

# ASSESSMENT OF THE ANGIOGENESIS AND LAMININ EXPRESSION IN BLIGHTED OVA

Erdener ÖZER, M.D.

Dokuz Eylül University, Faculty of Medicine, Department of Pathology, İzmir, Turkey  
Gazi Medical Journal 1998; 9 : 14-18

## SUMMARY :

**Purpose :** We aimed to compare the vascularization and laminin (an extracellular matrix protein) expression in blighted ova materials in first trimester legal abortions. **Methods :** This retrospective histopathological study included archival materials of 32 women with first trimester miscarriages and legal abortions obtained from the files of Dokuz Eylül University Hospital, Department of Pathology, İzmir. Using immunohistochemistry and stereology, two vessel parameters including vascular surface density per unit tissue volume (VSD) and the number of vessels per mm<sup>2</sup> (NVES) were estimated. Immunohistochemical deposition of laminin antibodies was also evaluated by a semi-quantitative scoring. **Results :** Most legal abortions exhibited strongly positive staining for laminin (%69). However, fewer blighted ova were strongly positive (31%). VSD and NVES showed a statistically significant decrease in blighted ova (Mann-Whitney U test,  $p < 0.05$ ). **Conclusion :** Decidual vascularization is an important component in the maintenance of the first trimester gestation, and the presence of laminin plays a role in enhancing physiological trophoblast adhesion to decidua. Thus, both parameters might explain the pathogenesis of first trimester spontaneous abortions.

**Key Words:** Blighted Ovum, Angiogenesis, Laminin, Trophoblast Adhesion.

## INTRODUCTION

Abortion is defined as the spontaneous or operative termination of pregnancy before fetal viability. Spontaneous abortions are common, and their exact incidence is difficult to assess, because most are clinically unapparent. The classification of spontaneous abortion is based on simple macroscopic and microscopic findings, given in table 1 (1).

Three most important components in the development and maintenance of the gestation are trophoblast function, fetal circulation, and

maternal circulation. The evidence of data suggests that the single most important factor is the integrity of the trophoblast. Trophoblast function dictates implantation, fetal angiogenesis and physiological adaptation of the uterine vasculature (1). One of the key mechanisms underlying various steps in trophoblast implantation is the attachment to the basement membrane, most likely by binding to laminin. The expression of cell adhesion molecules and secretion of metalloproteinase by trophoblasts also play key roles (2-4).

**Group 1: Blighted ova**

- a. most villi show hydropic change
- b. intermediate between (a) and (c)
- c. most villi show stromal fibrosis and vascular obliteration

**Group 2: Fetal death with maceration**

- a. embryo present
- b. embryo absent

**Group 3: Non-macerated fetuses**

- a. embryo present
- b. embryo absent

Table 1 : Pathologic classification of spontaneous abortion.

The goal of this study is to demonstrate the factors in the mechanism of spontaneous abortion. We, therefore, assessed here the deposition of laminin protein in the decidua, the uterine vasculature, and the histological appearance of the villi consistent with the extent of vascularization.

### MATERIAL AND METHODS

Paraffin blocks of the archival materials of 32 late first trimester abortions with maternal ages ranging between 17 and 44 years were studied. Of these, 13 were with blighted ova and 19 were with legal abortions (control group). No history of chromosomal abnormality or recurrent miscarriages were present. All tissues were originally fixed in formalin. The blocks were sectioned at polylysine-coated slides. The vascular endothelium in the decidua was labelled immunohistochemically by antibodies against the factor-VIII-associated antigen. The antibodies to laminin were also used. A single representative area of the section consisting of well-vascularized decidua was selected and marked for the analysis. Histological appearance of the chorionic villi was also evaluated in haematoxylin-eosin stained sections retrieved from the files.

#### *Immunohistochemistry*

The avidin-biotin-peroxidase method was performed using the primary monoclonal antibodies against the laminin (1:30, DAKO Corp, Denmark) and the factor-VIII-associated antigen (1:50, DAKO Corp, Denmark). Briefly, the sections were deparaffinized, endogenous peroxidase activity was blocked using 0.3% solution of hydrogen peroxidase, and primary

antibodies were applied for 30 minutes at room temperature. After a phosphate-buffered saline (PBS) wash, linking antibody and streptavidin-biotin-peroxidase complex (DAKO LSAB kit, Denmark) was added for 10 minutes at room temperature and washed in PBS. The peroxidase activity was visualised with diaminobenzidine (Sigma Chemical Co, USA), applied for 5 minutes. Positive controls were also substituted for the primary antibody.

#### *Stereologic Measurements*

The microscopic image obtained at x 10 objective was projected by a CCD camera (Sony, Japan) to a monitor (Sony, Japan) attaching to a microscope (Nikon, Japan). The representative area of the image was superimposed with a transparent grid containing 11 horizontal and 11 vertical test lines with known total test line length ( $Lr=1.1$  mm). The vascular surface density (VSD), equivalent to the vascular surface per unit tissue volume and the number of vessels per  $mm^2$  stroma were computed according to (5):

$$VSD = \frac{\sum In \cdot 2 \cdot 121}{Istr \cdot Lr} \quad NVES = \frac{N \cdot 121}{Istr}$$

where Istr = the number of the grid points superimposed on decidual stroma, In = the counts of intersections between test lines and vessel walls, and N = the number of vessels within the measuring field. The coefficient error of the method was kept equal to or less than 5%. Statistical analysis was done by Mann-Whitney U test using a computer software (SPSS). The probability level of 0.05 or less was chosen to represent statistical significance.

#### *Evaluation of Immunohistochemical Staining*

The degree of positive staining for laminin antibody was evaluated by scoring on a scale of 1 to 4 for intensity (I) and distribution (D). Tissues with I x D less than or equal to 4 were considered weakly positive, and those with I x D greater than 4 were designated strongly positive. Reproducibility of evaluation was tested by comparison of data obtained respectively without knowing the first assessment, and the reproducibility was found to be 0.87.

### RESULTS

Table 2 shows the immunohistochemical staining profile of the laminin in the 32 abortion

materials. Approximately one third of cases with blighted ova stained strongly positive for laminin, while 62% of the cases were weakly positive. However, most of the cases with legal abortion (69%) exhibited strongly positive staining (Fig. 1).

Assessment of the VSD and NVES yielded statistically significant results (Table 3). In the cases with blighted ova, the average number of microvessels was found to be  $9.3 \pm 1.4 \text{ mm}^{-2}$  (Fig. 2). The microvessel number increased significantly in the control group ( $p=0.0002$ ). The assessment of VSD also resulted in a statistically significant

	Blighted Ovum (n=13)	Legal Abortion (n=19)
Negative	7% (1)	5% (1)
Weakly positive	62% (8)	26% (5)
Strongly positive	31% (4)	69% (13)

Table 2 : Immunohistochemical staining profiles of the cases for laminin antibody.

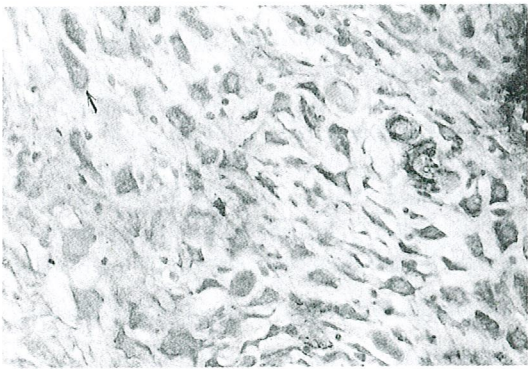


Fig - 1 : Representative sections showing extensive strongly positive peridecidual staining. Immunoperoxidase staining for laminin antigen (black arrow); original magnification x100.

	Blighted Ovum (n=13)	Legal Abortion (n=19)	p**
VSD ( $\text{mm}^{-1}$ )	$4.4 \pm 1.0$	$6.2 \pm 1.6$	$p=0.0019$
NVES ( $\text{mm}^{-2}$ )	$9.3 \pm 1.4$	$13.3 \pm 3.2$	$p=0.0002$

\* Data are presented by arithmetic mean + 1 standard deviation.

\*\* The right column indicates p value obtained from analysis of statistically significant difference computed by Mann-Whitney U test.

Table 3 : Statistical analysis of vessel parameters\* in the study.

increase ( $p=0.0019$ ).

The results of histopathological examination of the chorionic villi are shown in table 4. Most cases of blighted ova (85%) showed hydropic villi (Fig. 3). However, most of the control group was found to be histologically within the limits of normality.

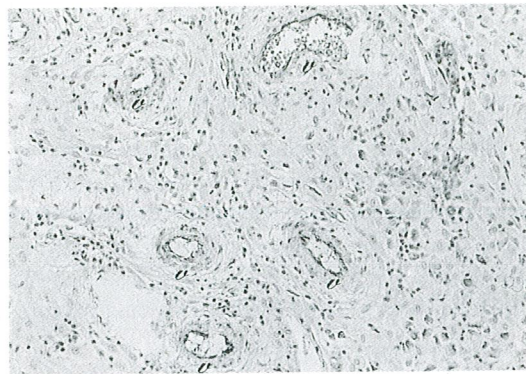


Fig - 2 : Microphotograph of a blighted ovum. VSD = 3.58 and NVES = 10.52. Immunoperoxidase staining for factor-VIII-associated antigen expressed by endothelial cells lining arterioles (v); original magnification x400.

	Blighted Ovum (n=13)	Legal Abortion (n=19)
Hydropic villi	85 % (11)	90 % (17)
Fibrotic villi	15 % (2)	10 % (2)

Table 4 : Histopathological findings of the chorionic villi in the study.

## DISCUSSION

The conventional explanation for spontaneous abortion is abnormality of the conceptus, but this fails to explain a mechanism for expulsion of the gestation. The establishment and maintenance of

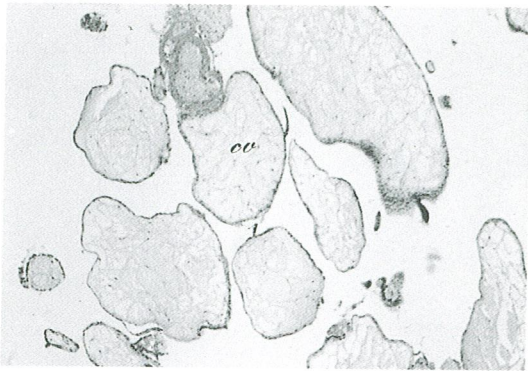


Fig - 3 : Histological section of chorionic villi (cv) showing extensive hydropic change. Haematoxylin-eosin staining; original magnification x400.

pregnancy demand a complex set of hormonal and anatomical interrelations between fetal and maternal tissues. Expulsion of a conceptus represents a final common pathway, which may be initiated by a failure of any of these factors (1).

Development of human embryo depends on the ability of first trimester cytotrophoblastic stem cells to differentiate and invade the uterus. In normal human pregnancy, invasion of the uterus and the uterine arteries by cytotrophoblasts extends through the entire decidua and the adjacent third of the myometrium, and is accompanied by a marked change in the expression of cell adhesion molecules by trophoblasts, the most relevant being the family of integrins (6, 7). Key mechanisms underlying various steps in trophoblast invasion are expressing integrins that bind to the basement membrane, most likely to laminin and the breakdown of basement membrane components, mediated by secretion of metalloproteinase (4, 8). Decidual stromal cells, like trophoblast, express integrins that bind certain glycoproteins that support outgrowth and attachment of trophoblast (9). With abnormalities of the conceptus, such as in spontaneous abortion, any of these mechanisms may be defective. At the time of implantation, the extracellular matrix proteins such as laminin, are abundant in the decidua and are distributed pericellularly around each individual cell (2). In this study, a significant decrease in laminin expression was found consistent with its important role in the maintenance of pregnancy.

A reduced trophoblastic penetration into the stroma and spiral arteries seem to be related to untimely initiation blood flow in the intervillous space which in turn is associated with arrest of pregnancy and eventual expulsion (10). Maternal circulation is one of the components in the development and maintenance of gestation. Major changes in vascularization would be expected during the latter part of the first trimester. Our findings showed that there was a statistically significant decrease of decidual vascularization in the cases with blighted ova. This suggests that the changes are a result of inadequacy of the conceptus, failing to induce the appropriate changes in the decidua.

The relative amount of villous oedema or stromal fibrosis on histological examination depends on the extent to which the fetal circulation had become established prior to fetal death (1). In blighted ova, chorionic villous vascularization seems to be deficient (11). The histological appearance of the villi that fail to develop are two types: villi in which trophoblasts become attenuated and the stroma is hydropic, and villi in which the trophoblasts initially appear normal and the stroma is fibrotic. The first type of the villus is typical of the blighted ova in which there is no evidence of any embryonic development. Most of the chorionic villi in this study appeared to be in the former group compatible with the deficiency in chorionic villi vascularization.

Blighted ova are one of the basic categories of spontaneous abortion. Although our understanding of the pathophysiology of abortion is poor, this study concluded that in blighted ova, the decidual vascularization was significantly poorer and the decrease in laminin expression might cause deficient trophoblastic adhesion. However, it should not be disregarded that laminin expression and uterine vasculature are only two factors in complex mechanisms of spontaneous abortion.

Correspondence to : Dr.Erdener ÖZER  
Dokuz Eylül Üniversitesi Tıp Fakültesi  
Patoloji Anabilim Dalı  
İnciraltı  
35340 İZMİR - TÜRKİYE  
Phone : 232 - 330 69 77  
Fax : 232 - 259 05 41

## REFERENCES

1. Knowles S : Spontaneous abortion and the pathology of early pregnancy. In. Keeling JW (ed): Fetal and Neonatal Pathology. 2nd ed. London: Springer Verlag. 1993: 87-110.
2. Burrows TD, King A, Smith SK, Loke YW : Human trophoblast adhesion to matrix proteins: inhibition and signal transduction. Hum Reprod 1995; 10 : 2489-2500.
3. Burrows TD, King A, Loke YW : Expression of integrins by human trophoblasts and differential adhesion to laminin or fibronectin. Hum Reprod 1993; 8 : 475-484.
4. Fisher SJ, Damsky CH : Human cytotrophoblast invasion. Semin Cell Biol 1993; 4 : 183-188.
5. Weibel ER : Principles and methods for the morphometric study of the lung and the other organs. Lab Invest 1963; 12 : 131-155.
6. Zhou Y, Damsky CH, Chiu K, Roberts JM, Fisher SJ : Preeclampsia is associated with abnormal expression of adhesion molecules by invasive trophoblasts. J Clin Invest 1993; 91 : 950-960.
7. Damsky CH, Fitzgerald ML, Fisher SJ : Distribution patterns of extracellular matrix proteins and adhesion receptors are intricately modulated during first trimester cytotrophoblast differentiation along the invasive pathway, in vitro. J Clin Invest 1992; 89 : 210-222.
8. Graham CH, Lala PK: Mechanisms of placental invasion of the uterus and their control. Biochem Cell Biol 1992; 70:867-874.
9. Ruck P, Marzusch A, Kaiserling E, Horny HP, Dietl J, Geiselhart A, Handgretinger R, Redman JW : Distribution of cell adhesion molecules in decidua of early human pregnancy. An immunohistochemical study. Lab Invest 1994; 71 : 94-101.
10. Hustin J, Janiaux E, Schaaps JP : Histological study of the materno-embryonic interface in spontaneous abortion. Placenta 1990; 11 : 477-486.
11. Meegdes BH, Ingenhous R, Peters LL, Exalto N : Early pregnancy wastage: relationship between chorionic vascularization. Fertil Steril 1988; 49:216-220.