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INVITED EDITORIAL
Fetal DNA in Maternal Plasma: The Plot Thickens and the Placental Barrier Thins

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In this issue of the *Journal,* Lo et al. (1998) describe their experiences with the novel application of a real-time quantitative PCR assay designed to measure the concentration of fetal DNA in maternal plasma and serum samples. Their system, which uses a 5′ nuclease and fluorescent primers, permits continuous optical monitoring of the progress of an amplification reaction, and it is sensitive enough to detect the DNA in the equivalent of 1 male fetal cell against a background of 12,800 female-genome equivalents. The data presented in that article demonstrate the reproducibility of this system.

Lo et al. (1998) used SRY, a single-copy Y chromosome–specific sequence, to quantify the number of genome equivalents per milliliter of blood when the pregnant woman carries a male fetus. SRY was chosen because of the obvious lack of this sequence in the mother. SRY sequences were never detected in nonpregnant women but were detected in all pregnant women carrying male fetuses, as early as 7 wk of gestation. These findings are in agreement with the previous reports of the other groups, who used less-sensitive PCR methods (Thomas et al. 1995). Furthermore, Lo et al. did not detect any evidence of SRY when a prior child had been male but the current fetus was female.

The results of this study demonstrate surprisingly high mean concentrations of fetal DNA (3.4%–6.2%) in total maternal plasma DNA, with the mean fetal DNA concentrations increasing 12-fold over the course of pregnancy. The fractional concentration of fetal DNA in serum was significantly less because of the increased amount of total DNA in serum, presumably because of cellular lysis occurring during coagulation. These results validate and extend the prior work of Lo et al. (1997), which showed that fetal DNA could be reliably detected in as little as 10 μl of maternal plasma.

How do the results compare with those reported in previous studies? Almost all prior investigations have focused on complete and intact fetal *cells* in the maternal circulation, so that their nuclei are available for either cell culture or interphase cytogenetic analysis using chromosome-specific probes. Many laboratory groups have attempted to quantify the amount of fetal cells in maternal blood (Ganshirt-Ahlert et al. 1990; Reading et al. 1995; Lewis et al. 1996; Sohda et al. 1997). These reports cannot be compared with each other because different methods of cell preparation were used. For example, Hamada et al. (1993) treated 2 ml of maternal peripheral blood with a hypotonic solution, followed by fixative and FISH. They performed manual observation of nuclei bearing a hybridization signal with a Y chromosome–specific probe (Hamada et al. 1993). They observed only 2 fetal cells in 770,000 cells examined in eight subjects carrying male fetuses at <15 wk of gestation. A significantly higher frequency was reported by Watchel et al. (1996), who used charge-flow–separation techniques and estimated that thousands of fetal cells were present in maternal blood samples.

Most PCR-based noninvasive prenatal diagnostic studies have also used DNA prepared from intact fetal and maternal cells. In the past, the plasma has been discarded. This was the approach taken in a previous study reported by Bianchi et al. (1997), in which a radioactive quantitative PCR was employed in an assay to measure the number of fetal cell DNA equivalents in 16 ml of maternal blood samples (Bianchi et al. 1997). The mean number of male DNA equivalents in samples obtained from women carrying karyotypically normal male pregnancies was 19, or ~1.2/ml of whole blood. Lo et al. (1998) expressed their results as copies of SRY per milliliter and demonstrated a mean of 25.4 copies in the maternal plasma samples obtained early in pregnancy.

Many mechanisms could explain the notable findings of Lo et al. (1998). One possibility is that there is continuous leakage or transfer of fetal cells across the placenta but that the maternal immune system rapidly destroys the material, leaving DNA remaining in the plasma. This would imply that investigators who isolate fetal cells from maternal blood are seeing only a limited fraction of what was once there. An alternative expla-

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nation is that there is active remodeling of the placenta at the fetal/maternal interface, with continuous cell lysis and release of fetal DNA into the maternal circulation. A third explanation, as Lo et al. (1998) suggest, is that developmentally associated apoptosis of fetal cells may be occurring. A combination of several factors may explain the results. In any event, it is intriguing that the fetal DNA remaining from the disintegrating cells is not further metabolized and can be detected by this assay.

In reviewing the data presented in Lo et al.'s (1998) current study, one cannot help but notice the rather sharp increase in the amount of fetal DNA detected during the last 8 wk of pregnancy. The data shown demonstrate an overall increase in fetal DNA concentration as gestation advances, but it is the sharp increase depicted in Lo et al.'s figure 2 that is remarkable. Does this correlate with the peak phase of fetal growth (in the third trimester) or does this imply that, at the microscopic level, the placental barrier is breaking down in anticipation of the upcoming delivery?

The normative values established by Lo et al. (1998) for the amount of fetal DNA present in maternal plasma and serum across gestation will permit a determination of the specific factors that affect these values. Will, for example, the intrauterine growth–restricted fetus have less DNA in the maternal plasma? Will the infected placenta permit transfer of more fetal DNA into the mother? Will measurement of fetal DNA in maternal plasma ultimately be used as an indicator of fetal or placental well-being? In a previous study, a variation in the number of fetal cells detected among pregnant women carrying karyotypically normal male fetuses was noted (Bianchi et al. 1997). A statistically significant increase in the number of fetal cells detectable in maternal blood when the fetus had Down syndrome was also noted, leading to the hypothesis that there are placental abnormalities associated with aneuploidy. It would be very interesting to know whether fetuses with Down syndrome have increased amounts of fetal DNA in their maternal plasma as well.

The authors envisage the primary clinical utility of this assay to be determination of the presence of paternally inherited polymorphisms associated with disease, such as beta-globin mutations or rhesus D in a sensitized mother (Camaschella et al. 1990; Lo et al. 1993; Cheung et al. 1996). Although fetal aneuploidy might be suggested by increased amounts of fetal DNA present in maternal plasma, cytogenetic confirmation using intact nuclei will ultimately be necessary. The major advance of the Lo et al. (1998) study is that it demonstrates the presence of relatively large amounts of fetal DNA in the mother. This forces all of us to rethink the traditional teaching that the fetal and maternal circulations are separated by a generally impermeable chorionic barrier. Future experiments must be directed toward understanding the maternal clinical implications of this extensive exposure to "foreign" fetal DNA. The development of sensitive methods that allow detection of previously unrecognized amounts of fetal DNA in the maternal circulation has, indeed, caused the plot to thicken.

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