

1. McDonald CC, Stewart HJ. Fatal myocardial infarction in the Scottish adjuvant tamoxifen trial. *BMJ* 1991;303:435-7.
2. Stampfer MJ, Colditz GA, Willett WC. Menopause and heart disease: a review. *Ann N Y Acad Sci* 1990;592:193-203.
3. Ritterband AB, Jaffe IA, Densen PM, Magagna JF, Reed E. Gonadal function and the development of coronary heart disease. *Circulation* 1963;27:237-51.
4. Manchester JH, Herman MV, Gorlin R. Premenopausal castration and documented coronary atherosclerosis. *Am J Cardiol* 1971;28:33-7.
5. Wuest JH, Dry TJ, Edwards JE. The degree of coronary atherosclerosis in bilaterally oophorectomized women. *J Am Heart Assoc* 1953;7:801-9.
6. Parrish HM, Carr CA, Hall DG, King TM. Time interval from castration in premenopausal women to development of excessive coronary atherosclerosis. *Am J Obstet Gynecol* 1967;99:155-62.
7. Barrett-Connor E. Putative complications of estrogen replacement therapy: hypertension, diabetes, thrombophlebitis, and gallstones. In: Korenman SG, ed. *The menopause*. Norwell, Mass.: Serono Symposia, 1990:199-209.

### FETAL FIBRONECTIN AND PRETERM LABOR

*To the Editor:* We applaud Lockwood et al. (Sept. 5 issue)<sup>1</sup> for their multicenter study aimed at identifying a biochemical marker for preterm labor and delivery. However, it seems that the authors may have done themselves and the readers a disservice by not appreciating the implications of our previous collaborative efforts on this topic. For example, the cellular sources of "fetal" fibronectin had previously been unclear, but our studies revealed that the fibronectin was probably not fetal in origin. Specifically, using monoclonal antibody FDC-6 as an immunohistochemical probe, we found that oncofetal fibronectin is deposited by anchoring trophoblasts and chorionic trophoblasts in the extracellular matrix of the placental-uterine junction and chorion.<sup>2,4</sup> Our studies further identified the FDC-6-reactive epitope on fibronectin that was actively synthesized, secreted, and deposited in the extracellular matrix by cultured human trophoblasts.<sup>2,3</sup> These combined results gave us new insight into the possibility that the chorionic trophoblasts in the extracellular matrix might be an important source of cervicovaginal "fetal" fibronectin.<sup>4</sup>

The authors drew certain conclusions about the molecular form of the FDC-6-reactive material present in positive cervicovaginal samples obtained by swabbing. We, as well as the authors, had previously wondered whether the ROMcheck immunoassay truly detected fibronectin molecules, rather than simply small, degraded protein fragments. We answered these questions in our laboratory using the same samples collected and studied by Lockwood et al.<sup>1</sup> and sent to us by Drs. Lockwood and Garite.<sup>4</sup> By analyzing these clinical samples with the same Western immunoblot approach described for trophoblasts,<sup>2,3</sup> we identified both intact and degraded forms of oncofetal fibronectin (Fig. 1). These results demonstrated that large molecular forms of fibronectin could leak into the cervix and vagina and suggested that the pathophysiologic process of preterm labor might involve the proteolytic degradation of oncofetal fibronectin in the chorion.<sup>4</sup>

Although the degradation and release of oncofetal fibronectin from the fetal membranes may be a marker for preterm delivery, the general clinical usefulness of an assay for this marker remains in question. For example, how will it be possible to distinguish FDC-6-positive material derived from the chorion from that derived from the amniotic fluid, where oncofetal fibronectin is present in high concentrations? In addition, since there is apparently substantial oncofetal fibronectin in third-trimester maternal serum,<sup>1</sup> it is hard to understand how some patients who were found on gross examination to have blood in the vagina could test negative on the authors' sensitive immunoassay. A further consideration not addressed experimentally by the authors is the specificity of FDC-6 binding to fibronectin isoforms circulating in the plasma of pregnant and nonpregnant women. We should be cautious not to overinterpret the clinical importance of material in cervicovaginal secretions found to be positive by ROMcheck assay. However, on the basis of what we have learned about oncofetal fibronectins, we

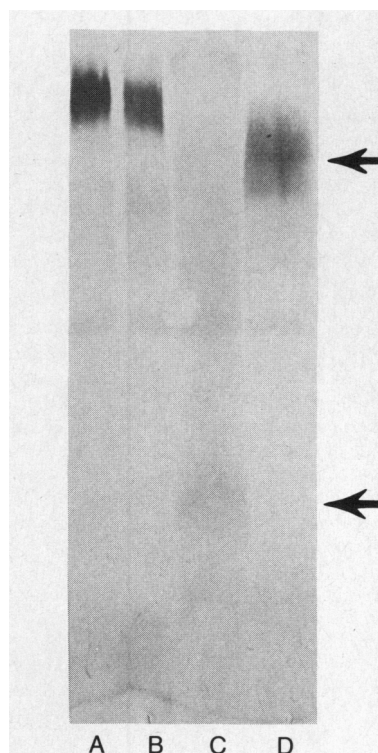


Figure 1. Immunoblotting with Monoclonal Antibody FDC-6 of Cervicovaginal-Secretion Samples from Four Patients Who Delivered Prematurely, with (Lanes A and B) or without (Lanes C and D) Spontaneous Rupture of Membranes

In lanes A and B 10  $\mu$ l of ROMcheck sample was loaded, and in lanes C and D 50  $\mu$ l of ROMcheck sample was loaded. The samples were electrophoresed in 6 percent sodium dodecyl sulfate-polyacrylamide gels under reducing conditions, electrotransferred to nitrocellulose filters, and assayed for the presence of oncofetal fibronectin with the monoclonal antibody FDC-6, as previously described.<sup>3</sup> Patients with ruptured membranes generally had intact oncofetal fibronectin (lanes A and B), whereas patients with intact membranes had intact fibronectin (data not shown) as well as degraded moieties reactive with the antibody (lanes C and D, indicated by arrows). Other antifibronectin antibodies that are not specific for the oncofetal domain reacted with these samples in a similar pattern.

should encourage the pursuit of earlier and more specific markers for preterm inflammatory processes in the chorionic membrane.

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1. Lockwood CJ, Senyei AE, Dische MR, et al. Fetal fibronectin in cervical and vaginal secretions as a predictor of preterm delivery. *N Engl J Med* 1991;325:669-74.
2. Feinberg RF, Kliman HJ, Lockwood CJ. Is oncofetal fibronectin the trophoblast "glue" for normal human implantation? Proceedings of the 38th Annual Meeting of the Society for Gynecologic Investigation, March 20-23, 1991, San Antonio, Texas. Washington, D.C.: Society for Gynecologic Investigation, 1991:107. abstract.
3. *Idem*. Is oncofetal fibronectin a trophoblast glue for human implantation? *Am J Pathol* 1991;138:537-43.
4. Lockwood CJ, Feinberg RF, Kliman H, Garite TJ, Senyei A. Cervico-vaginal oncofetal fibronectin in preterm labor patients: a result of chorion extracellular matrix degradation. *Am J Obstet Gynecol* 1991;164:Suppl:374. abstract.

*To the Editor:* Lockwood et al. reported that the presence of cervicovaginal fetal fibronectin at concentrations above 0.05  $\mu\text{g}$  per milliliter identifies a subgroup of women at increased risk for preterm delivery. They found that the mean ( $\pm$ SD) fetal-fibronectin concentrations in maternal plasma were  $2.0 \pm 2.3 \mu\text{g}$  per milliliter in the second trimester and  $3.5 \pm 2.2 \mu\text{g}$  per milliliter in the third trimester. Hence, maternal plasma levels of fetal fibronectin are 40 and 70 times greater than the cervicovaginal threshold value in the second and third trimesters, respectively. We are concerned that the presence of fetal fibronectin in cervicovaginal secretions may simply reflect contamination by maternal blood, which is frequently present in the secretions obtained from patients admitted with preterm contractions. Although the authors excluded patients with clinically apparent vaginal bleeding, they did not exclude those with occult blood. Using the data presented in this study, we have calculated that secretions with a hematocrit of 1 percent or less, in which blood would not be clinically apparent, would nevertheless contain enough maternal plasma to yield a positive test for fetal fibronectin, regardless of the contribution of the secretions themselves. We suggest, therefore, that only the simultaneous measurement of blood and fetal fibronectin in cervicovaginal secretions will exclude the possibility that the presence of fetal fibronectin merely reflects the presence of maternal blood, which itself could define a subgroup of women at high risk for preterm delivery.

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The authors reply:

*To the Editor:* Drs. Feinberg and Kliman question the origin of cervical and vaginal fetal fibronectin that we found to be associated with preterm delivery. They contend that previous reports<sup>1-3</sup> localizing a substance reactive to FDC-6 to the placental and membrane-uterine junction demonstrated an extravillous-trophoblast source of fetal fibronectin. Such a conclusion appears unwarranted, since maternal decidual cells were not excluded as a potential site of synthesis. Our study addressed this deficiency, since it minimized antigen masking<sup>4</sup> potentially present in the earlier study and used parallel immunohistochemical staining for cytokeratin and vimentin to indicate that the cells surrounded by FDC-6-reactive material had the immunogenic properties of cytotrophoblasts, not decidual cells.<sup>5</sup> Furthermore, we showed that decidua-free regions of dichorionic membranes displayed intense chorionic FDC-6 immunoreactivity. We do concur that the finding of fetal-fibronectin release by cultured trophoblasts from full-term infants<sup>3</sup> is suggestive of a trophoblastic source.

Drs. Feinberg and Kliman interpret their previously reported immunoblot data<sup>1</sup> as consistent with the proteolytic degradation of the chorionic extracellular matrix in some patients. However, that study did not control for exposure to cervical and vaginal protease activity. Such a control is crucial to their interpretation, since fetal fibronectin may be released intact from the chorion and subsequently degraded by cervical and vaginal proteases.

Drs. Sadovsky and Friedman suggest that occult vaginal blood could serve as a source of cervical and vaginal fetal fibronectin. We believe that this is not likely to be the case. Levels of cervical and vaginal fetal fibronectin were reported as micrograms per milliliter of extraction buffer, not blood. Therefore, even pure maternal blood with a hematocrit of 30 percent and a whole-blood fetal-fibronectin level of 2  $\mu\text{g}$  per milliliter would, after a minimal dilution of 1:6 in buffer, result in a maximal fetal-fibronectin concentration of 300 ng per milliliter of buffer. Thus, a sample buffer would have to have a visually apparent hematocrit of more than 5 percent for blood to account for the fetal fibronectin detected.

In addition, we analyzed 843 cervical and vaginal samples obtained from 81 women between 24 and 34 weeks of gestation for both free hemoglobin<sup>6</sup> and fetal fibronectin. The threshold for detecting hemoglobin was 0.05  $\mu\text{g}$  per milliliter. Of the 843 samples,

2.0 percent were positive for both blood and fetal fibronectin, whereas hemoglobin alone was detected in 6.3 percent and fetal fibronectin alone in 11.1 percent. Thus, 85 percent of samples with fetal fibronectin contained no detectable hemoglobin. Moreover, the presence of hemoglobin was not predictive of preterm delivery. This discordancy indicates that gross and occult blood are not primary sources for cervical and vaginal fetal fibronectin.

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1. Lockwood CJ, Feinberg RF, Kliman H, Garite TJ, Senyei A. Cervico-vaginal oncofetal fibronectin in preterm labor patients: a result of chorion extracellular matrix degradation. *Am J Obstet Gynecol* 1991;164:Suppl:374. abstract.
2. Feinberg RF, Kliman HJ, Lockwood CJ. Is oncofetal fibronectin the trophoblast "glue" for normal human implantation? Proceedings of the 38th Annual Meeting of the Society for Gynecologic Investigation, March 20-23, 1991, San Antonio, Texas. Washington, D.C.: Society for Gynecologic Investigation, 1991:107. abstract.
3. *Idem*. Is oncofetal fibronectin a trophoblast glue for human implantation? *Am J Pathol* 1991;138:537-43.
4. Folkvord JM, Vidars D, Coleman-Smith A, Clark RAF. Optimization of immunohistochemical techniques to detect extracellular matrix proteins in fixed skin specimens. *J Histochem Cytochem* 1989;37:105-13.
5. Yeh IT, Kurman RJ. Functional and morphologic expressions of trophoblast. *Lab Invest* 1989;61:1-4.
6. Lijana RC, Williams MC. Tetramethylbenzidine — a substitute for benzidine in hemoglobin analysis. *J Lab Clin Med* 1979;94:266-76.

#### CIRCADIAN VARIATION IN VASCULAR TONE

*To the Editor:* The possibility of a circadian variation in the incidence of stroke, myocardial infarction, and sudden death has intrigued investigators in recent years.<sup>1-3</sup> Although some workers have implicated the sympathetic nervous system in this purported temporal pattern of heart disease, others have dismissed such an association. The reasons include the findings that catecholamine secretion does not increase during spontaneous episodes of ST-segment elevation<sup>4</sup> and that ischemic changes in ST waves during early-morning sleep are not preceded by increases in heart rate and do not appear otherwise to involve increased adrenergic activity.<sup>5</sup>

The report by Panza et al. (Oct. 3 issue)<sup>6</sup> that the  $\alpha$ -adrenergic-receptor antagonist phentolamine caused greater forearm vasodilation in the morning than in the afternoon or evening, but that sodium nitroprusside, a nonspecific antagonist, was equally active at all three times raises again the question of the participation of the adrenergic axis in cardiovascular disease. Two points, however, render the study questionable. First, phentolamine is not a specific  $\alpha$ -adrenergic antagonist. Second, the finding that sodium nitroprusside induced an equivalent reduction in vasomotor tone at all three times of day provides no insight into tone-generating mechanisms, adrenergic or otherwise.