Microscopic study of edema in hydatidiform mole

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Estudo microscópico de edema em mola hidatiforme

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Abstract

Objectives: the purpose of this study is to use light microscopy and scanning electron microscopy to determine the effect of edema on the structure of the molar vesicle.

Methods: samples were taken from the complete hydatidiform mole and processed using conventional light and scanning electron microscopy techniques and an observation protocol that identified four variables: factors underlying the development of edema; the condition of the trophoblast basement membrane, development of the villi, accumulation and degeneration of sulphated mucosubstances at stromal level.

Results: light microscopy showed a permeable trophoblastic basement membrane, a swollen syncytium, edematous regions disorganizating the stromal region and causing ischemic necrosis of cells. Using scanning electron microscopy, the basement membrane was found to be distended and thickened, with large irregular holes for the entry and movement of liquid, leaving a wide range of fluids during the influx process and depriving stromal cells of nutrition.

Conclusions: a new three-dimensional view of the changes brought about by the entry of fluids into the stroma of molar hydropic vesicles was provided by scanning electron microscopy and confirmed by light microscopy, thereby explaining the changes occurring at the level of the stroma as an effect of the edema.

Key words *Hydatidiform mole, Edema, Microscopy, Scanning electron microscopy*

Resumo

Objetivos: este estudo visa utilizar a microscopia ótica e eletrônica de varredura para determinar o efeito de edema na estrutura da vesícula molar.

Métodos: amostras foram obtidas da mola hidatiforme completa e processadas por técnicas de microscopia ótica convencional e eletrônica de varredura e por um protocolo de observação para identificar quatro variáveis relacionados à evolução de edema; a condição da membrana trofoblástica basal, a evolução dos vilos, a acumulação e a degeneração de mucosubstâncias sulfatadas ao nível stromal.

Resultados: a microscopia ótica revelou uma membrana trofoblástica basal permeável, um sincítio inchado, regiões edematosas desorganizando a região stromal e provocando a necrose isquêmica das células. Com a microscopia eletrônica de varredura, foi observado que a membrana basal foi distendida e espessada, com grandes buracos irregulares para a entrada e movimento de líquidos, deixando uma larga variedade de fluidos durante o processo de influxo e privando as células stromais de nutrição.

Conclusões: a microscopia eletrônica de varredura providenciou uma nova visualização tridimensional das alterações provocadas pela entrada de fluidos na stroma das vesículas molares, que foi verificada pela microscopia ótica, assim explicando as alterações que ocorreram ao nível da stroma como um efeito da edema.

Palavras-chave *Mola hidatiforme, Edema, Microscopia, Microscopia eletrônica de varredura*

Introduction

A hydatidiform mole is characterized by a cystic swelling of the placental villi accompanied by varying trophoblastic proliferation.[1] This anomaly of the villi is classified within the so-called gestational trophoblastic diseases as an entity with nonspecific pathophysiological aspects and accurate assessment of its form is important to prevent and detect the disease.[2] Clinically, it covers benign alterations.[3]

Worldwide, the incidence of hydatidiform mole in trophoblastic disease is 85%, but the frequency varies, from 1 per 1500 pregnancies in the United States to 1 per 947 in Venezuela.[4]

In Venezuela, the frequency also varies depending on the region, being 1 per 423 pregnancies in San Cristobal[5] and 1 per 1066 in the Caracas University Hospital.[6] Palacios Concepción Maternity reports that one in every approximately 40 moles will lead to choriocarcinoma,[7] while this only occurs in one in 150,000 normal pregnancies.[8] A scanning electron microscopy study of the molar villi showed that, during normal development of the villus in pregnancy, the basement membrane tends to thicken and become impermeable, not allowing the passage of substances through the basement membrane found in normal villi. In the case of hydatidiform moles this leads to hypoxia or ischemia and the tendency is for the basement membrane to thicken further. This membrane also has two or three layers of cells which may contribute to the formation of more material in them. The edema could be explained by the fluid entering from the trophoblast in the direction of the central cistern to the development of molar villi and stopping the cistern expanding with an impermeable membrane.[9,10]

The clinical consequences of this syndrome include vaginal bleeding, early embryonic death, undue uterine enlargement, high hCG levels, hyperemesis and preeclampsia. This structure may invade the uterus and destroy portions of it as an invasive mole. The consecutive development of this structure may lead to fatal choriocarcinoma. This article will discuss the mechanisms that intervene in the inflow of fluids into the molar vesicle and describe the degenerative changes that occur in the molar stroma under the influence of edema.

Methods

The material was taken from molar pregnancies at 8 and 10 weeks respectively, in the form of two full moles, in two hospitals whose patients were notified of the investigation and gave informed consent after the nature of procedure had been fully explained. The protocol was carried out in accordance with our institutional ethical requirements and the principles outlined in the Helsinki Declaration. The research discussed here was institutionally reviewed and approved. Two hundred and fifty vesicles were obtained from both moles for processing using light microscopy and 20 vesicles for scanning microscopy. The analysis and scope of the results was based on a descriptive basic cross-sectional study, using non-probabilistic sampling, in which samples obtained were processed using conventional light microscopy (LM) and scanning electron microscopy (SEM) techniques.

Vesicles were placed in buffered 10% formaldehyde, then washed and dehydrated in different concentrations of ethanol, further cleaned using xylene and set in paraffin from which 3 to 5 micron tissue sections were obtained for every piece. These cuts were mounted on glass slides to be stained with hematoxylin and eosin. Fifteen molar tissue slices were obtained and these were viewed and photographed using the light microscope at an objective resolution of 40x.

The material obtained for SEM, was placed in a mixture of 4% formaldehyde and 1% glutaraldehyde in a phosphate buffer and then washed to remove excess phosphate crystals and 1% osmium added for postfixation. Dehydration was subsequently carried out using an ascending series of alcohols followed by three ethanol-amyl acetate mixtures and the samples were dried to the critical point using an HCP-2 Hitachi desiccator. The sample obtained was placed in the sample holder or aluminum Stub attached with colloidal silver paste to be taken to an Eiko IB3 ion coater to be covered with platinum (Pt) and palladium (Pd) and viewed under a Hitachi S2300 SEM.

After following the normal procedures for histological slides using light microscopy, the samples were immediately viewed using a scanning electron microscope, by placing ten histological slides in xylene for three days to dislodge object covers. The glass behind the histological sample was then cut with a diamond-tipped scalpel to produce a 7x7mm area which was double-taped to the SEM specimen holder using Scotch tape. The specimens were then coated with platinum-palladium and finally viewed using a Hitachi S2300 scanning electron microscope. Analysis of the edema of molar vesicles considered several factors, using an observation protocol that contributed to understanding of the origin and development of this edema as the trophoblast basement membrane, the state of development the villi and the

accumulation and degeneration of sulfated substances by stromal cells. Degenerative changes affecting the organization of the molar villous stroma were evaluated using these factors.

Results

Light Microscopy

There were areas where the surface of the molar vesicle was altered and a syncytium was found separating liquid infiltrate as a disorganized structure in several segments of cytoplasm. Basement membrane debris was observed to be fragmented or divided into layers. Blank spaces between degenerated trophoblast regions corresponded to edematous spaces. The sub-trophoblastic zone lacked cells and was notably blurred (Figure 1).

The basement membrane was no longer impermeable and was found to be swollen with no normal intercellular contacts of myofibroblasts. Large fluid-filled vesicles were found under the basement membrane and stromal regions, which were observed to be separated by the dispersion of liquid in that area. It was evident that the liquid streams disorganized stromal tissue cells and necrotic residues of myofibroblasts were observed in a clear matrix (Figure 2).

Figure 1

The syncytium with edematous spaces is separated from the layered and interrupted basement membrane. Cell necrosis debris can be seen. The sub-trophoblastic image is typical of tissue liquefaction produced by edema. H&E stain.

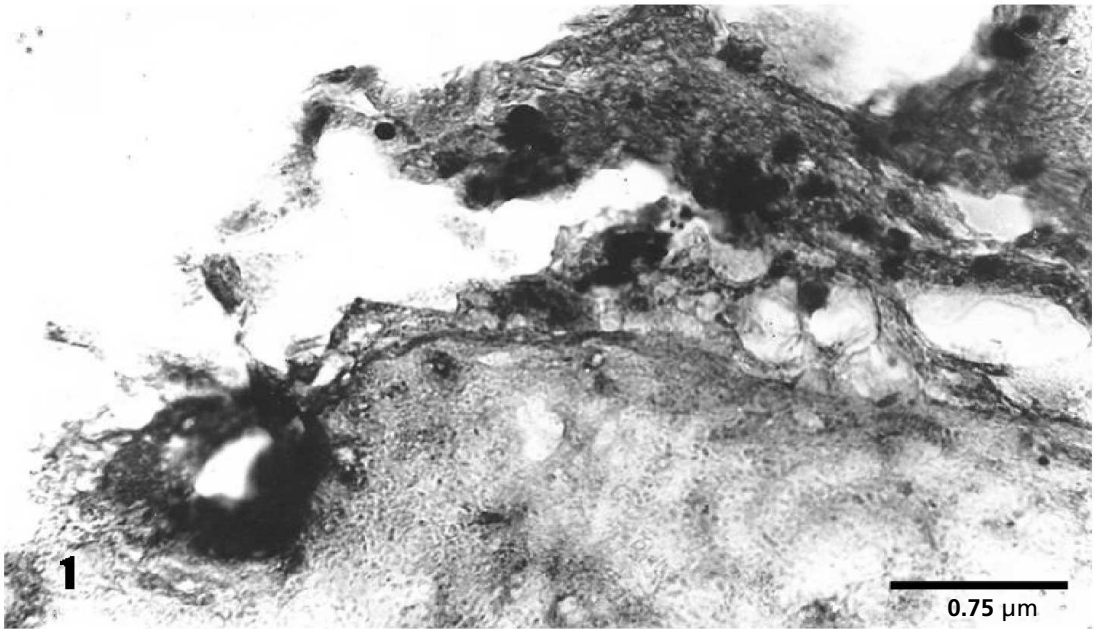
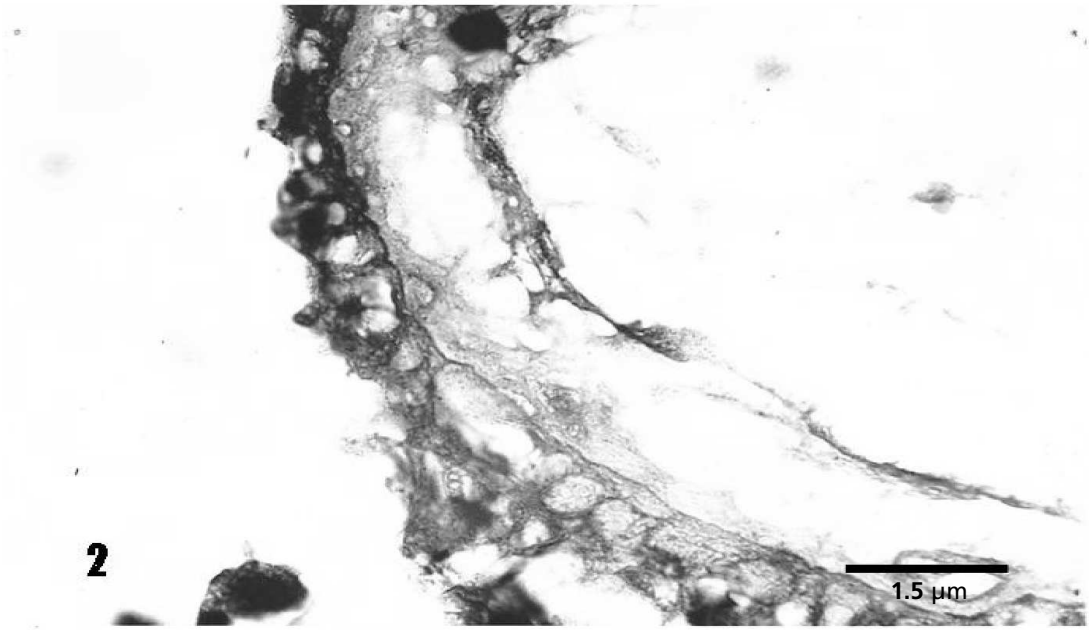


Figure 2

Remains of myofibroblasts associated with clumps of collagen fiber floating in a clear matrix devoid of stromal cells in a line parallel to the degenerating syncytium. H&E stain.



Internal flows of liquid flows were found to be putting pressure on the remains of collagen fibers and pushing them in the direction of the trophoblast (Figure 2). While some molar vesicles had a full stromal region, others lacked content and were recorded as empty. Severe edema destroyed the internal organization of the stroma and cells underwent necrosis.

Scanning Electron Microscopy

SEM enabled the observation of three-dimensional features in areas where liquid flows permitted orientation and location of the effects on the structure of the villi. Some areas of the trophoblast were interrupted leaving large channels through which liquid flows entered the stroma. These channels were of irregular trajectory (Figure 3).

Interrupted regions of the trophoblast showed the basement membrane to be distended and separated from it. Holes were found to have been made, contributing to the inflow of liquid (Figure 4).

The fact that the basement membrane was detached from the trophoblast, with a concave appearance inside, indicates that streams of fluid are driven from intervillous space into the stroma (Figure 5).

Large regions of the basement membrane of the syncytium were found to be separated from the syncytial cytoplasm and leave a wide area where interstitial fluid accumulated between basement membrane and the cytoplasmic basal surface of the syncytium. In vivo, this accumulation of liquid separated the stroma from the trophoblast preventing adequate nutrition of the cell elements in the stroma. These cells located in the stroma are known as myofibroblasts which come into contact with one another when they are not affected by edema.

Other molar villi affected by edema were found to be shaped by a hyperplastic trophoblast, tight stromal cells and fibers which could be flooded in vivo with liquid coming from the interstitial space containing maternal blood.

To penetrate the internal cistern fluid flows pushed a layer of stroma to the periphery as they entered the streams of liquid and vessels were found to have collapsed and tended to degenerate.

Figure 3

Fractured regions of the syncytium, through which fluid may be entering. Histological section viewed using scanning electron microscopy.

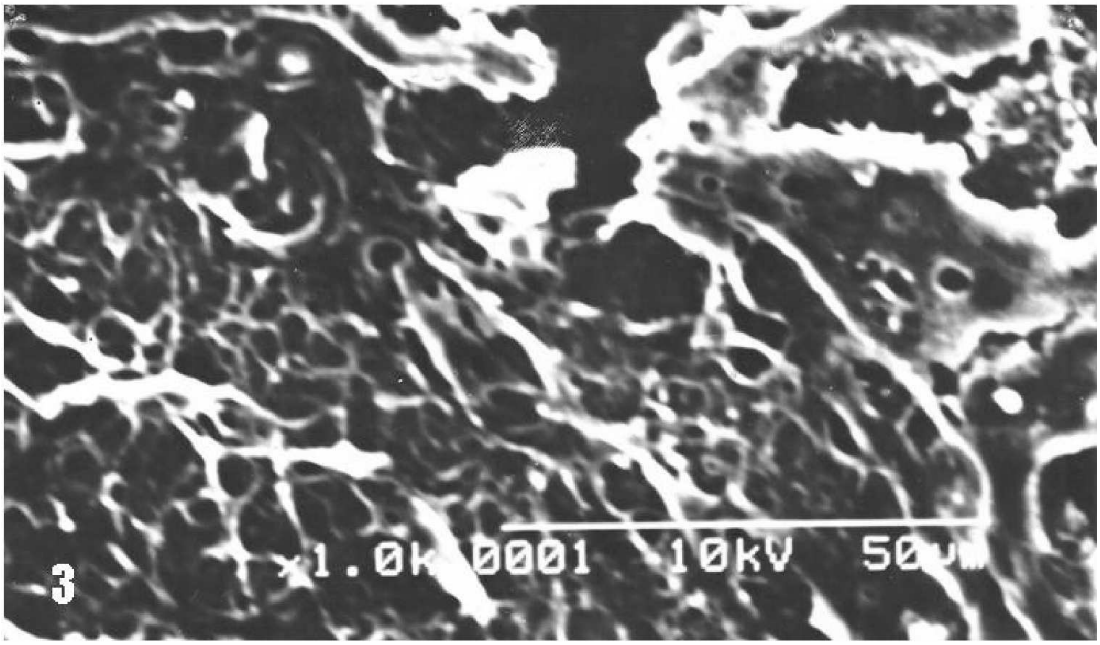


Figure 4

In the central and lower scanning electron microscopy, noticeable holes can be seen in the basement membrane of the trophoblast. Arrows indicate trophoblastic regions.

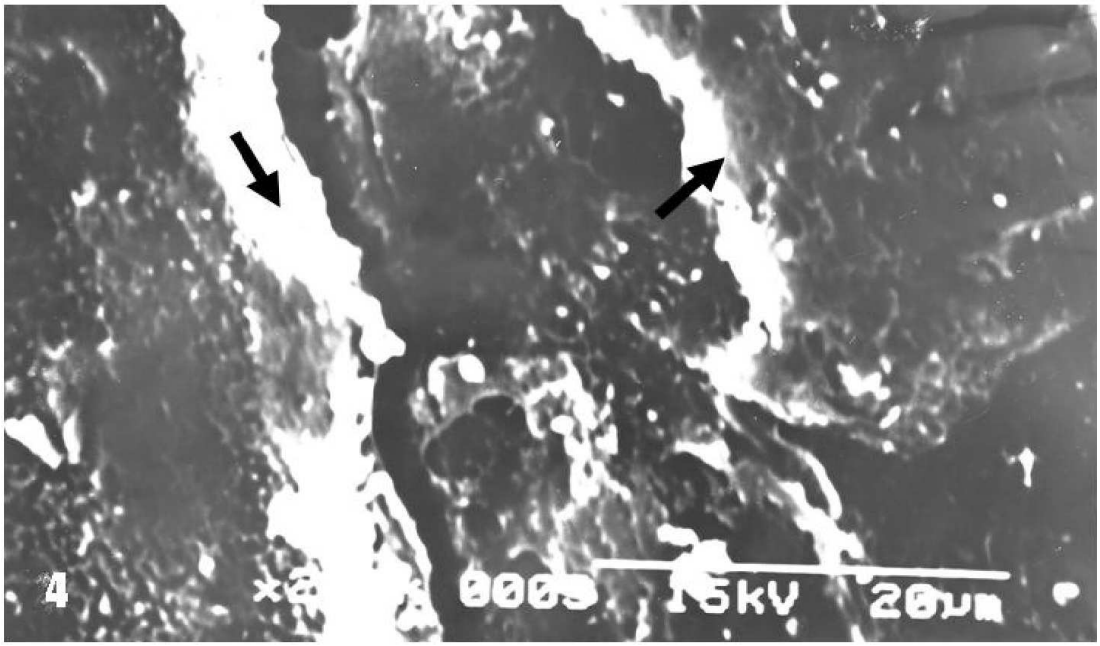
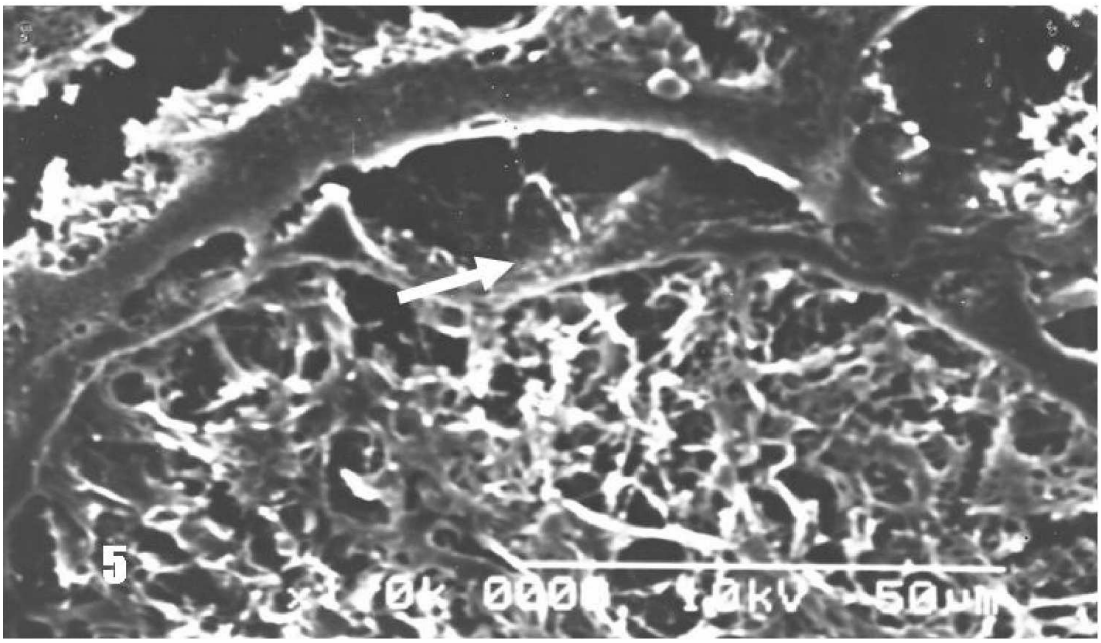


Figure 5

The laminar zone of the basement membrane (arrow) separated from the syncytial cytoplasm, with a concavity towards the center of the stroma. Histological section view using scanning electron microscopy.



Discussion

The pathophysiology of hydropic change following damage to the embryo or fetus, with or without chromosomal abnormality in the hydatidiform mole, is still not known. The liquid infiltrate observed in the syncytium results from the continued transport of liquid by the trophoblast, which may produce changes in basement membranes and produce a subtrophoblastic area of edema.[11] Streams of liquid streams helped to form the central cistern and severe edema has deprived the placental villi of a stromal region.[12]

In vivo, it is probable that, as the liquid flows in, the intervillous space is simultaneously reduced and produces some pressure, owing to the compression exerted on the vesicles in each villus, causing hydropic transformation and liquids are forced in via the trophoblast layer.[11] Cracks, tears or channels, resulting from the degeneration of the trophoblast plasma membrane in this hypoxic environment partly explain the regions through which the liquid flows.[10]

Large channels of irregular trajectory were found by SEM, suggesting how the liquids may have been absorbed by the trophoblast. Very small interruptions of the syncytial plasma membrane may be present but these could only be viewed using a transmission electron microscope, which was not available for this study. The influx of liquid was facilited by holes formed in the basement membrane. The trophoblast normally has 20 nm trans-trophoblastic channels connecting the intervillous space to the stromal region.[11] Aquoporins or molecular complexes located in the plasma membrane of the trophoblast during degeneration may have contributed to the influx of liquid from the intervillous space.[9] Such structures can only be viewed using a transmission electron microscope.

The changes observed here caused by the syncytium, which was extensively vacuolated by cytoplasmic degenerative changes and interrupted basement membrane, which was altered, sometimes layered, hydrated or swollen, suggest a massive transfer of liquid to the stromal region. which undergoes morphological changes. It is possible that such changes in the basement membrane are similar to those observed with normal placental villi but more pronounced.[12] Those occurring at the stromal level are similar to those the seen in a condition known as mesenchymal dysplasia of the placenta, as demonstrated by scanning electron microscopy.[13] The wide clear regions of stroma observed under light microscopy are stromal areas devoid of structure

which are now occupied by edema. The influx of liquid has displaced the structures of the stromal region, leaving the necrotic debris of myofibroblasts or primitive vessels.[10]

SEM images were able to demonstrate that the degenerated basement membrane enables the inflow of substantial streams of fluid, eventually detaching the surface base of the syncytium underlying the stromal region and giving rise to a layer of edema around the peripherical stroma. The multiple holes or perforations of the basement membrane observed confirm that degenerative changes enabled fluid to flow into the stromal region. This region remains structured or well-shaped but is soon disorganized by the pressure of the interstitial edema that moves stromal regions in layers to the periphery of the trophoblast. Alterations in the basement membrane in these cases of hydropic transformation have been demonstrated using histochemical techniques and the aforementioned material has been identified using SEM in mesenchymal hyperplasia,[13] as well as its relation to the malignancy of this condition.[14] These studies have demonstrated the loss of basement membrane impermeability.

The formation of a cistern observed in these cases may be well compared to what happens with placental mesenchymal dysplasia, which is similar to the molar formation because it produces hydatidiform vesicles, like the partial mole, with which it is often confused; the malformation maintaining the normal fetus, different from the partial mole which produces fetal congenital adrenal hyperplasia.[13]

The hyaluronan glycosaminoglycan type is a component of the extracellular matrix, in which the cells that comprise the stroma are inserted. The degeneration of these macro molecules form the region of the cistern,[10] shown here as an empty space. Fibroblasts have been considered to be involved in the production of metalloproteinases,[15] which may initiate digestion of the extracellular matrix, thereby causing the onset of the cistern. Moreover, probable sustained compression between vesicles, as described here, may trigger a mechanism that dissolves the stromal matrix, similar to that which occurs when Wharton's jelly dissolves in the umbilical cord, reducing the mechanical effect of compression. This hyaluronan would be responsible for the compressibility of the cistern, as occurs in cartilage and may be associated with polycations such as Na^+ and K^+.[16,17] This property attracts water trapped inside this macromolecule and, as this may be associated with proteoglycans that interact with stromal collagen fibers, the result is a complex that may retain large quantities of water.

It is not known whether the trophoblast produces similar stromal elements, such as myofibroblasts, to synthesize massive amounts of hyaluronan or whether the microscopic organization of the trophoblast cell membrane prevents the passage of fluid from the intervillous space. All these speculations require further study. The pathophysiology of hydropic transformation is only beginning to be understood.[10] According to SEM studies, internalized fluid appears to be associated with a blood plasma fibrin mesh. The pressure variations created in the intervillous space lead to negative pressure within the cistern and the influx of blood. Under conditions of hypoxia or ischemia, fluid enters the space between the trophoblast and the central cistern and accumulates, expanding the central cistern surrounded by a waterproof basement membrane.[10] Placental villi which have been filled with a large amount of fluid are called hydropic. As the enlarged central cistern fills with liquid, the stromal folds are arranged in the form of a peripheral band surrounding the underlying area of the trophoblast, as also occurs in villi that have no trophoblast hyperplasia, characteristic of some miscarriages.[18] This molar fluid contains procoagulant substances that are very likely capable of causing clotting when they spill into the intervillous blood space and this may cause local villous infarction that spreads to the decidual vessels, producing localized necrosis and hence uterine bleeding. It is important to be aware of this pathology, since a pregnancy under such conditions is not viable and there is a high risk of hemorrhage. Treatment involves evacuation of the uterus and prolonged follow-up to measuring hCG serum concentrations is essential. Besides chemotherapy, some drug combinations do increase the risk of late onset cancer.[12]

In conclusion, a new three-dimensional view of the changes brought about by the entry of fluids into the stroma of the molar vesicle has been revealed by SEM and confirmed by LM. This also explains the changes in the stromal region, indicating damage caused by the influx of liquid and subsequent edema.

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