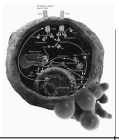
Medicine, 2015/16



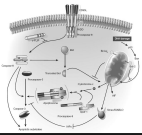
Trophoblast physiology

“Live et Let Die”

prof. Damir Roje

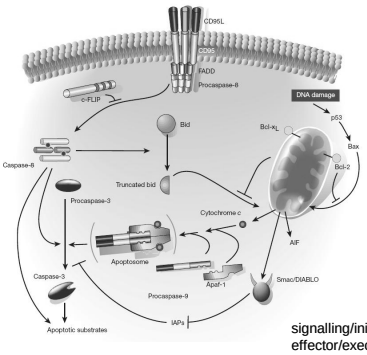
Apoptosis in a few words

- programmed cell death - energy utilization process
- organised and precisely designed ireversibile process mediated by cascade like activation of the caspase enzymes that leads cell (step by step) into death
- final product - apoptotic body - absence of inflammatory or immune local or systemic reactions !!!!



Cell dies by exactly planned protocol for the "higher interests".

INITIATION OF APOPTOSIS

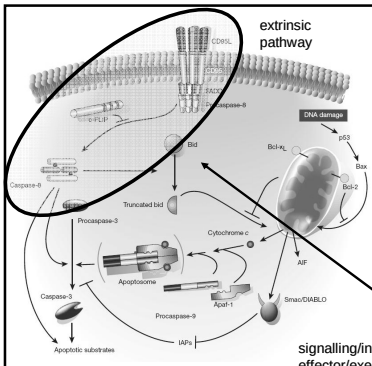


signalling/initiator caspases: 2,8,9,10
effector/execution caspases: 3,6,7

Hengartner MO. The biochemistry of apoptosis. Nature 2000.

INITIATION OF APOPTOSIS

extrinsic pathway



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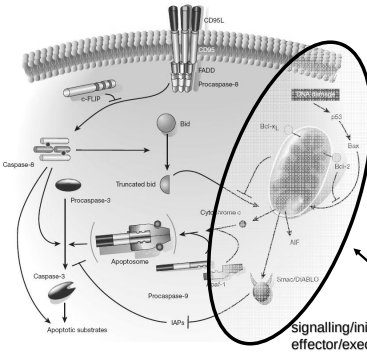
INITIATION OF APOPTOSIS

intrinsic pathway



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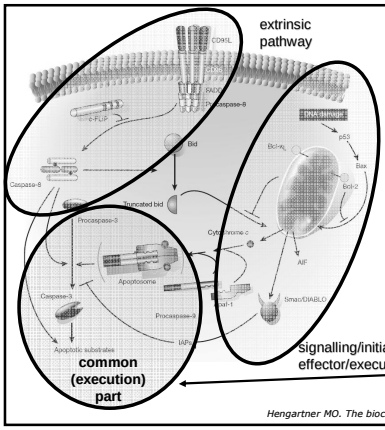
APOPTOSIS

extrinsic pathway

intrinsic pathway

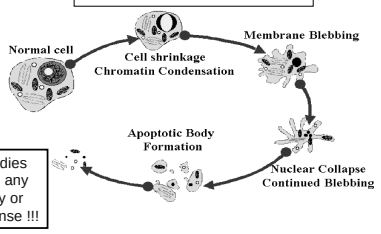


common (execution) part

signalling/initiator caspases: 2,8,9,10
effector/execution caspases: 3,6,7

Hengartner MO. The biochemistry of apoptosis. Nature 2000.

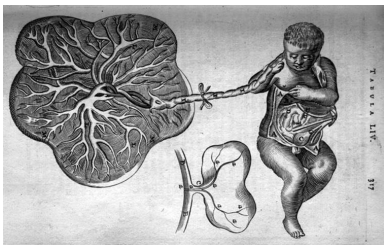
APOPTOSIS



Apoptotic bodies do not cause any inflammatory or immune response !!!

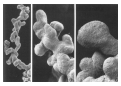
~ 24 hours

PLACENTA



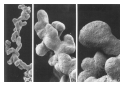
Thomas Bartholin (1616-1680): Anatome ex omnium veterum recentiorumque observationibus, 1673.

TROPHOBLAST / PLACENTA:

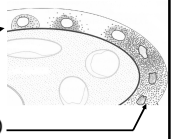


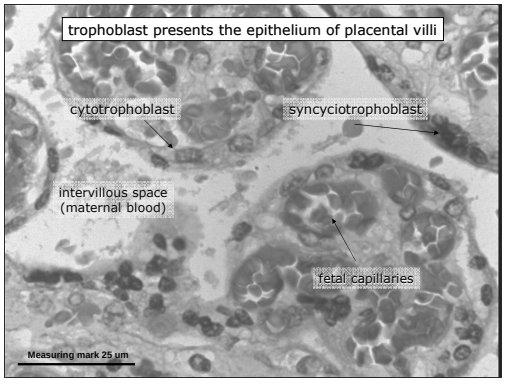
- trophoblast presents the epithelium of placental villi

TROPHOBLAST / PLACENTA:



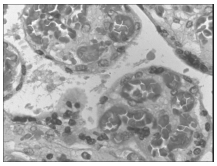
- trophoblast presents the epithelium of placental villi
- trophoblast has two layers
  1. single cell layer
     (basal membrane): cytotrophoblast (CTB)
  2. multinuclear layer: syncyciotrophoblast (STB)
     (in direct contact with maternal blood)





Trophoblast as the epithelium

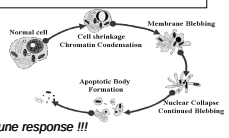
- each epithelium releases aged and/or dead cells over time
- IMPORTANT: in the trophoblast case, that means releasing cells into intervillous space (maternal circulation!)



Trophoblast as the epithelium

- each epithelium releases aged and/or dead cells over time
- IMPORTANT: in the trophoblast case, that means releasing cells into intervillous space (maternal circulation!)

Apoptosis as the "trick solution"



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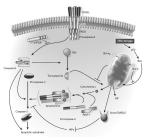
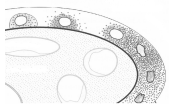


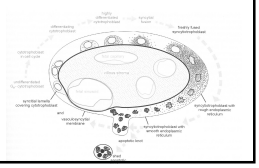


Apoptosis - trophoblast

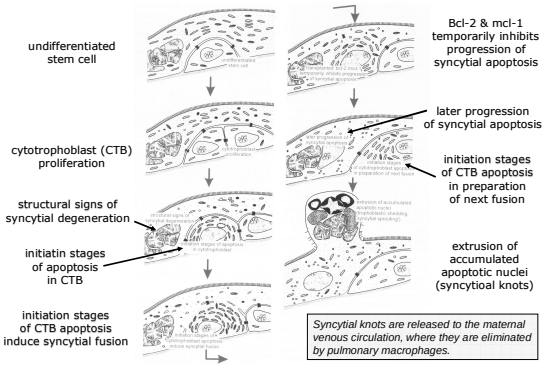
Syncyciotrophoblast is apoptotic tissue generated from cytotrophoblast

Apoptosis - trophoblast

- trophoblast life cycle begins with the cytotrophoblast (CTB) stem cell proliferation ⇨ activation of apoptosis in some of the newly formed cells by activation of caspase 8 ....
- ... what is precondition for their further differentiation and fusion in the syncytiotrophoblast (STB) structure

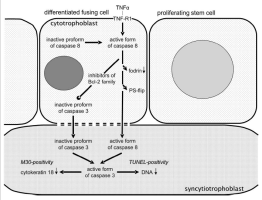


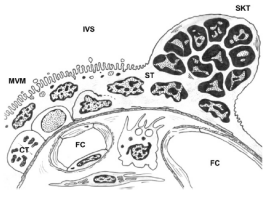
TROPHOBLAST LIFE CYCLE



Huppertz B, i sur. Apoptosis and its role in the trophoblast. Am J Obstet Gynecol 2006.

Trophoblast life cycle presents slow apoptotic process lasting for few weeks instead ~24 hours



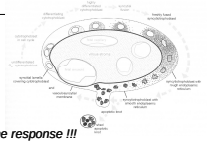


Extremely strong antiapoptotic activity!

Trophoblast apoptosis

- trophoblast apoptosis does not represent (only) its **death**
- trophoblast apoptosis is specific because it is the very basis of trophoblast formation, development and function; in a word, it presents the trophoblasts **life**

Apoptosis as the "trick solution"



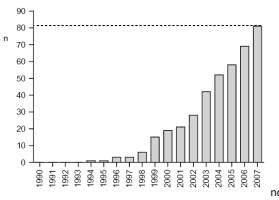
Apoptotic bodies do not cause any inflammatory or immune response !!!

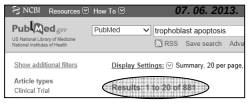
Trophoblast life cycle presents slow apoptotic process lasting for few weeks instead ~24 hours

… dies for living

… lives through dying

No. of published papers
(PubMed – key words: trophoblast, apoptosis)



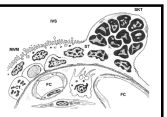


Heazell AE, Crocker IP. Live and let die – regulation of villous trophoblast apoptosis in normal and abnormal pregnancies. Placenta 2008.

TERM "APOPTOSIS" FOR THE FIRST TIME IN THE LITERATURE: Kerr JF. i sur. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972.

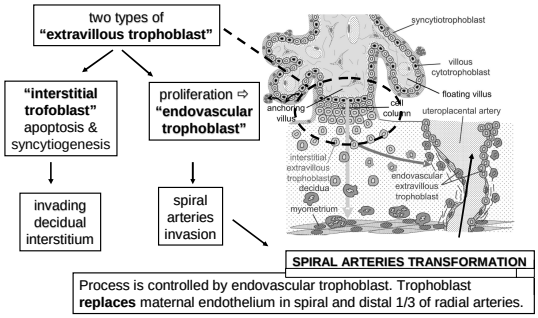
TERM "TROPHOBLAST APOPTOSIS" FOR THE FIRST TIME IN THE LITERATURE : Sakuragi N. i sur. Differentiation-dependent expression of the BCL-2 proto-oncogene in the human trophoblast lineage. J Soc Gynecol Investig. 1994.

Trophoblast apoptosis:



- spiral arteries remodeling *(by trophoblast invasion)*
- preeclampsia and/or HELLP sindrome
- IUGR
- Antiphospholipid syndrome

Extravilous trophoblast invasion and apoptosis



Process is controlled by endovascular trophoblast. Trophoblast **replaces** maternal endothelium in spiral and distal 1/3 of radial arteries.

Huppertz B et al . AJOG 2006.

☐Preeclampsia & HELLP syndrome trophoblast apoptosis

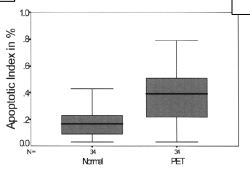
Increased placental apoptosis in pregnancies complicated by preeclampsia

Danny N. Leung, MD,[a] Stephen C. Smith, DM,[b] K.F. To, PhD,[c] Daljit S. Sahota, PhD,[a] and Philip N. Baker, DM[b]
Hong Kong, China, and Nottingham, United Kingdom

Placentas were obtained at delivery from 34 pregnancies complicated by preeclampsia and from 34 uncomplicated pregnancies. The incidence of apoptotic nuclei was significantly greater ($P < .01$) in the placentas from the pregnancies complicated by preeclampsia. (Am J Obstet Gynecol 2001;184:1249-50.)

Key words: Placenta, apoptosis, preeclampsia

☐ ☐ YES!



Am J Obstet Gynecol 2001.

Inappropriate trophoblast apoptosis *vs*. preeclampsia & HELLP syndrome

- **DIRECT CAUSE (I. & II. trimester - extravillous trophoblast):** impaired placentation resulting in hypoxic, mechanical, and ? trophoblast damage
- **INDIRECT CAUSE (II. & III. trimester - villous trophoblast):** enhanced, accelerated and incomplete trophoblast apoptosis:
  ⇨ **"aponecrosis"** ⇨ release of biologically "non inert" material in the intervilous space (mothers bloodstream) ⇨ ….
  ⇨ systemic inflammatory and immune response ⇨ ….
  ⇨ preeclampsia, HELLP syndrome, eclampsia, ...

Kaufmann P et al. Biology of Reproduction 2003.

Inappropriate trophoblast apoptosis *vs*. preeclampsia & HELLP syndrome

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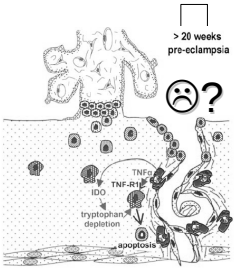
Kaufmann P et al. Biology of Reproduction 2003.

TNF-α is one of the factors that can initiate apoptosis by extrinsic pathway



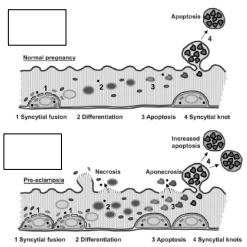
WHO IS TO BLAME?

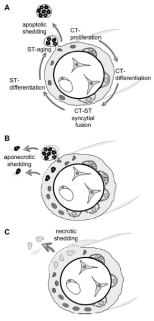
Is the **specificity of mother's inflammatory response** real cause of the spiral arteries transformation efficacy that consequently determinates the incidence of pre-eclampsia? (Genetic factor of preeclampsia?)

Macrophages from the spiral arteries walls **(through TNF-α)** encourage trophoblast apoptosis reducing its activity

Kaufmann P et al. Biology of Reproduction 2003.

Inappropriate trophoblast apoptosis *vs*. preeclampsia & HELLP syndrome ⇨ INDIRECT CONSEQUENCE



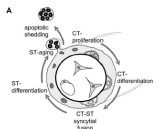


Huppertz B et al . AJOG 2006.

Inappropriate trophoblast apoptosis *vs*. preeclampsia & HELLP syndrome ⇨ INDIRECT CONSEQUENCE

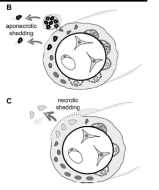
Physiology:

- 150 000 syntitial knots / day
- 3,1g trophoblast
- 3,4% of total trophoblast mass
  (90g in average term placenta)



Preeclampsia:

- ⇧ syncyciotrofoblast microparticles in mothers bloodstream
- ⇧ free fetal DNA in mothers bloodstreem
  - *changes macrophages activity in the mothers lungs in promotion of immune tolerance*
  - *strongly encourages mothers systemic inflammatory response*



Basic structure of the Villous Trees. U: Benirschke K, Kaufmann P, Baergen R. (Ur): Pathology of the Human Placenta. New York. Springer. 2006:50-120.

Inappropriate trophoblast apoptosis *vs*. preeclampsia & HELLP syndrome ⇨ INDIRECT CONSEQUENCE

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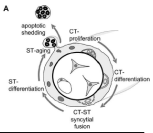
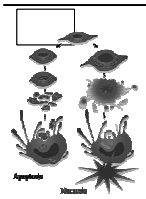
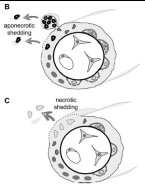




physiology : preeclampsia

trofoblast apoptosis ▪ trophoblast necrosis (aponecrosis)



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AOGS
ACTA Obstetricia et Gynecologica Scandinavica

MAIN RESEARCH ARTICLE

Apoptosis, proliferation and Fas ligand expression in placental trophoblast from pregnancies complicated by HELLP syndrome or pre-eclampsia

IVANA KUZMIC PRUSAC[1], SANDRA ZEKIC TOMAS[2] & DAMIR ROJE[3]

[1]*Department of Pathology, Forensic Medicine and Cytology, Clinical Hospital Centre Split,* [2]*School of Medicine, University of Split, and* [3]*Department of Obstetrics and Gynecology, Clinical Hospital Centre Split, Split, Croatia*

HELLP syndrome : preeclampsia

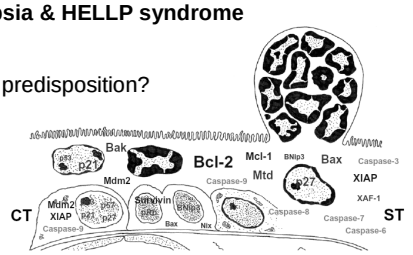
Conclusion:

- increased trophoblast apoptosis
- increased cytotrophoblast proliferation
- reduced FasL expression in trophoblast
- … could be involved in pathophysiological mechanisms of preeclampsia.

Acta Obstet Gynecol Scand 2011.

Inappropriate trophoblast apoptosis *vs*. preeclampsia & HELLP syndrome

Genetic predisposition?



- blue – antiapoptotic factors
- red – proapoptotic factors

Bigger letters mean higher impact!!

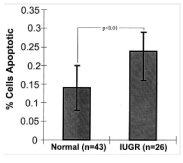
Heazell AEP et al, Placenta 2009.

Trophoblast apoptosis and IUGR

Increased placental apoptosis in intrauterine growth restriction * ** * **

Stephen C. Smith, MB, ChB, Philip N. Baker, DM, E.Malcolm Symonds, MD

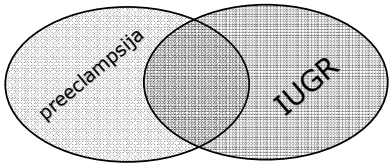
Northwestern Institut Kinzstrom



Yes!

Am J Obstet Gynecol 1997.

TROPHOBLAST APOPTOSIS PREECLAMPISA AND/OR IUGR



Trophoblast apoptosis preeclampia and/or IUGR

- increased apoptotic index value in trophoblast in placentas from pregnancies complicated by preeclampsia / IUGR (0,12%-4,2%) : (0,06% – 0,74%)

Smith SC, i sur Am J Obstet Gynecol 1997.
Axt R, Kordina AC, i sur. Clin Exp Obstet Gynecol 1999.
Allaire AD, i usr. Obstet Gynecol 2000.
Leung DN, i sur. Am J Obstet Gynecol 2001.
Erel CT, i sur. Int J Gynaecol Obstet 2001.
Levy R, i sur. Am J Obstet Gynecol 2002.
Ishihara N, i sur. Am J Obstet Gynecol 2002.
Austgulen R, i sur. J Reprod Immunol 2004.
Barrio E, i sur. J Pediatr Endocrinol Metab 2004

Trophoblast apoptosis – IUGR

- As IUGR and preeclampsia frequently coincide, most studies have not considered them in separate way, but investigated them as a unit.

Barrio E, et al. Intrauterine growth retardation: study of placental apoptosis. J Pediatr Endocrinol Metab 2004.
Ishihara N, et al. Increased apoptosis in the syncytiotrophoblast in human term placentas complicated by either preeclampsia or intrauterine growth retardation. Am J Obstet Gynecol 2002.
Endo H, et al. Frequent apoptosis in placental villi from pregnancies complicated with intrauterine growth restriction and without maternal symptoms. Int J Mol Med 2005.

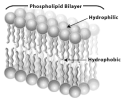
Trophoblast apoptosis – idiopatic IUGR

- The proliferative and apoptotic events in the trophoblast of placentas with idiopathic IUGR do not differ from physiologic ones.

Roje D, et al. Trophoblast apoptosis in human term placentas from pregnancies complicated with idiopathic intrauterine growth retardation. J Matern Fetal Neonatal Med. 2011.

"Urbi et orbi"

- After the initiation of apoptosis, cells announce to the others that a process started
- One of the most important signs is a change in the membrane structure ...
- ... *negatively charged phospholipids are moved to the outside of the membrane and are "exposed" (phosphatidyl serine)*



What about heparin in this situation?

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Heparin prevents programmed cell death in human trophoblast

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Heparin is used clinically for the prevention of pregnancy complications associated with prothrombotic disorders, especially antiphospholipid antibody syndrome. Recent studies have suggested that heparin may exert direct effects on placental trophoblast, independently of its anticoagulant activity. We now demonstrate that heparin abrogates apoptosis of primary first trimester villous trophoblast in response to treatment with the pro-inflammatory cytokines interferon (IFN)-γ and tumour necrosis factor (TNF)-α. This multifunctional glycosaminoglycan also inhibited apoptosis induced by other agents, including staurosporin, broad-spectrum kinase inhibitor and thrombin. Furthermore, heparin attenuated caspase-3 activity, a hallmark of apoptosis, in human first trimester villous and extravillous trophoblast cell lines treated with peptidoglycan, a Toll-like receptor-2 agonist isolated from *Staphylococcus aureus*. The ability of heparin to antagonize cell death induced by such diverse apoptotic signals suggested that it acts as a survival factor for human trophoblast. We demonstrate that heparin, like epidermal growth factor (EGF) and heparin-binding EGF (HB-EGF), elicits phosphorylation of the EGF receptor and activation of the phosphatidyl inositol 3-kinase (PI3K)-, the extracellular signal-related kinase 1/2 (ERK1/2)- and the c-Jun NH2 terminal kinase (JNK)-signal transduction pathways in primary villous trophoblast. In summary, we have demonstrated that heparin activates multiple anti-apoptotic pathways in human trophoblast. Our results suggest that heparin may be useful in the management of at-risk patients, even in the absence of an identifiable thrombophilic disorder.

Key words: heparin/apoptosis/trophoblast/survival

Hills FA, i sur. Heparin prevents programmed cell death in human trophoblast. Mol Hum Repr 2006.

Heparin – apoptosis – anticoagulant?

- Heparin in vitro prevents apoptosis induction by trophoblast antiphospholipid antibodies and increases trophoblasts invasiveness

Bose P. i sur. Adverse affects of lupus antikoagulant positive blood sera on placental viability can be prevented by heparin in vitro. Am J Obstet Gynecol 2004.

- Although the same anticoagulant effect of similar anticoagulant drugs (hirudin and fondaparin) only heparin reduces the risk of spontaneous abortion in mice,

Girardi G. i sur. Heparin prevents antiphospholiipid antibody-induced fetal loss by inhibiting complement activation. Nat Med 2004.

"Other" anti-inflammatory and immunomodulatory biological heparin effects:

- NK (natural killer) cells supression
- cutting down neutrophils adhesion ability
- antagonistic effect on the interferon gamma signaling effect
- heaprin inhibits complement activation
- heparin amplifies the effect of several growth factors *(hepatocyte growth factor, epidermal growth factor, heparin binding and fibroblast growth factor)* that have significant role in the invasiveness of trophoblast

Hills FA, i sur. Heparin prevents programmed cell death in human trophoblast. Mol Hum Repr 2006.

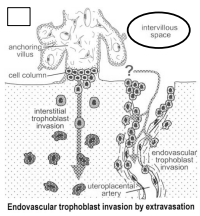
Apoptosis in the peri-implantation period

- **apposition:** inhibited apoptotic activity of the endometrium around embryo ⇨ embryo secretes antiapoptotic factors
- **adhesion (*vice versa*):** amplification of endometrial apoptotic activity around ⇨ embryo secretes proapoptotic factors

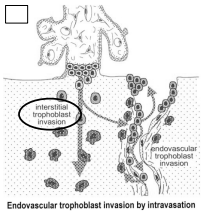
Modulating the secretion of pro and anti apoptotic factors embryo controls decidual apoptotic activity at the paracrine level

Straszewski-Chavez SL et al. Endocrine Reviews 2005.

Where from endovascular trophoblast comes in the spiral arteries lumen?



Endovascular trophoblast invasion by extravasation



Endovascular trophoblast invasion by intravasation

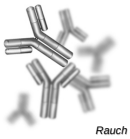
Possibly

Probably

Kaufmann P et al. Biology of Reproduction 2003.

Connection between apoptosis and antiphospholipide syndrome

- apoptotic cells **as antigen** for existing antiphospholipid antibodies
- apoptotic cells **as immunogen** for the development of antiphospholipid syndrome



Rauch J i sur. Apoptosis and the Antiphospholipid syndrome. Journal of Autoimunity 2000.

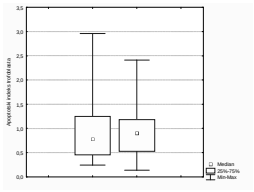
Trophoblast apoptosis – preeclampia and/or IUGR

- Numerous studies have shown that the trophoblast apoptotic activity is more pronounce in the placentas from:
  - preeclampsia
    and/or
  - IUGR

Allaire AD, at al. Placental apoptosis in preeclampsia. Obstet Gynecol 2000.
Erel CTet al. Apoptosis in the placenta of pregnancies complicated with IUGR. Int J Gynaecol Obstet 2001.
Austgulen Ret al. Pre-eclampsia associated with increased syncytial apoptosis when the infant is small-for-gestationalage. J Reprod Immunol 2004.
Leung DN, et al. Increased placental apoptosis in pregnancies complicated by preeclampsia. Am J Obstet Gynecol 2001.

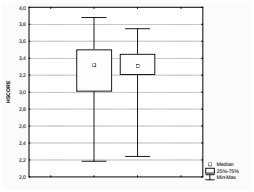
Trophoblast apoptosis and idiopathic IUGR

M30 immunoreactivity
apoptotic activity



p=0,613

Bcl-2 immunoreactivity
apoptitic activity



p=0,634

NO!

Roje D, et al. J Matern Fetal Neonatal Med 2011.

Trophoblast apoptosis and idiopathic IUGR

Excess Syncytiotrophoblast Microparticle Shedding is a Feature of Early-onset Pre-eclampsia, but not Normotensive Intrauterine Growth Restriction

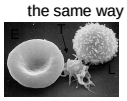
D. Goswami[a], D. S. Tannetta[b], L. A. Magee[c,d], A. Fuchisawa[a], C. W. G. Redman[b], I. L. Sargent[b] and P. von Dadelszen[a,d,*]

Placenta 2006.

How does aspirin works?

- *Aspirin inhibits nitrogen (II) oxide (NO) synthesis*
- *Because oxidative stress is the initiator and promoter of trophoblast apoptosis, ...*
  ... mechanism of aspirin action is probably in the regulation of redox processes associated with placental apoptosis.

Aspirin action is not exclusively related to platelet activity! ...
... although activated platelet changes the structure of its membrane the same way as the apoptotic cell does.



Bose P st all. Heparin and aspirin attenuate placental apoptosis in vitro: implications for early pregnancy failure. Am J Obstet Gynecol 2005.