Edward E. Wallach, M.D.
Associate Editor

Transcervical retrieval of fetal cells in the practice of modern medicine: a review of the current literature and future direction

Anthony N. Imudia, M.D.,[a] *Sanjeev Kumar, M.D.,*[a] *Michael P. Diamond, M.D.,*[a] *Alan H. DeCherney, M.D.,*[b] *and D. Randall Armant, Ph.D.*[a,b,c]

[a] Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, Michigan; [b] Program in Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland; [c] Department of Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, Michigan

Objective: To review published methods for transcervical collection of fetal cells and to assess the potential of this approach for application in prenatal diagnosis.
Main Outcome Measure(s): Retrospective analysis of efforts at prenatal diagnosis with trophoblast cells shed into the lower uterine pole that accumulate within the cervical mucus at the level of the internal os.
Result(s): Minimally invasive techniques that include cervical mucus aspiration, cervical swabbing, and cervical or intrauterine lavage can be used to retrieve trophoblast cells during the first trimester for diagnostic purposes, including for prenatal genetic analysis. Fetal cells have been identified in these specimens with success rates that vary from 40% to 90%. The disparity in reported success rates can be a function of gestational age, collection method, operator variability, detection sensitivity, or pregnancy status. Molecular approaches have been devised to determine fetal sex and identify aneuploidies. Antibody markers have proven useful to select trophoblast cells for genetic analysis and to demonstrate that the abundance of recoverable fetal cells diminishes in abnormal gestations, such as in ectopic pregnancy or blighted ovum.
Conclusion(s): Transcervical collection of fetal cells offers several avenues for prenatal diagnosis that with further refinement could one day provide valuable information for the management of ongoing pregnancies. (Fertil Steril® 2010;93:1725–30. ©2010 by American Society for Reproductive Medicine.)

Key Words: Cervical mucus, ectopic pregnancy, fetal cells, genetic analysis, immunological markers, intrauterine lavage, prenatal diagnosis, transcervical cell collection, trophoblast

It is thought that due to changing demographics, increased exposure to environmental toxins, and intervention in the reproductive process, developmental abnormalities may be on the rise (1). The risk to any pregnant couple of having a liveborn infant with a chromosomal abnormality or structural defect has been previously estimated to be between 3% and 5% (2). Because of this considerable risk, much effort has been expended in recent decades to identify pregnancies at risk of chromosomal anomalies and genetic disorders at an early gestational age. The current standard of care involves screening maternal analytes and ultrasound markers, each alone or in combination, to identify at risk pregnancies, followed by referral for definitive diagnostic tests that include amniocentesis and chorionic villous sampling. Although the former screening modalities have considerable rates of false-positive and false-negative results, the latter diagnostic tests are invasive and carry a substantial risk of fetal loss. Indeed, Mujezinovic and Alfirevic (3) conducted a systematic analysis of 45 studies and reported a fetal loss rate of 1.9% for amniocentesis and 2% for chorionic villous sampling. Therefore, the search to develop safer methods to obtain genetic material from the fetus is ongoing and desperately needed.

Another alternative for prenatal diagnosis is preimplantation genetic diagnosis (PGD), which involves screening for chromosome abnormalities or single gene disorders in an embryo before implantation (4). The main advantage is avoidance of elective pregnancy termination while offering a high likelihood that the fetus will be

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Reprint requests: D. Randall Armant, Ph.D., Department of Obstetrics and Gynecology, Wayne State University School of Medicine, CS Mott Center, 275 East Hancock Avenue, Detroit, MI 48201-1405 (FAX: 313-577-8554; E-mail: darmant@med.wayne.edu).

free of a specific disorder. Although PGD is an attractive method for prenatal diagnosis, it is an adjunct of assisted reproductive technology that requires in vitro fertilization, which has its own risks and high costs. Thus, PGD is not feasible as a universal diagnostic tool for genetic abnormalities in the general population.

Identification of fetal cells in maternal serum has been attempted, but this approach has been hindered by the relative rarity of fetal cells in maternal blood (one fetal cell per 10^6–10^7 maternal cells) and associated difficulties in their isolation and analysis. Overall, the projected clinical efficacy has been disappointing (5). Nevertheless, recent discovery of fetal nucleic acids in maternal plasma has introduced several new possibilities for noninvasive prenatal diagnosis of chromosomal aneuploidies (6). Anomalies are revealed after the first 11 weeks of gestation by measuring the allelic ratio of single nucleotide polymorphisms in the coding region of placental messenger RNA (mRNA), analysis of DNA fragments with different patterns of DNA methylation between fetal and maternal DNA, enrichment of the fractional concentration of fetal DNA in maternal plasma using physical or chemical methods, and the development of more precise digital methods based on polymerase chain reaction (PCR) for fetal nucleic acid analysis (6, 7). Specific inheritable diseases also could be diagnosed with fetal DNA (8). These new approaches for prenatal diagnosis using maternal plasma are challenging for practical application because they require sophisticated and expensive technology. Presumably, large scale clinical trials will soon be initiated to validate the accuracy and safety of these approaches for routine clinical practice.

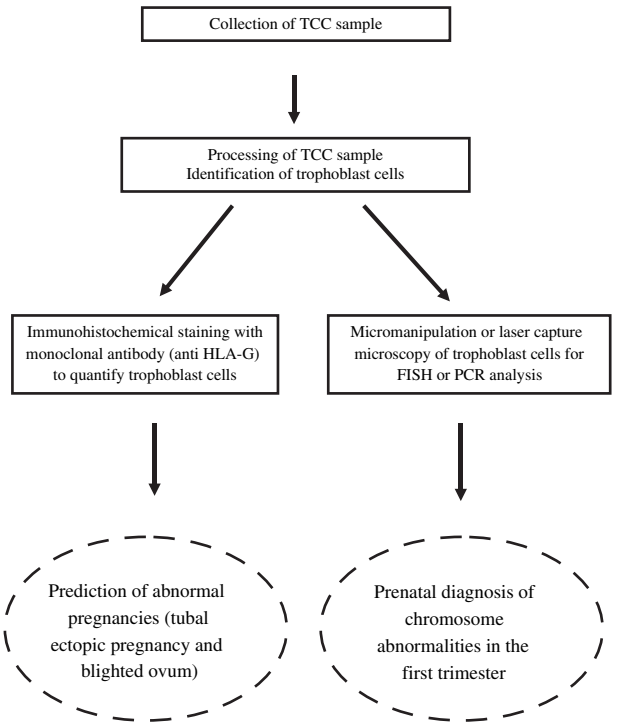
Before 13 to 15 weeks of gestation, it is believed that small areas of erosion allow trophoblast cells to cross the decidua capsularis and reach the uterine cavity (9). This process becomes less likely after the amniochorionic membrane seals the uterine cavity and the internal cervical os, which is thought to occur at 3 months of gestation. In 1971, Shettles (10) suggested that during early pregnancy a similar shedding occurs into the uterine cavity, making chorionic cellular elements from the degenerating villi available in the endocervical canal. The possibility of capturing fetal cells from accessible regions of the reproductive tract suggests new approaches for early prenatal diagnosis. The isolation of fetal cells from the cervix and the endometrial cavity offers an attractive noninvasive alternative for very early (6 to 14 weeks, possibly as early as 5 weeks [11]) diagnosis. Since the procedure's first description, several investigators have reported the feasibility of isolating fetal cells from the cervical mucus or from fluid obtained by lavage of the endometrial cavity with varying degrees of success. The existing literature suggests that the present status of transcervical cell (TCC) sampling in prenatal diagnosis is experimental but carries excellent potential for both genetic diagnosis and prediction of pregnancy outcome (Fig. 1) as laboratory methods are refined and standardized. The publication of both encouraging and discouraging reports of TCC sampling reliability warrants a reexamination of this topic, which has been previously surveyed (9, 12, 13). Here, we review the pertinent literature that describes TCC collection, the isolation of fetal cells and their subsequent analysis, and the applicability of the technique in the practice of modern clinical medicine.

RETRIEVAL OF TROPHOBLAST CELLS FROM THE CERVIX

The ideal method, which would reliably yield fetal cells in appreciable quantity, should have no negative impact on the ongoing pregnancy and should be free from infectious or traumatic complications. It should also be simple to perform and cost effective, with minimal interobserver variability. A number of techniques

FIGURE 1

Transcervical cell analysis and potential clinical applications by quantification of trophoblast cells or genetic analysis using fluorescence in situ hybridization (FISH) or the polymerase chain reaction (PCR).



Imudia. Transcervical sampling for trophoblast cells. Fertil Steril 2010.

have been devised to retrieve TCC samples from the endocervical canal and the endometrial cavity, including smears obtained with cotton swabs or a cytobrush (14–16), aspiration of cervical mucus with a catheter (17–19), endometrial biopsy with a Pipelle (20), and lavage of the endocervical canal (21–24) or the uterine cavity (14, 22, 25), all with variable success (Table 1).

At present, the existing literature differs vastly and is often contradictory in projecting the relative efficacy of the various methods for retrieving fetal cells. Previously, emphasis has been placed on the feasibility of obtaining fetal cells and establishing their diagnostic utility, rather than on a direct comparison of the relative efficacy of the various methods in randomized control trials, as recently reported elsewhere (14, 22). It has been noted that the postcollection processing of the TCC samples has tremendous variation from one study to another (26), which directly affects the yield of useful information. The techniques used to identify the fetal cells and the diagnostic end points (fetal sex vs. gene disorders) also have differed, yielding heterogeneous groups for comparison with nonuniform results. Thus, there is a lack of information on well-described techniques for sample collection and analysis, resulting in considerable dependence on the technique and skill of individual operators.

In the landmark 1971 report by Shettles (10), identification of the Y chromosome was used to determine fetal sex from midcervical mucus samples obtained with cotton swabs. A limitation of using cotton swabs to retrieve TCC samples is the entrapment of cells within the cotton, which may reduce yield (9, 27). The use of a cytobrush for cervical mucus retrieval or lavage of the endocervical canal with normal