Acta Dermatovener (Stockholm) 63: 185–189, 1983

PRENATAL EXCLUSION OF HERLITZ SYNDROME BY ELECTRON MICROSCOPY OF FETAL SKIN BIOPSIES OBTAINED AT FETOSCOPY

L. Löfberg,[1] I. Anton-Lamprecht,[2] G. Michaëlsson[3] and B. Gustavii[1]

[1]*Department of Obstetrics and Gynecology, University Hospital, S-221 85 Lund, Sweden,*
[2]*Institut für Ultrastrukturforschung der Haut, Universitäts-Hautklinik, D-6900 Heidelberg, Federal Republic of Germany, and* [3]*Department of Dermatology, University Hospital, S-751 22 Uppsala, Sweden*

Abstract. Two women had each borne a child who had died of Herlitz syndrome, i.e., epidermolysis bullosa atrophicans generalisata gravis. In subsequent pregnancies, the women requested prenatal diagnosis. Samples of skin from the two fetuses were obtained at fetoscopy in the 19th week of gestation. In both cases the disorder could be ruled out prenatally on the basis of ultrastructural demonstration of the regular presence of normal hemidesmosomes with well-developed sub-basal dense plates at the dermo-epidermal junction. The infants were subsequently born and had normal skin, the sites of fetal skin biopsies showing no scarring.

Key words: Prenatal diagnosis; Fetoscopy; Epidermolysis bullosa; Electron microscopy

The first reports of a successful prenatal diagnosis of inborn skin diseases appeared in 1980. The disorders in question were ichthyosiform erythroderma (7), the Herlitz syndrome (13), and Harlequin ichthyosis (5).

This paper reports two cases of prenatal *exclusion* of the Herlitz syndrome, i.e., epidermolysis bullosa atrophicans generalisata gravis. It also describes the technical difficulties of fetal skin sampling.

MATERIAL AND METHODS

Case 1

Neither the woman nor her husband were aware of any skin disease in their families. Their first child, born 1974, is a healthy boy without any skin abnormalities. The second child, a girl born 1979, had blisters around the nails and umbilicus at birth. She also had oral mucosal lesions. New blisters appeared continuously. Herlitz syndrome was diagnosed on the basis of the electron microscopic findings of junctional blister formations and hypoplasia of hemidesmosomes. Death of the infant within 19 days of birth, from sepsis, despite massive antibiotic medication, confirmed the grave prognosis of the Herlitz syndrome. Post-mortem skin biopsies again revealed junctional cleavage.

Both parents must be regarded as heterozygous carriers of the Herlitz gene, which is transmitted in an autosomal recessive pattern. The parents were informed of the 1 : 4 risk of their having a child affected by the disease.

In the third pregnancy, the woman requested prenatal diagnosis. In the 19th week of gestation (menstrual weeks), fetal skin specimens were obtained at fetoscopy. A preliminary report on this case has been published (11).

Case 2

This case resembled case 1, with a female baby suffering from Herlitz syndrome and succumbing at the age of 5 months. In the 19th week of a second pregnancy, fetal skin specimens were taken at fetoscopy.

Controls

The fetuses of 5 women who were to undergo elective abortion by hysterotomy in the 16th to 21st week of gestation served as controls. Immediately prior to th hysterotomy, fetal skin biopsy specimens were taken at fetoscopy.

The examinations were done after obtaining informed consent from the women and the approval of the Ethical Committee at the University Hospital in Lund.

Fetal skin sampling

A sharp trocar and cannula (diameter 2.2 mm) were introduced percutaneously into the uterus under local anesthesia (Fig. 1). The trocar was withdrawn and, in the 2 cases of diagnosis, 20 ml of amniotic fluid was aspirated for chromosomal analysis and α-fetoprotein determination. A 1.7 mm diameter "Needlescope" (Dyonics, Inc.) was inserted through the cannula. A suitable site for skin biopsy was chosen (thigh, buttock or back). The cannula was gently placed against the fetal skin at the selected site. The fetoscope was withdrawn, a biopsy forceps (2×2 mm jaws) was passed down the cannula and a biopsy specimen was obtained "blind".

Electron microscopy

The samples were placed immediately in fixation solution freshly prepared according to Peracchia & Mittler (12).

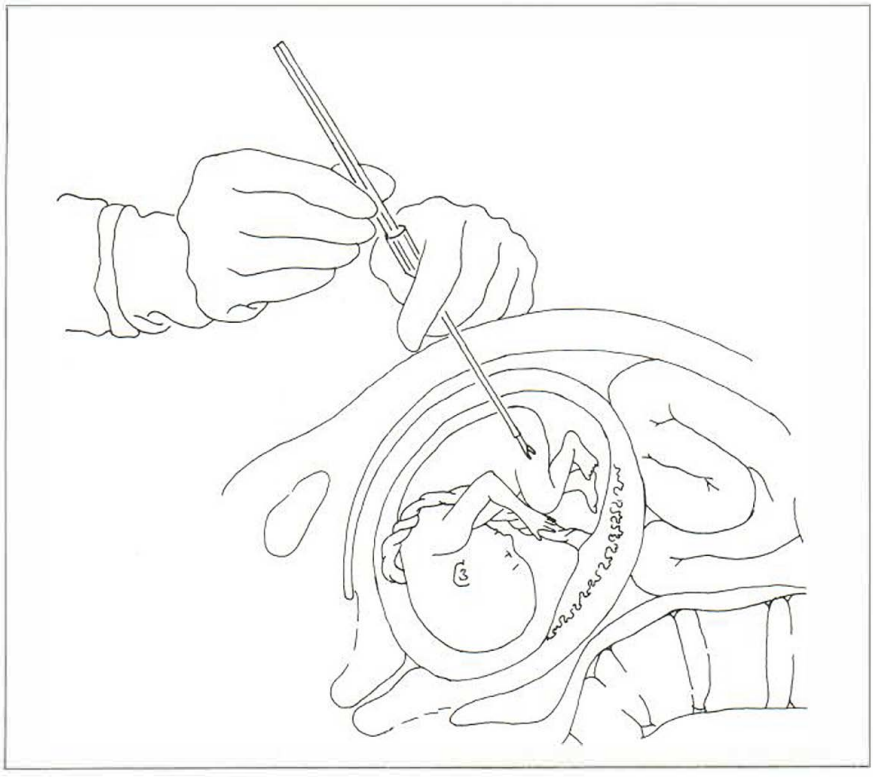

Fig. 1. Fetal skin biopsy sampling by the "blind" technique.

This consisted of 3% glutaraldehyde in 0.1 mol cacodylate buffer solution, pH 7.4, partially oxidized by adding 6 drops of 30% hydrogen peroxide to 25 ml buffered glutaraldehyde solution and stirring for 10 min before use. Fixation was performed at room temperature for 2 h in this solution and continued after changing to 3% buffered glutaraldehyde without hydrogen peroxide at room temperature. The biopsies were sent to Heidelberg and there processed as described elsewhere (1).

RESULTS

Controls

Of 17 biopsy specimens obtained from the control group, only 2 consisted of skin, one from each of the 2 fetuses in the 21st week of gestation (see Table I). Of the remaining 15 biopsy specimens, 9 consisted of fetal membranes; 3, of myometrium; and 3, of trophoblast. The electron microscopy examination in the control group was thus confined to only two skin specimens.

Hemidesmosomes, fully developed or still developing, were found along the dermo-epidermal junction. This region is of special interest since blisters form within that area in most types of epidermolysis bullosa. The ultrastructure of the dermo-epidermal junction was consistent with that in postnatal skin, although not fully developed in all details.

Case 1

Altogether 8 biopsy samples were obtained from the thighs and buttocks at fetoscopy in the 19th week of gestation. Two of the specimens turned out to be fetal membranes. Thus 6 biopsies of fetal skin were available for electron microscope examination.

Completely normal conditions comparable to those in control skin were found. There was no indication of junctional separation. At the dermo-epidermal junction, the basal lamina was thus continuous. The hemidesmosomes showed their normal ultrastructure, including well-developed subbasal dense plates. Complete hemidesmosomes were less common than after birth. This was not different from the control samples studied in parallel, indicating that the dermo-epidermal junction

Table 1. *Fetal skin sampling*

Case no.[a]	Gestational age (weeks)	No. of biopsies	Tissue obtained			
			Skin	Fetal membranes[b]	Myometrium[c]	Trophoblast[c]
Controls						
1	17	3	0	2	1	0
2	21	5	1	2	0	2
3	16	3	0	2	1	0
4	21	5	0	3	1	1
5	21	1	1	0	0	0
Diagnostic cases						
6	19	8	6	2	0	0
7	19	8	2	3	2	1

[a] Cases numbered in order in which they were studied.
[b] This specimen was either amnion alone or both amnion and chorion.
[c] In most cases, this specimen included fetal membranes.

at the 19th to 21st week of gestation is still undergoing development. The constant demonstrability of well-developed sub-basal dense plates in all hemidesmosomes cut perpendicularly was taken as evidence of the normal, non-Herlitz condition of the fetus. The time needed for processing the biopsies and for diagnostic electron microscopy was 7 days (9–16.7.1980).

After an uneventful pregnancy, a healthy male infant was born in due course (41st week of gestation). After birth, he was carefully examined; no scars or sequelae were visible at the sites of the fetal biopsies. For confirmatory studies, two biopsies were taken on day 2, one knife biopsy and one shave biopsy. Normal ultrastructural skin development for a newborn was found in both biopsies. The child is now 1 year 7 months old and is developing normally.

Case 2

Altogether 8 biopsy specimens were obtained from the thighs at fetoscopy in the 19th week of gestation. Examination under the light microscope showed that only 2 of the samples were of skin; the remaining 6 were fetal membranes, myometrium and trophoblast (see table).

No split or cleft formation was found between the epidermis and the connective tissue, and the dermo-epidermal junction showed normal ultrastructural features (Fig. 2) similar to those in case 1. The hemidesmosomes were present in the same frequency as in the control specimens, and they regularly presented a well-developed sub-basal dense plate. Thus, the Herlitz syndrome could be ruled out regarding this fetus too. The time needed for prenatal diagnosis was 7 days (5–12.5. 1981).

There were no early complications following the fetoscopy, but from the 26th week of gestation the woman was hospitalized because of intermittent leakage of amniotic fluid. Labour started in the 33rd week, and a healthy boy weighing 2.02 kg was born by cesarean section. No scars were seen at the sites of fetal biopsy. The infant is now 11 months old and is developing normally.

DISCUSSION

It is much easier to demonstrate a disease when specific changes such as blisters are present, than it is to exclude it with certainty. Although blister formation can occur in the Herlitz syndrome even during fetal life, most Herlitz babies are born with intact skin and develop blisters first some hours or even days after brith. Blisters appear in clusters in most epidermolyses, can occur in some regions only, or be fortuitously absent at the time and site of biopsy. Blisters can also be produced by the sampling of the fetal skin. Therefore, reliable criteria for abnormality or normality are a necessary basis for the safe exclusion of the disease in a high-risk pregnancy.

The Herlitz syndrome offers such criteria, the most important of which is the hypoplasia of hemidesmosomes which has been proved constantly demonstrable in homozygous carriers of this reces-

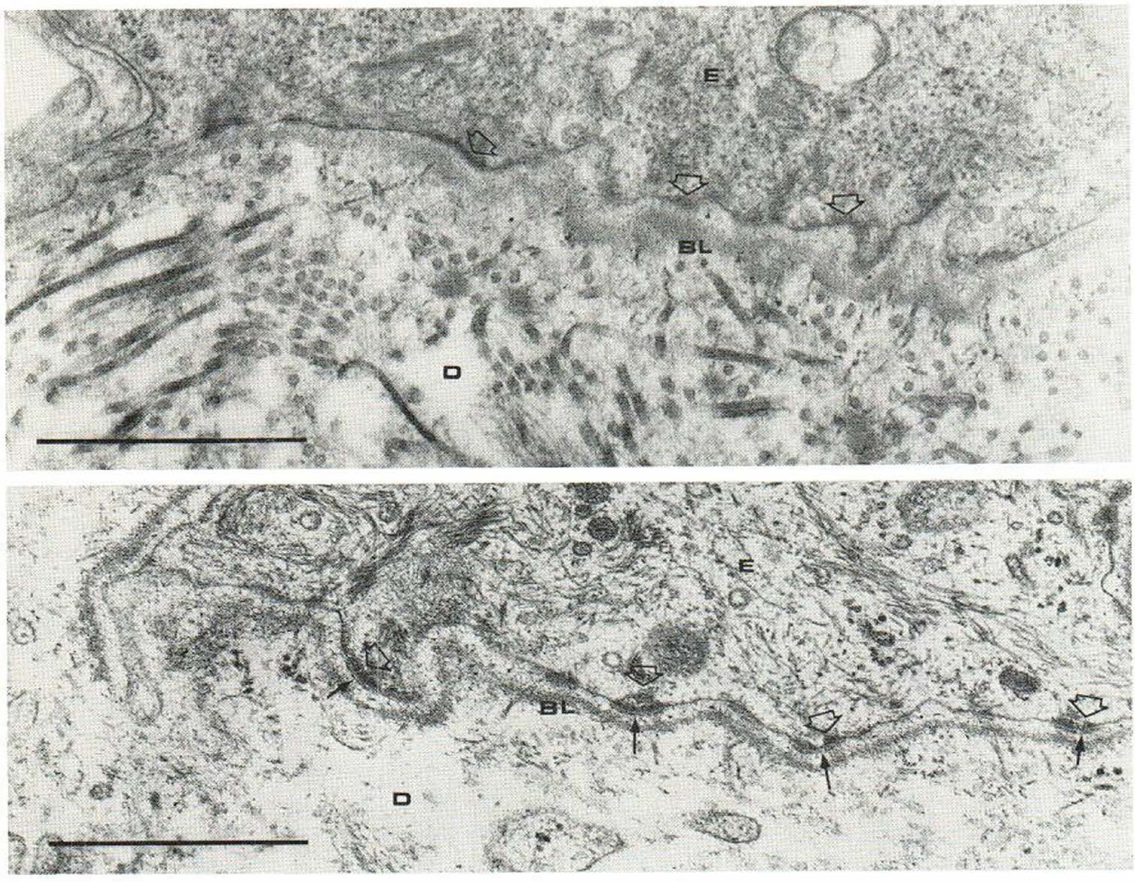

Fig. 2. Skin biopsy from a Herlitz baby (top) showing hypoplasia of hemidesmosomes (open arrows) and lack of sub-basal dense plates in dermo-epidermal junction without any signs of blister formation as compared with normal junction area (bottom) with well-developed hemidesmosomes and sub-basal dense plates (small arrows) in a skin biopsy obtained at fetoscopy in case 2. E = epidermis. BL = basal lamina. D = dermal connective tissue.

sive gene (3). This hypoplasia includes a numerical reduction of hemidesmosomes and complete absence of the sub-basal dense plates below the basal cell plasma membrane in the space of the lamina rara. Such hypoplastic hemidesmosomes represent points of minor resistance and explain the site of separation within the junction area, i.e., in the space of the lamina rara.

Hemidesmosomes appear at the dermo-epidermal junction at about the 12th week of gestation (9, 10). From that time of fetal development, the Herlitz syndrome—as well as the other genetic types of junctional epidermolyses with hypoplasia of hemidesmosomes (6)—should be demonstrable on the basis of their hypoplasia of the hemidesmosomes. However, since hemidesmosomes increase in both quantity and size during the subsequent weeks of fetal development (1, 9), prenatal diagnosis becomes more reliable with progressing pregnancy. Therefore, it would appear most advisable to do prenatal evaluation of a fetus at risk for Herlitz syndrome or related skin disorders between the 19th and 21st week of gestation. Even then sufficient time remains for legal termination in case of an affected fetus since, in our experience, the time needed for the preparation procedure and ultrastructural analysis is about 7 days after receipt of the biopsies.

The "blind" biopsy procedure is the conventional method for obtaining fetal skin specimens in utero (1, 2, 4, 5, 7, 8, 11). This method involves a risk of erroneously collecting fetal membranes (amnion,

chorion) or placental and uterine wall fragments instead of skin (see table). This can occur if the tip of the cannula inadvertently slips off the fetus and onto the fetal membranes lining the uterine wall or lining the placenta, from which the biopsy specimen is then removed. This happened especially in case 2. The damage to the amniotic sac caused by such unsuccessful biopsy attempts was probably responsible for the intermittent leakage of amniotic fluid that occurred in this woman from the 26th week of gestation and for her premature delivery in the 33rd week. Using the "blind" technique in a case for prenatal diagnosis, Elias et al. (5) obtained only one single biopsy specimen and even that turned out to be a piece of the amniotic membrane; 4 weeks later, they repeated the fetoscopy with success. Thus, there is an urgent need for a set of instruments that makes it possible to perform the biopsy under direct vision.

REFERENCES

1. Anton-Lamprecht, I.: Prenatal diagnosis of genetic disorders of the skin by means of electron microscopy. Hum Genet *59:* 392, 1981.
2. Anton-Lamprecht, I., Rauskolb, R., Jovanovic, V., Kern, B., Arnold, M. L. & Schenck, W.: Prenatal diagnosis of epidermolysis bullosa dystrophica Hallopeau-Siemens with electron microscopy of fetal skin. Lancet *ii:* 1077, 1981.
3. Anton-Lamprecht, I. & Schnyder, U. W.: Zur Ultrastruktur der Epidermolysen mit junktionaler Blasenbildung. Dermatologica *159:* 377, 1979.
4. Benzie, R. J. & Doran, T. A.: The "fetoscope": A new clinical tool for prenatal genetic diagnosis. Am J Obstet Gynecol *121:* 460, 1975.
5. Elias, S., Mazur, M., Sabbagha, R., Esterly, N. B. & Simpson, J. L.: Prenatal diagnosis of harlequin ichthyosis. Clin Genet *17:* 275, 1980.
6. Gedde-Dahl, T. Jr & Anton-Lamprecht, I.: Epidermolysis bullosa. *In* The Principles and Practice in Medical Genetics (ed. A. E. H. Emery & D. L. Rimoin). Churchill Livingstone, Edinburgh, 1982 (in press).
7. Golbus, M. S., Sagebiel, R. W., Filly, R. A., Gindhart, T. D. & Hall, J. G.: Prenatal diagnosis of congenital bullous ichthyosiform erythroderma (epidermolytic hyperkeratosis) by fetal skin biopsy. N Engl J Med *302:* 93, 1980.
8. Hobbins, J. C. & Mahoney, M. J.: Fetoscopy and fetal blood sampling: The present state of the method. Clin Obstet Gynecol *19:* 341, 1976.
9. Holbrook, K. A.: Human epidermal embryogenesis. Int J Dermatol *18:* 329, 1979.
10. Holbrook, K. A. & Odland, G. F.: Regional development of the human epidermis in the first trimester embryo and the second trimester fetus. J Invest Dermatol *80:* 161, 1980.
11. Löfberg, L., Anton-Lamprecht, I., Cordesius, E. & Gustavii, B.: Prenatal exclusion of Herlitz syndrome by fetal skin biopsy (in Swedish). Proceedings of the Swedish Medical Society, Hygiea *89:* 350, 1980.
12. Peracchia, C. & Mittler, B. S.: Fixation by means of glutaraldehyde-hydrogen peroxide reaction products. J Cell Biol *53:* 234, 1972.
13. Rodeck, C. H., Eady, R. A. J. & Gosden, C. M.: Prenatal diagnosis of epidermolysis bullosa letalis. Lancet *i:* 949, 1980.

Received July 26, 1982

L. Löfberg, M.D.
Department of Obstetrics and Gynecology
University Hospital
S-221 85 Lund
Sweden