure sedimentation of cells. Cerebral spinal fluid smears made by this method are superior to centrifugation in quality and quantity of cells recovered.

A cyto-centrifuge called the Cytospin (Shandon Southern Instrument, Inc., 515 Brood Street, Sewickley, PA) may be used to concentrate small quantities of low cellular fluid. The preparations are of excellent quality, and this is the concentration method of choice for small animal practice.

High Viscosity Fluids and Tissues

Thick viscous fluids and solid tissue aspirates require alternate techniques of spreading, crushing, imprinting, and

scraping on to slides. Thin and thick areas are usually present when small quantities of material are spread. Damage to cells is usually greater than with that encountered in fluid specimens. Recognition of cell damage and poor quality slides become apparent from slide examination. Thus, the slide examiner should prepare the slides for cytologic study in order to recognize when improvements in smear preparation techniques are necessary.

Common problems encountered with high viscosity fluids and solid tissue specimens are large clumps of cells. The Romanovsky stains do not penetrate the cell clusters and adjacent areas of such smears are generally much too thick for cytologic study. A common problem with tissue imprints is fluid covering the surface of the tissue preventing contact of the cells to the slides. Blotting the tissue to remove blood or fluid before touching a slide allows direct contact with glass and adherance. Impression smears made by this method allow judgments to be made regarding cell location and relationships.

Smears made from fluids and tissues are air-dried. Crenation changes induced by slow drying are not critical as they are with blood smears. The air-dried smear yields the best quality stained smears when staining is done shortly after smear preparation. Smears left unstained for several hours usually decrease in the quality of staining. This decrease in staining quality does not seriously reduce the diagnostic value of the specimen. The submission of air dried smears is more desirable than fixation of smears with methylalcohol. In the experiences of the authors, methylalcohol fixed stains are inferior to air-dried smears for staining with Wright's stain. Further, fixation with methylalcohol precludes the use of Gram and NMB stains.

Slide breakage of mailed specimens is relatively frequent for slides shipped in cardboard containers placed in unreinforced mailers. Rigid plastic mailers are much more satisfactory.

Specimens to be submitted to laboratories for cytologic examination by the Papanicolaou method need to be wet-fixed in special fixatives. When in doubt as to procedures to be followed in preparing specimens for submission to diagnostic laboratories, contact the laboratory for instruction.

Abstract

Weight gain in steer and heifer calves treated with zeranol or oestradiol 17β

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SUMMARY: Male and female Hereford and Hereford cross beef calves between 2.5 and 4.5 months of age were implanted with 36 mg zeranol, 24 mg oestradiol 17β or left untreated, and then weighed every 4 to 5 weeks. Calves treated with zeranol or oestradiol 17β gained 9.6 kg (8.2%) and 12.9 kg (11.0%) more weight respectively than untreated calves. Steer calves gained more weight than heifer calves. There was no response to either growth promotant in heifers or in zeranol-treated steers beyond 35 days after implantation, and the only significant further gains in the next 31 days were made by steers treated with oestradiol 17β. After this time growth of treated and control calves was similar. The lack of a continued response to the growth promotants was not associated with significantly lower growth rates in any weighing interval.

Possible reasons for the duration of response observed and the difference in response between the sexes are discussed.
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