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# MITOCHONDRIAL DYSFUNCTION AND APOPTOSIS IN TROPHOBLAST CELLS DURING PREECLAMPSIA: AN ULTRASTRUCTURAL STUDY

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## SUMMARY

**Objective:** The aim of this study was to examine the ultrastructure of the mitochondria in trophoblast cells of pregnant women with preeclampsia and to relate it with apoptotic events.

**Material and methods:** Placentas at term were obtained immediately after cesarean delivery. Three biopsies per placenta were taken from the maternal basal surface in the delivery room. Specimens from peripheral ramifications of the villous tree (especially immature intermediate and terminal villi) were processed through conventional procedures of transmission electron microscopy and opportunely compared with controls obtained from normal placentas.

**Results:** Ultrastructural degenerative changes in mitochondrial inner membrane and matrix of trophoblast cells (lysis of cristae and matrix vacuolization) were visualized. Swollen mitochondria in diverse degenerative grades were found. Two types of degenerated mitochondria in accordance with the mitochondrial matrix were detected in apoptotic trophoblast cells.

**Conclusion:** Mitochondrial ultrastructural changes appear to be the origin of trophoblast cell death by apoptosis. Subsequently, trophoblast debris would fall from placental villi into the intervillous space and could become not only corpuscles damaging the endothelium of the fetal-placental maternal unit but one of the stimuli for endothelial dysfunction and the maintenance of the pathogenesis of preeclampsia.

**KEY WORDS:** Hypoxia. Mitochondrial dysfunction. Trophoblast cell apoptosis. Pre eclampsia

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## RESUMEN:

**Objetivo:** Examinar la ultraestructura de la mitocondria en células trofoblásticas de embarazadas con pre-eclampsia y relacionarla con sucesos apoptóticos

**Material y métodos:** Fueron obtenidas placentas a término inmediatamente después de partos por cesárea. En la sala de partos, fueron tomadas tres biopsias de la superficie basal maternal en cada placenta. Los especímenes, correspondientes a ramificaciones periféricas del árbol veloso (en especial, velosidades inmaduras intermedias y terminales), fueron procesados mediante técnica convencional para microscopía electrónica de transmisión y comparados oportunamente con controles obtenidos de placentas normales a término.

**Resultados:** Se visualizaron cambios ultraestructurales degenerativos en la membrana interna mitocondrial (lisis de las crestas) así como en la matriz mitocondrial (vacuolización matricial). Fueron halladas tanto hinchazón mitocondrial de diversos grados cuanto dos tipos de degeneración mitocondrial, según el estado matricial, en las células trofoblásticas apoptóticas.

**Conclusión:** Los cambios ultraestructurales mitocondriales parecieran originar muerte celular trofoblástica por apoptosis. Con posterioridad, los fragmentos trofoblásticos caerían desde las velosidades placentarias hacia espacio interveloso y devendrían no sólo corpúsculos capaces de dañar el endotelio de la unidad feto-placentaria sino uno de los estímulos para la disfunción endotelial y el mantenimiento de la patogénesis de la pre-eclampsia.

**PALABRAS CLAVE:** Hipoxia. Disfunción mitocondrial. Apoptosis células trofoblásticas. Pre-eclampsia

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## INTRODUCTION

Preeclampsia has been correlated with mitochondrial dysfunction in obstetric and gynecological studies and characteristic features of this disorder may be explained by this inborn error in metabolism in which there is a failure in the aerobic energy production<sup>1-2</sup>.

The genesis of preeclampsia is clearly related to deficient trophoblast invasion and failure of uterine artery remodeling<sup>3-4</sup>. Defective spiral artery remodeling in preeclampsia likely results in reduced uteroplacental perfusion and foci of placental hypoxia or ischemia<sup>5</sup>. Bad placental perfusion could cause ultrastructural changes of preeclamptic placental tissue since chorionic hypoxia is a predisposing factor for preeclampsia<sup>6</sup>.

The ultrastructure of the trophoblast in pregnant women with preeclampsia, recently examined with special reference to the effects of hypoxia, revealed degenerative ultrastructural changes. Swollen mitochondria in diverse degenerative grade were seen with particles electron dense in their matrix<sup>7-8</sup>.

The mitochondrial damage may be a possible initial cause of trophoblastic apoptosis since hypoxia is apt to produce placental degenerative changes at mitochondrial level. Consequently, a mitochondrial injury could lead not only to the trophoblast cell death but to the detachment of its fragments from placental villous surface after the apoptotic changes associated to hypoxic injury.

Taking into account the aforesaid considerations, this paper reports mitochondrial ultrastructural alterations in apoptotic trophoblast cells of placentas obtained from preeclamptic patients and relates it with apoptotic events.

## MATERIAL AND METHODS:

Ten pregnant patients undergoing hypoxia during preeclampsia were strictly studied following basic ethical principles of the Declaration of Helsinki as well as the rules of the Ethical Committee of our institution.

Ten placentas at term were obtained immediately after cesarean delivery. Three biopsies per placenta were taken from the maternal basal surface in the delivery room. Two to five mm specimens from peripheral ramifications of the villous tree (especially immature intermediate and terminal villi) were instantly fixed in 4% glutaraldehyde - 0.1 M phosphate buffer, pH 7.4, at 4°C. After its division in one mm each, they were immersed in a fresh similar solution from 2 to 72 hours followed by a secondary fixation in 1% osmium tetroxide - 0.1 M phosphate buffer for 1 hour. Subsequently, samples were processed through the conventional procedures for transmission electron microscopy<sup>7</sup>. Electronic micrographs were selected and compared with those of normal placentas at term (controls).

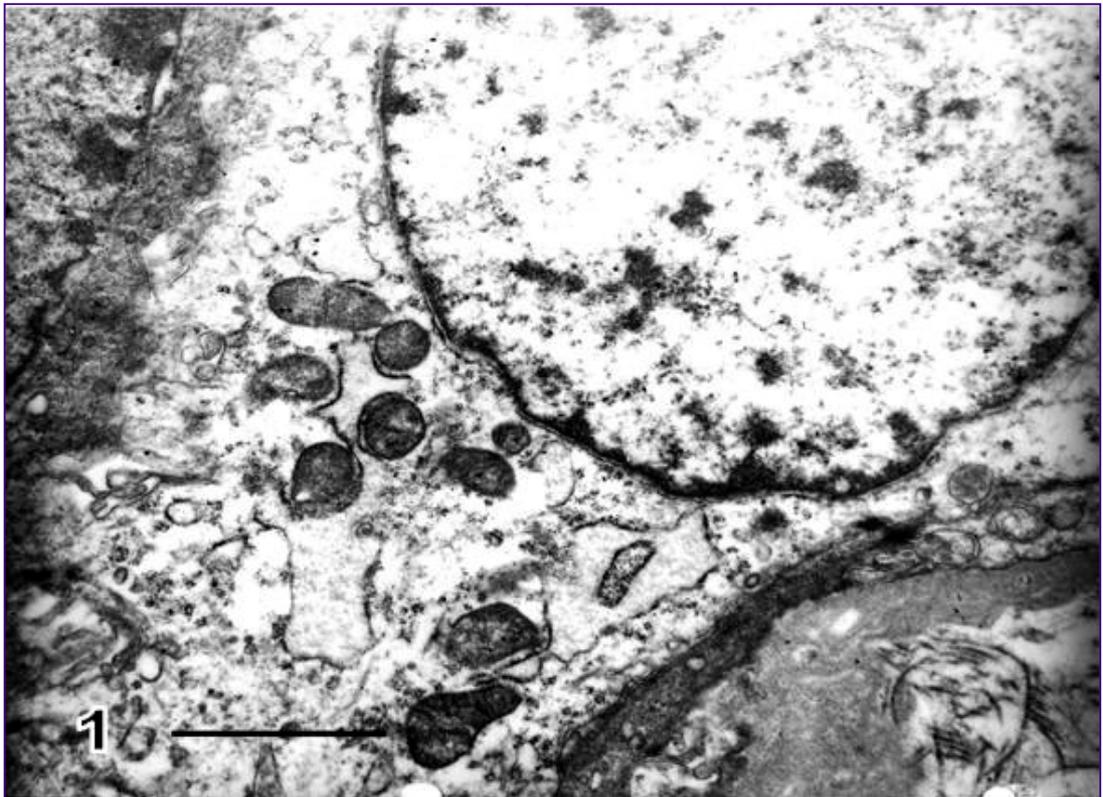
Criteria related with tissue hypoxia were complementary taken into account<sup>6</sup>.

## RESULTS

Presence of villous trophoblast with degenerative changes surrounding the peripheral area of placental villi was put into evidence. Increased syncytial knots, syncytial sprouts, infarction of villi, apoptotic trophoblast cells, intervillous thrombi and intervillous deposit of fibrinoids were also observed when semi-thin sections of biopsies were analyzed.

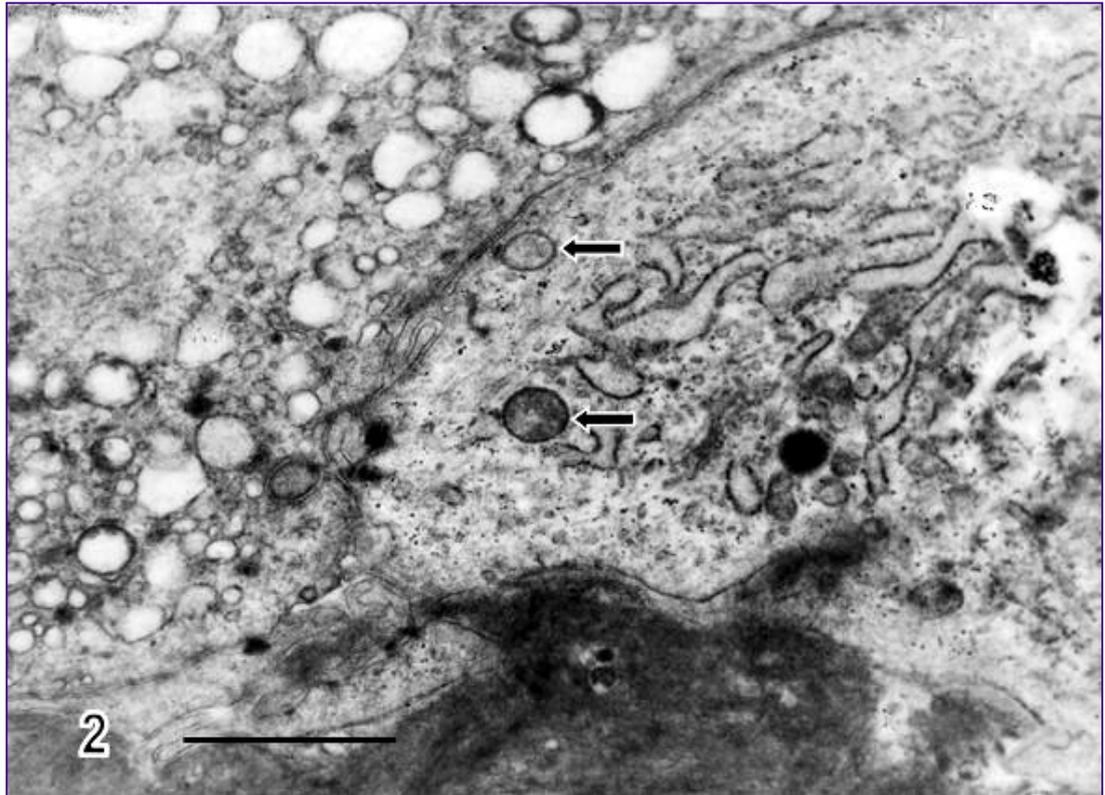
Regions of cytotrophoblast revealed dilated cisterns of rough endoplasmic reticulum. This organelle presented degranulation of ribosomes in its membranes. This was not seen in controls. A light hyaloplasmic and nucleoplasmic matrix was observed. In some cases scarce heterochromatin was noted associated to inner nuclear membrane. Interrupted cytoplasmic microfilaments were dispersed in the hyaloplasmic matrix of cytotrophoblast cell. Swollen electron-dense mitochondria were visualized with interruption of membranes, cristolysis and obscure mitochondrial matrix. In control specimens, the integrity of the mitochondrial ultrastructure was preserved.

Regions of syncytiotrophoblast in process of degenerative change were found. These regions were more abundant in preeclamptic specimens. Some cytoplasmic prolongations of cytotrophoblast cells were more obscure and located between the cytotrophoblast cell and basal membrane. This appeared thickened and penetrated by collagen fibers. Different thickness was noted in their trajectory (Fig 1).

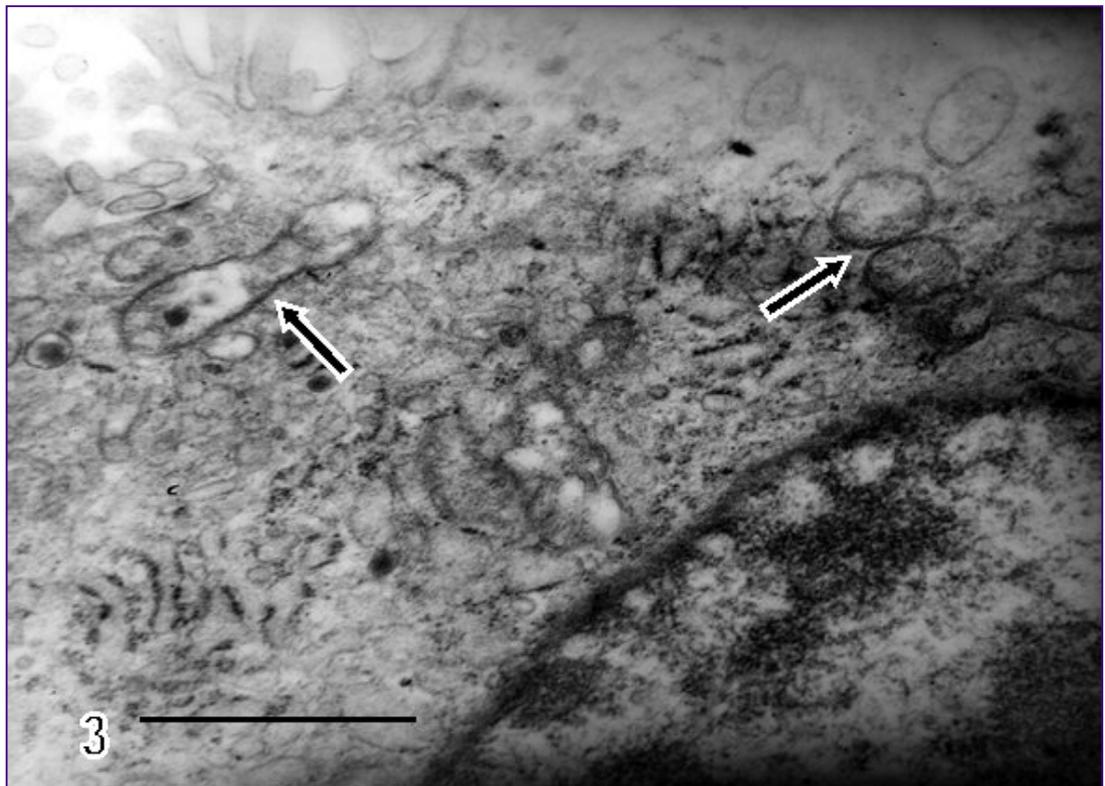


**Fig.1.-** Region of cytotrophoblast cells. Mitochondria show notorious electron density changes in mitochondrial matrix probably due to mitochondrial matrix protein aggregation responsible for its osmiophilic property. Collagen fibers are seen incrustated in trophoblast basement membrane in the lower right-hand side of the micrograph. Bar: 1  $\mu$ m

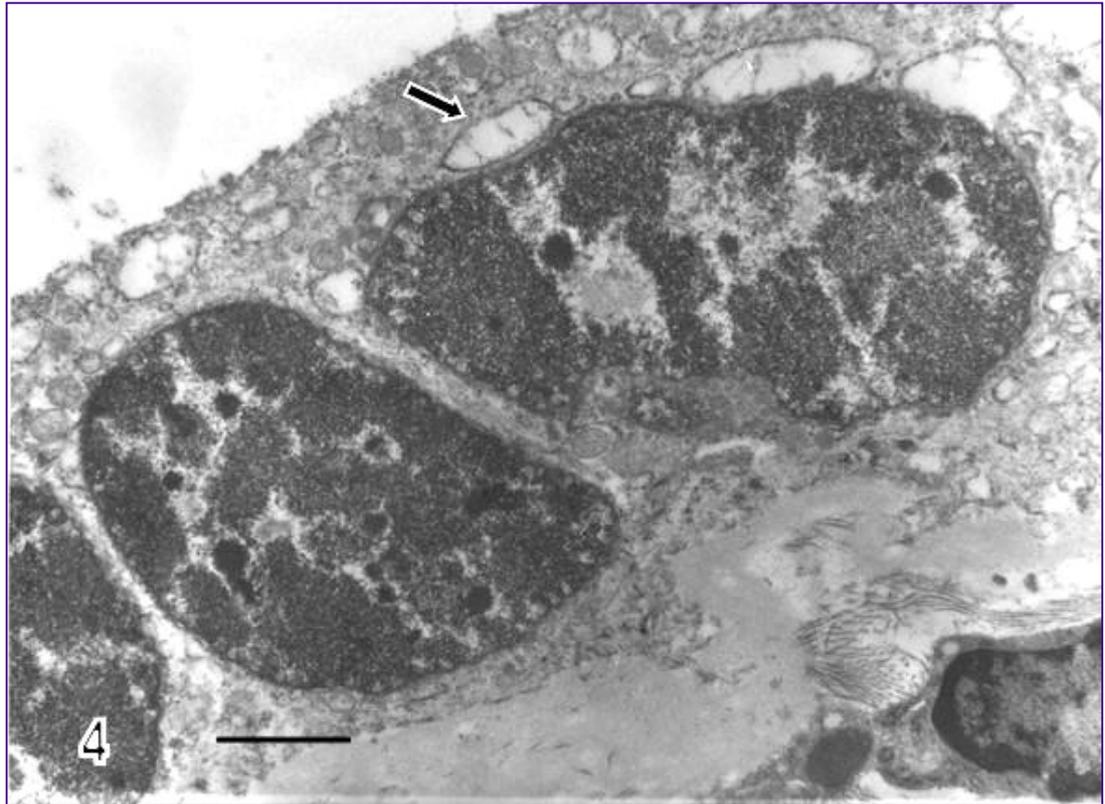
There are zones of cytotrophoblast cells that contain two types of mitochondria, ones are of clear mitochondrial matrix and others of electron dense mitochondrial matrix observed in degenerating trophoblast cells (Fig 2). The syncytiotrophoblast also showed swollen mitochondria with strongly osmiophilic intramitochondrial granules. These mitochondria had interrupted cristae and exhibited vacuolar zone in their light matrix (Fig 3) whilst in controls images of mitochondria preserved the normal ultrastructure. The syncytiotrophoblast presented an interrupted plasma membrane, a vacuolar cytoplasm and apoptotic nucleus (Fig 4). These features were not observed in most of control specimens.



**Fig.2.- Thickened and disorganized basal membrane is observed in regions of trophoblast cells. The cytotrophoblast contains two types of mitochondria (arrows) which differ in electron density from the mitochondrial matrix. A region of vacuolated syncytiotrophoblast is visualized in the upper left-hand side of the micrograph. Bar: 1  $\mu$ m**

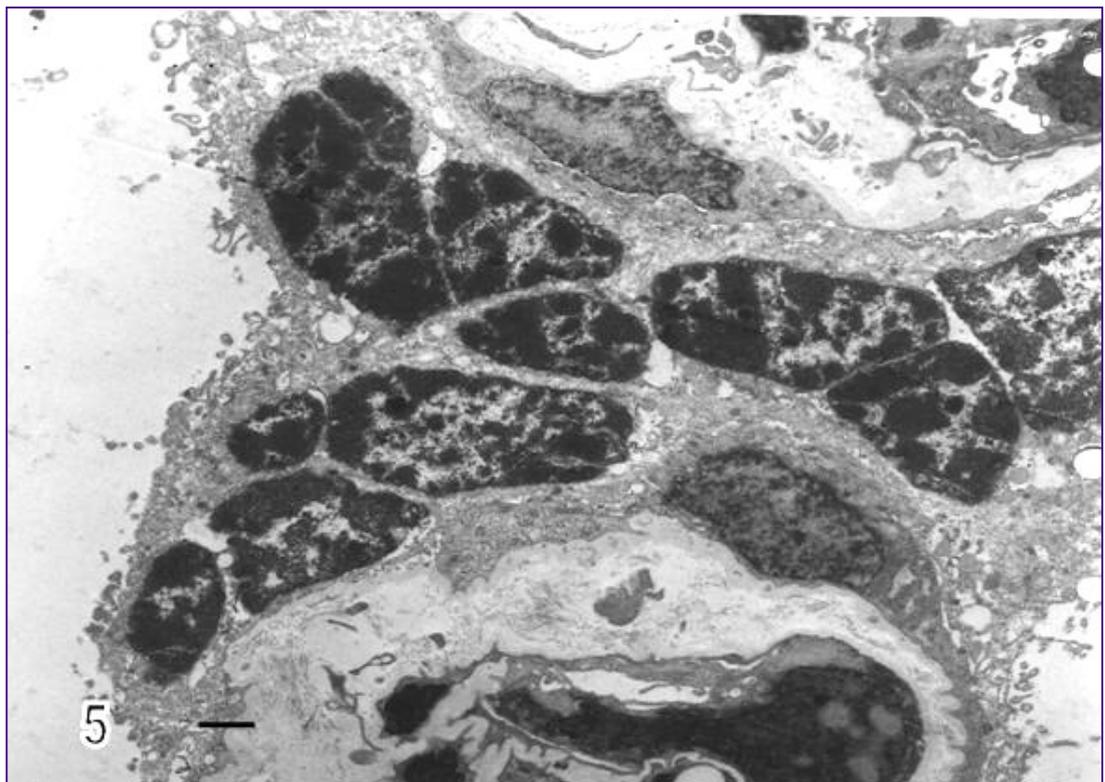


**Fig.3.- Clearly swollen mitochondria (arrows) in degenerative process are observed in this micrograph of the syncytiotrophoblastic zone. Bar: 1  $\mu$ m**



**Fig.4.- Syncytiotrophoblastic region with apoptotic nucleus and cytoplasmic region showing ghost mitochondria (arrow).  
Bar: 1  $\mu$ m**

Syncytial knots were observed with plasma membrane showing microvilli in blebs, filamentous or battledore form (Fig 5). These were increased in preeclamptic specimens when compared with controls. Zones detached of trophoblast cell were seen in placental villi which presented subtrophoblastic edema (Fig 6). Large areas of degenerating trophoblast appeared to be delivered to the intervillous space (Fig7). Fragments of syncytiotrophoblast were expelled to the maternal circulation by a process of subtrophoblastic edematous change (Fig 8).



**Fig.5.- A syncytial knot contain a notably modified plasma membrane and organelles with degenerative changes  
Bar: 1  $\mu$ m**

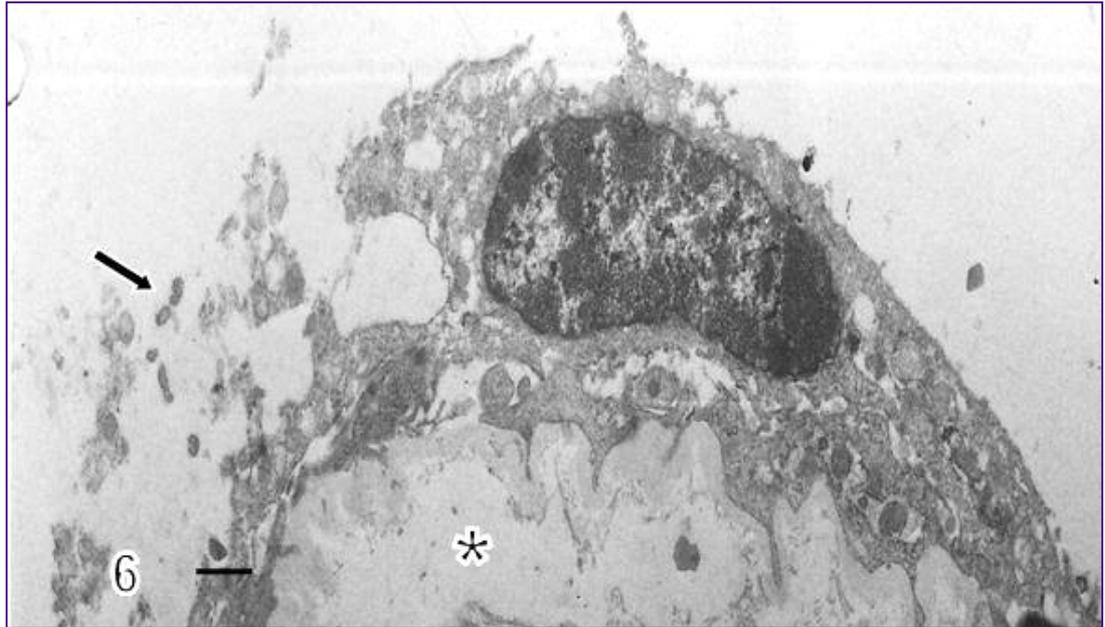


Fig. 6.- A syncytiotrophoblastic zone has been detached from the syncytiotrophoblast (arrow). Edema below the basement membrane is observed (\*).  
Bar: 1  $\mu$ m

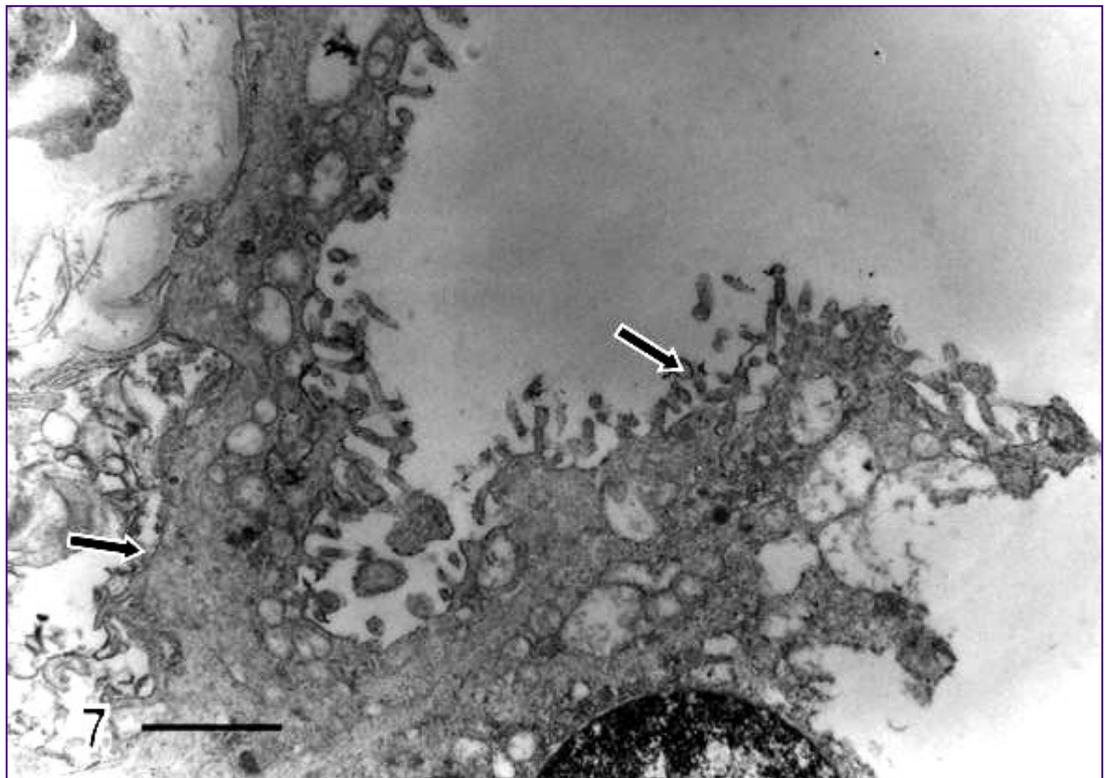


Fig. 7.- Areas of degenerative syncytiotrophoblast (arrows) are near to be detached from the surface of the placental villi.  
Bar: 1  $\mu$ m

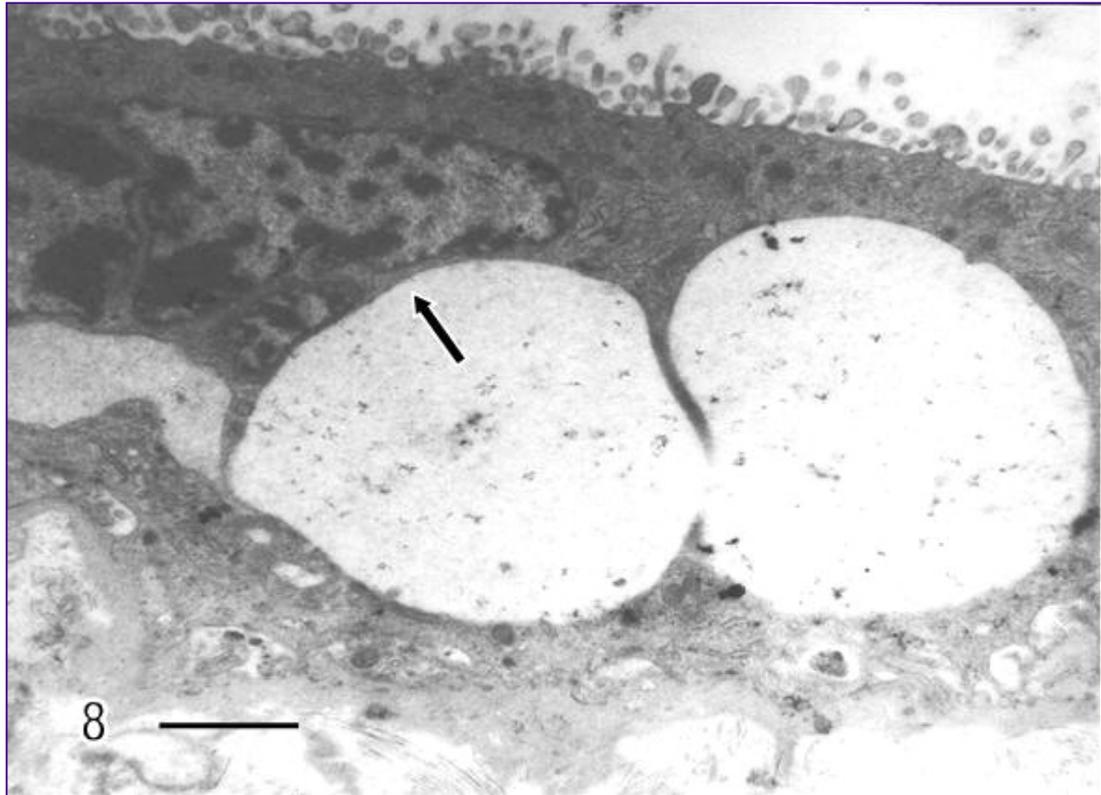


Fig. 8.- Regions of syncytiotrophoblast (arrow) detached from cytotrophoblast cells and large spaces with edema are visualized.  
Bar: 1  $\mu$ m

#### DISCUSSION AND CONCLUSION

Several electron microscopic studies have suggested that lesions of placental tissue in preeclampsia could be discussed on the basis of changes in endothelial cells or reduced perfusion by maternal blood<sup>9-13</sup>.

No characteristic findings were found associated to preeclampsia. Mitochondrial changes have been seen in vessels, myometrial smooth muscle, myometrial interstitial cells, circulating leukocytes, epidermal and dermal cells and in hepatocytes of preeclamptic pregnant women<sup>14</sup>. The mitochondria of these preeclamptic tissues showed a central disruption. Similar structural studies of mitochondria in cerebral edema and brain ischemia or anoxia have also been reported<sup>15</sup>. The injured mitochondrial patterns are related with nerve cell death and postulated as markers of lethal nerve cell injury. Mitochondrial injury relates with hypoxic-ischemic processes and precedes trophoblastic apoptosis<sup>6,9</sup>.

Reduced utero - placental flood flow has been recognized in cases of severe preeclampsia with hypertension<sup>6</sup>. In these cases, histological changes as decreased number of syncytial microvilli, proliferation of cytotrophoblastic cells, focal syncytial necrosis, thickening of trophoblastic basement membrane and narrowing of fetal capillaries have been demonstrated<sup>8</sup>.

In this study, mitochondrial degenerative changes occur in trophoblast cells undergoing apoptosis. Chronic hypoxia or alternate periods of hypoxia/re-oxygenation within intervillous space is expected to trigger tissue oxidative stress and increase placental apoptosis<sup>16</sup>. Mitochondria contain the enzymatic systems which generates ATP through the Krebs cycle and oxidative phosphorylation. The formation of ATP requires oxygen which diminishes in preeclampsia. In this condition, it is suggested that trophoblast cells undergo apoptotic events and fragments or debris are delivered into the intervillous space<sup>7</sup>.

Previous reports pointed out that an increased release of syncytiotrophoblasts microparticles (STBM) of 0.2 to 2 $\mu$ m in size formed by plasma membrane blebbing during apoptosis are triggered in excess into the maternal circulation<sup>17,18</sup>. The present findings are congruent with those reported by Bachmaier et al<sup>19</sup>. They also found swollen and degenerating mitochondria in hypoxia condition at 34 mmHg of oxygen pressure according to their model of hypoxic dual in vitro placental perfusion.

Thus, mitochondrial death induces trophoblastic apoptosis in degenerating placental villi. The role of the mitochondria in apoptotic cell death is well known. Hypoxia acting as an external stimulus changes the mitochondrial function. Mitochondria discharge cytochrome c, this protein activates the system of caspases and the trophoblast die by apoptosis. The increased apoptosis in preeclampsia leads to greater number of syncytiotrophoblastic fragments being shed into the maternal blood<sup>20</sup>. These detached fragments, debris of trophoblast, microvilli from syncytium or syncytial microparticles could be considered as causing endothelial damage, among other factors. Syncytiotrophoblastic detritus detached from the trophoblastic surface and resulting from a continuous process of regeneration, reparation or apoptosis are increased during preeclampsia. Being a consequence of a poor blood supply subsequent to a spiral artery failure occurring in placental implantation, it constitutes the stimulus for a permanent inflammatory response characteristic in this common and dangerous pregnancy complication<sup>21</sup>.

During preeclampsia, the machinery maintaining the intracellular respiration and the biosynthesis of ATP in the trophoblast is damaged. The trophoblast is then transformed in a tissue with tendency to be fragmented in smaller particles passing initially to the intervillous space and subsequently to the endothelium of the fetal-placental maternal unit. These fragments of tissue contacting maternal endothelium could stimulate the process of disseminated intravascular coagulation, typical of preeclampsia. Lapaire et al. (2007) reported that Schmorl in 1893 was one of the first researchers in recognizing the placental origin of preeclampsia<sup>22</sup>. These results obtained from transmission electron microscopy support it 118 years after.

In conclusion, these findings could be pointing out that in preeclampsia the trophoblast undergo apoptosis caused by mitochondrial damage, transforming it in a tissue with tendency to be fragmented. Such detritus detached from their surface could become the stimulus for endothelial dysfunction during the maintenance of the analyzed pregnancy complication.

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Comment of the reviewer Alberto Enrique D'Ottavio PhD. Professor and Researcher, Faculty of Medical Sciences, Rosario National University, Rosario. Argentina.

Olivar Castejón focuses his study in the ultrastructural examination of the mitochondria in trophoblast cells of pregnant women with preeclampsia and in its relationship with apoptotic events, complementing previous ones published during 2008. Taking into account what the author have already reported and reports here on this matter as well as the satisfactory corrections made to the original, the paper may be considered acceptable for publication.

Comment of the reviewer Larisa Ivón Carrera PhD. Professor and Researcher, Faculty of Medical Sciences, Litoral National University, Santa Fe. Argentina.

This paper centers the analysis in mitochondria of preeclamptic trophoblast cells of pregnant women and relates it with apoptosis, describing damages linked to hypoxemia. Primarily descriptive and secondarily hypothetic it results acceptable for publication. Furthermore, the relevance of the topic faced here renders an additional reason for this approval.

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