staging of endometrial cancer and possibly in infertility practice. For cervical lesions, this modality does not appear to be satisfactory at present.

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First-trimester endocervical irrigation: Feasibility of obtaining trophoblast cells for prenatal diagnosis

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We sought to determine the feasibility of obtaining trophoblast cells for first-trimester prenatal diagnosis using endocervical irrigation. We studied 20 pregnant patients between 7–10.5 weeks' gestation who presented for elective pregnancy termination. Under ultrasound guidance, a specially designed plastic catheter was advanced to the level of the internal cervical os. Gentle flushing and aspiration was performed with 3 mL of normal saline. The material obtained was fixed and stained. A placental pathologist identified trophoblast cells using light microscopy. In another five cases, we attempted to culture the endocervical washings. Trophoblast cells were identified by microscopy after staining the cultured material with an anti-alpha-hCG-antibody bound stain. In ten of 20 cases (50%), trophoblast material was retrieved on irrigation. Of the five additional cases on which culture was attempted, trophoblast was successfully cultured in one, the results were equivocal in two, and culture was unsuccessful in the other two. Trophoblast cells for prenatal diagnosis can be obtained in a significant percentage of cases by first-trimester endocervical irrigation. The advantages of irrigation include technical simplicity, brief duration (less than 3 minutes), and suitability to first-trimester diagnosis. Further testing is necessary to determine the risks. *(Obstet Gynecol 1995;85:461–4)*

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There has been a surge in the use of prenatal diagnosis services over the last decade. This increase is largely because of growing patient awareness of the burden of genetic disease, the greater number of disorders that are diagnosable prenatally, and the now widespread availability of sampling techniques that were formerly confined to pioneering university centers.

Noninvasive methods of prenatal diagnosis are highly desirable. Advantages of such methods include negligible risk of pregnancy loss or other fetal harm, easily mastered skills for obtaining a specimen, and lower procedural costs. Using the method described herein, there is the possibility of early prenatal diagno-

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sis at less than 9 weeks, when reproductive choices such as pregnancy termination could be pursued with maximum safety. Even if noninvasive methods are introduced into clinical practice, invasive sampling may still be required for confirmation of abnormal results. Ironically, while progress in ultrasound technology and serum analyte screening promises earlier identification (in the first trimester) of fetuses at risk for aneuploidy, fetal safety concerns support a delay in invasive testing. Therefore, we set out to determine the feasibility of obtaining trophoblast cells useful for prenatal diagnosis, using a minimally invasive technique.

Method

Twenty consecutive women presenting for elective first-trimester termination of pregnancy agreed to participate in the first phase of the study. The study protocol was approved by the Human Investigation Committee of the Yale University School of Medicine, and informed consent was obtained from each woman. Ultrasound confirmation of fetal cardiac activity at the time of termination was a prerequisite for inclusion.

The patient was prepared for pregnancy termination in the usual fashion and placed in the dorsal lithotomy position. After placement of a speculum, the vagina and cervix were cleaned with povidone iodine. A paracervical block was performed with a total of 10 mL of 1% lidocaine, after which a tenaculum was applied to the anterior cervical lip for stabilization. Under continuous ultrasound guidance, a uterine sound was inserted to determine the direction of the cervical canal, as is done in transcervical chorionic villus sampling (CVS). We used a modified CVS catheter (Concord-Portex, ME) with a blunt end to reduce the possibility of direct placental biopsy, but with proximally placed holes on the side for irrigation and aspiration. The catheter was advanced under continuous ultrasound guidance to a level just beyond the internal cervical os. If the catheter tip was judged to be too high relative to the internal os, it was withdrawn to this level before irrigation. Three milliliters of normal saline were slowly injected at low pressure and immediately aspirated. The material obtained was immediately fixed in Bouin solution and light microscopy was subsequently performed.

In a later phase of the study, we attempted to culture trophoblast cells from another five cases. The culture technique was the same as that used for CVS samples. The cells were cultured on a coverslip in Alpha MEM media (Gibco BRL, Gaithersburg, MD) with 20% fetal bovine serum and 1% kanamycin. Harvesting was performed 5–7 days later. Trophoblast cells from culture were identified after fixing the material to a microscope slide and then by staining with an anti–alpha-hCG-antibody bound stain. Microscopy was performed by a single experienced placental pathologist. No nonpregnant endocervical specimens were utilized as controls in this study.

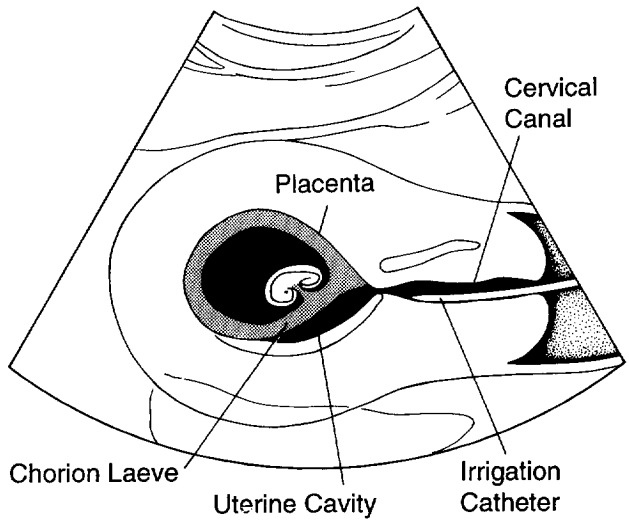

Figure 1. Sonographic appearance of an endocervical catheter placed at the internal os at 9 weeks' gestation.

Experience

Figure 1 illustrates the ultrasound appearance of the catheter placed at the internal cervical os. The gestational age of the pregnancies ranged from 7–10.5 weeks. In the first phase of the study, we were able to identify syncytial clumps or fragments of villi on microscopy in ten of 20 (50%) cases. In the next phase, we successfully cultured trophoblast cells in one of five cases. In two, the results of the anti-hCG stain were equivocal, defined as light staining of the cells. In the remaining two cases, no trophoblast could be identified.

Neither placental location nor gestational age clearly affected the chances of successful retrieval of trophoblast cells. None of the patients developed complications after endocervical irrigation and abortion.

Comment

The development of an acid elution test for the identification of fetal erythrocytes[1] and the identification of cells with a 46,XY karyotype in maternal blood[2] confirmed the transfer of fetal cells into the maternal circulation. Capitalizing on this information and combining it with advances in the technology of fluorescence-activated cell sorting[3] and genetic diagnosis methods such as the polymerase chain reaction and fluorescent in situ hybridization, great strides have been

made towards achieving the goal of noninvasive prenatal diagnosis.[4–6] Prenatal diagnosis by this method is still in its infancy; however, this technique will probably need to be combined with one of the more invasive methods of sampling for confirmation of abnormal results.

The techniques of irrigation and swabbing of the internal cervical os promise minimally invasive methods of prenatal diagnosis that potentially could be combined with the extraction of fetal cells from the maternal circulation. At about 4 days after fertilization, certain cells of the blastocyst differentiate to form trophoblast. The trophoblast proliferates and eventually occupies the entire circumference of the chorion. Between 8–13 weeks, much of this branching trophoblast gradually disappears except for the area destined to become placenta. Shettles surmised that some of the exfoliated trophoblastic cells would accumulate at the level of the internal cervical os (Shettles LB. Use of the Y-chromosome in prenatal sex determination [letter]. Nature 1971;230:52–3). He performed endocervical swabbing and quinacrine staining of the cells retrieved in an attempt to identify Y chromosomes. A male fetus was thought to be present when more than 3% of cells obtained had a positive Y-chromosome signal. Shettles accurately predicted fetal sex in a series of patients, thus confirming his theory. Subsequent reports both confirmed and refuted his findings.[7–10] Contamination by semen up to several days after intercourse was shown to be a significant source of error with endocervical swabbing.

Our study confirms that material for genetic analysis of the fetus can be obtained by endocervical irrigation. The available literature on the subject is limited[9,11] (Coleman DV. Endocervical lavage in early pregnancy [letter]. Am J Obstet Gynecol. 1982;142:18–9), with only one study[11] reporting more cases than the current authors. The studies suggest the potential utility of this technique. In the literature, there were minor differences in the equipment used for irrigation. Usually, a simple plastic catheter was used. The most complicated design was that used by Rhine et al.[11] Their "Antenatal Cell Extractor" consisted of a plastic tube, 2.5 mm outer diameter, that contained a plunger and a side arm that permitted washing and aspiration of endocervical material.

We were successful in retrieving trophoblast cells in half the cases. Use of currently available technology, such as fluorescence-activated cell sorting or magnetic activated cell sorting, to reduce maternal cell contamination and fluorescence in situ hybridization or polymerase chain reaction techniques for identification of a chromosome or gene of interest, might have enhanced our success rate. In addition, in identifying trophoblast cells in culture, confirmation depended on an alpha-hCG-antibody bound stain. This stain will not bind to mesenchymal cells of the trophoblast because they do not produce hCG. Therefore, it is also possible that we underestimated our ability to culture cells successfully.

We have been concerned about the possibility of uterine infection with cervical irrigation. A risk may exist in continuing pregnancies. Interestingly, Rhine et al[11] reported performing pregnancy termination 8 days after cervical irrigation in a group of patients. No interim side effects such as spotting, bleeding, cramping, or temperature elevation were reported.

Because our study was done immediately before pregnancy termination, local anesthesia was routinely used. Rhine et al[11] reported performance of cervical irrigation without anesthesia and with minimal patient discomfort. We found the procedure to be technically simple. Sonographic localization of the tip of the catheter proved to be the critical step, requiring expertise well within the competence of an average sonographer. Furthermore, the duration of the actual procedure was brief, requiring less than 3 minutes for completion.

A question that might be raised is whether this procedure represents a "mini"-CVS. Although inadvertent placental biopsy cannot be completely ruled out, we took precautions to minimize this possibility. The likelihood of direct biopsy was reduced by catheter design (specifically, a sealed tip) combined with the technique of delaying irrigation until the desired placement just above the internal os. The previous studies were performed blindly, and steps were not taken to ensure that direct placental biopsy did not occur. Both the improvement in design and ultrasound-guided localization minimized the likelihood of direct placental trauma. This could prove beneficial in reducing complications such as pregnancy interruption. With low-pressure irrigation, it is possible that fragments of exposed trophoblast fronds can be dislodged. However, the extent of placental trauma that occurs with CVS is unlikely to occur with endocervical irrigation.

Endocervical irrigation has some advantages over diagnosis based on extraction of fetal cells from maternal blood. Fetal cells or trophoblasts can be found in the maternal circulation only in extremely minute proportions at any gestational age. In contrast, trophoblast can be obtained in much larger numbers with potentially less contamination by maternal cells. Endocervical irrigation permits retrieval of metabolically active cells,[11] which could facilitate diagnosis of metabolic disorders. In addition, high-resolution karyotype is achievable in the cells obtained, permitting identification of more subtle chromosome alterations than aneuploidy, such as rearrangements, deletions, and duplications. This is not the case when using fetal cells from the maternal

blood. At present, results from the cells in the maternal circulation are not definitive, but they do indicate an increased likelihood of aneuploidy.[12] Abnormal findings on maternal blood testing could potentially be confirmed by cervical irrigation or swabbing rather than amniocentesis or CVS.

Important issues regarding endocervical irrigation remain to be addressed. First among these is the problem of maternal cell contamination of the specimens obtained. A high degree of trophoblast cell purity must be achieved before polymerase chain reaction techniques can be used for diagnosis of single gene disorders. A second concern is the possibility of mosaic abnormalities in trophoblastic cells. This risk is unlikely to be greater than the situation currently encountered with CVS. Because many of the cells from irrigation are dead or dying, identification of trophoblast cells by using fluorescence in situ hybridization techniques rather than attempts at culturing would have increased the yield of trophoblast retrieved.

In summary, we attempted to determine whether the technique of cervical irrigation just above the level of the internal os in the first trimester could provide trophoblast cells sufficient for prenatal diagnosis. In this preliminary study we were successful in 50% of cases. At present, this success rate is not high enough to allow clinical application of this method. More sophisticated techniques, such as the use of cell sorting equipment to minimize maternal cell contamination and fluorescence in situ hybridization or polymerase chain reaction technology for genetic analysis, must be refined to make cervical irrigation a clinically reliable and complementary method to studies of fetal cells in maternal blood. Issues of safety and ultimately clinical usefulness can only be addressed after more extensive testing.

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