TECHNIQUE FOR OBTAINING PURE ENDOMETRIAL FLUID FROM RHESUS MONKEYS

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This communication describes a simple technique for obtaining sufficient quantities of pure endometrial fluid (uterine cavity fluid) for microchemical analysis. We believe this technique has several advantages over other methods of sampling uterine fluid.

Various constituents of endometrial fluid have been measured in flushings of the uterine cavity obtained through the endocervical canal. These measurements are valuable in a qualitative way, but are not indicative of actual cavity concentrations because the volume of such fluid obtained by flushing is not known. Since endometrial fluid volume may vary from time to time in the same animal, and volume recovery may differ from flushing to flushing, only major variations in measured substances from flushings taken at different times in the same animal may be compared. Direct aspirates of significant volumes of cavity fluid usually produce a fluid contaminated with blood since catheter insertion and suction traumatizes the endometrium.

Various techniques were tried in our laboratory in an attempt to collect pure endometrial fluid from monkeys. At first, fluid was collected by capillary action into indwelling catheters inserted either through the cervical canal or through a uterocutaneous abdominal fistula. The catheters were left in place over several hours or days, but the catheter tips induced haemorrhage (even when the tips consisted of soft silastic tubing), and this produced bloody contamination of the fluid collected as well as clot formation so that flow in the catheters was blocked. In addition, the viscosity of endometrial fluid itself often prevented flow in the catheters. Another disadvantage to very gradual collection of fluid is that analysis of labile constituents in endometrial fluid requires a fresh sample, not one which has been collected over hours or days.

The purpose of this communication is to report a method of direct aspiration of pure uterine cavity fluid from rhesus monkeys allowing quantitative analysis of constituent concentrations. The purity of this fluid was inferred from its gross appearance and viscous nature, both of which were similar to that found in the cavity of uteri removed at surgery or autopsy. The abdominal uterocutaneous fistula model of rhesus monkeys as previously described (Good & Moyer, 1966; Shaw, Moyer, Poon & Nogueira, 1972) was employed in this study. Both castrated, hormonally supplemented and non-castrated, naturally

menstruating animals were used. Animals having visible uterine secretions at the abdominal stoma of the fistula were found to be most productive of fluid.

Under sterile conditions, a 1.5-mm olivary-tipped probe is gently inserted through the fistula into the uterine cavity 2 days before aspiration. This serves to dilate the fistula and determine the angulation between it and the uterine cavity in each monkey. For aspiration, 60 cm of polyethylene catheter with an internal diameter of 0.38 mm and an outer diameter of 1.09 mm is applied to a 26-gauge needle attached to a 1-ml tuberculin syringe. The free end of the catheter is fitted approximately 2 mm inside a 3-mm length of silastic tubing, the latter having an internal diameter of 0.52 mm. The silastic tubing segment provides a very soft tip which produces minimal trauma inside the cavity. At the time of insertion, the anterior abdomen of the monkey is gently sterilized, and any secretions at the fistula stoma are wiped away. The silastic tipped catheter is then gently threaded through the fistula into the uterine cavity. As long as resistance is not encountered, the tip of the catheter is inserted deeply into the cavity, frequently to the level of the internal cervical os. Gentle suction is immediately applied by slowly extracting the plunger of the tuberculin syringe. Excess suction results in bloody fluid. During aspiration, the tip of the catheter is slowly withdrawn until it reaches the fundal end of the cavity. If blood has not appeared in the catheter by this time, the tip may be gently reinserted toward or to the level of the internal cervical os, and this is followed by repeated aspiration and slow catheter withdrawal. Sometimes, short segments of blood may appear in the catheter followed by segments of endometrial fluid. As soon as a solid column of blood appears, a clamp is applied to the catheter just above the level of the advancing endometrial fluid. This prevents suction of the fluid into the syringe when the catheter is withdrawn from the fistula. After withdrawal, the catheter is laid flat on a cutting board. A fresh Bard-Parker scalpel or razor blade is used to cut away segments of the catheter containing blood. After this, the length or lengths of catheter containing pure cavity fluid are measured. The length (in mm) of catheter containing pure fluid multiplied by 0.1134 (the value for πr^2 since the inner radius (r) of the catheter is 0.1900 mm) yields the volume in μl of fluid aspirated. Using this method, a maximum volume of 60 μl fluid has been aspirated from the same animal within a few minutes. Most animals with visible secretions at the fistula stoma initially will produce 10 to 30 μl .

Once the volume of aspirated fluid is known, an appropriate volume of diluent is pipetted into a test-tube or vial so that the uterine fluid may be mixed with it. The latter step is accomplished by attaching a 26-gauge needle to a 1-ml tuberculin syringe; withdrawing the plunger to fill the syringe with air; inserting the needle tip into one end of the catheter or catheter segments; and air-injecting the sample into its diluent with the syringe plunger. The catheter or its segment is then rinsed with diluent by numerous partial withdrawals and reinsertions of the syringe plunger. Thus, a known volume of aspirated fluid may be quantitatively transferred to a given volume of diluent. In this way, the catheter or segment serves as a 'to contain' or wash-out pipette.

This technique should be useful for any measurements which can be performed on μl quantities of fluid. We believe the technique may be applicable to

aspiration through the cervical canal in primate species having a less tortuous approach to the uterine cavity than that of the rhesus monkey. Using this method, we have collected fresh endometrial fluid for the assay of labile protease activity including plasmin, plasminogen activator, and plasminogen. A separate report on these data is in preparation.

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