PERIODICUM BIOLOGORUM
VOL. 116, No 2, 167–172, 2014

UDC 57:61
CODEN PDBIAD
ISSN 0031-5362

Original scientific paper

Bcl-2 and Bax immunoreactivity in placentas from pregnancies complicated with intrauterine growth restriction and hypertension

MARINA KOS[1,2]
EDUARD MATKOVICH[1,2]

[1]Clinical Department of Pathology
„Ljudevit Jurak", Clinical Hospital Center
„Sestre milosrdnice"

[2]Institue of Pathology, University of Zagreb
Medical School

Correspondence:
Marina Kos, MD, PhD
Clinical Department of Pathology
„Ljudevit Jurak", Clinical Hospital Center
„Sestre milosrdnice", Vinogradska 29, 10 000 Zagreb
E-mail: marina.kos@kbcsm.hr

Key words: apoptosis, placenta, IUGR, preeclampsia

Received September 18, 2013.

Abstract

Because placental apoptosis associated protein imbalance is thought to contribute to the pathogenesis of intrauterine growth restriction (IUGR) and preeclampsia (PE), morphological features and expression of Bcl-2 and Bax were studied on samples from 10 human placentas from pregnancies complicated with IUGR and PE and 10 placentas from uncomplicated term pregnancies.

In 9/10 placentas from the IUGR/PE group, the findings were consistent with hypoxic damage and in 1/10 placentas chronic villitis was found. In both groups Bax showed strong diffuse expression (3+) in amnionic epithelium, blood vessel wall and endothelium in the chorionic plate and placental villi and the villous stroma in all placentas. The extravillous trophoblast (EVT) in the basal decidua showed 3+ Bax expression in 8/10 placentas and 2+ expression in 2/10 placentas in both groups. In the IUGR/PE group the Bax expression in both cyto (CT) and syncytiotrophoblast (ST), was 3+ in 8/10 placentas, and 2+ in 2/10 placentas. In the control group Bax expression was 3+ in both CT and ST in all 10 placentas. The 3+ Bcl-2 expression in both groups was found only in CT and ST. The syncytial knots in both groups showed 3+ positivity for Bax and Bcl-2. These findings do not confirm other authors' findings about the increased expression of proapoptotic factors in the third trimester placentas with IUGR/PE, and the conclusion is that for the pathogenesis of IUGR and PE, the attention should be shifted to inappropriate apoptosis during the process of implantation.

INTRODUCTION

For the appropriate placentation and development of the placenta the extravillous trophoblast derived from the villi that anchor the developing blastocyst to the underlying decidua plays the most important role. It is the conversion of spiral arterioles into the uteroplacental blood vessels, resulting in the development of a high flow, low resistance vessels able to perfuse the intervillous space. At the surface of the placental villi, the syncytium is formed from underlying mitotically active mononucleated cytotrophoblasts that differentiate and undergo fusion. The anatomical integrity of the syncytiotrophoblast is critical, as a variety of receptors and enzymes are strategically positioned on the maternal blood facing membrane and regulate maternal–fetal exchange *(1)*. The villous

trophoblast bilayer is delimited from the villous stroma which contains branching fetal blood vessels in a connective tissue matrix by a basement membrane. Placental function is critical at all stages of pregnancy, but nutrient transport demands on villous trophoblasts are highest as the fetus triples in weight during the third trimester of gestation *(2)*. In preeclamsia (PE) a characteristic change of the spiral arteries, firstly described and named „acute atherosis" can be seen in the basal decidua layer, as well as in the free placental membranes *(3)*. Acute atherosis develops only in the spiral arteries that have not undergone physiologic changes, and the reason for this incomplete change still remains a mystery. Some authors have found a reduction in the extent of trophoblast invasion in severe preeclampsia, both in the spiral arteries and the myometrium *(4)*. Poor trophoblast invasion and remodeling of uterine spiral arteries have been suggested to lead to hypoperfusion, hypoxia, reperfusion injury, oxidative stress and signs of villous tree maldevelopment in the second half of the pregnancy. Hypoxic environment in cases of impaired uteroplacental blood flow, causes necrosis of superficial, syncytiotrophoblastic layer with subsequent discontinuities. In normal circumstances, within the villous trophoblast, proliferation is restricted to the cytotrophoblasts, and apoptosis is almost exclusively localised to the syncyciotrophoblast *(5)*. Bax, one of the proapoptotic members of the Bcl-2 family, is present in the villous trophoblast in the first and third trimester. In the first trimester, it is localized to the cytoplasm of the cytotrophoblast, but in the third trimester it is expressed in the syncytiotrophoblast *(6-8)*. The levels of apoptosis within tertiary villi increase with gestation, and are the greatest over 40 weeks of gestation *(9)*. Insults that result in villous trophoblast injury may not be accompanied by a compensatory increase in cytotrophoblast proliferation and differentiation *(10, 11)*. Because of the extreme importance of apoptosis regulation, cultured trophoblasts exposed to hypoxia show a marked upregulation of p53 activity, enhanced expression of the pro-apoptotic Mtd-1 and decreased expression of the anti-apoptotic Bcl-2, all of which promote apoptosis *(8, 12, 13)*. In contrast to hypoxia alone, hypoxia-reoxygenation results in more marked apoptosis regulated by other proteins such as the increased expression of the pro-apoptotic Bax and Bak *(13-15)*. Expression of Bax is often associated with areas of trophoblast damage, or degeneration, such as fibrinoid deposits and syncitial knots *(16, 17)*.

With all the data from the literature, we hypothesized that the expression of proapoptotic Bax protein will be enhanced in the tissue of placentas from the pregnancies complicated with PE and intrauterine growth restriction (IUGR), in comparison to the placentas from uncomplicated term pregnancies. We also expected the expression of anti-apoptotic Bcl-2 protein to be strong in the areas of villous trophoblast damage, and in the areas of fibrinoid deposition. This study is a pilot study of a larger study with the same hypotheses and aim which was to assess the expression of Bax and Bcl-2 proteins in different compartments of the placentas from the pregnancies complicated with IUGR and PE, and to comapare the results with the expression of Bax and Bcl-2 in the corresponding compartments of the placentas from uncomplicated term pregnancies.

MATERIALS AND METHODS

The material consisted od 10 samples of human placental tissue from the pregnancies complicated with IUGR and PE confirmed by serial clinical follow up. The control group consisted of 10 samples of human placental tissue from the uncomplicated term pregnancies. The tissue in both groups was retrieved from the archive of the Clinical Department of Pathology „Ljudevit Jurak", Clinical Hospital Center „Sestre Milosrdnice" in Zagreb, where they were collected during the scientific project of the Ministry of science, education and sport of the Republic of Croatia, No. 108-1081870-1940. The samples contained full thickness of the placenta, from the chorionic plate membranes to the basal decidua, that were taken from the unfixed placental tissue during routine pathological examination. The samples were routinely fixed in formalin and embedded in paraffin. Three sections of every sample were cut at 5 μm; one was stained routinely with hematoxylin-eosin (H-E) and examined by light microscopy to assess the morphology; the other two were analyzed after immunohistochemical staining for Bax (polyclonal rabbit anti-human, Code A3533, Dako Denmark) and Bcl-2 (monoclonal mouse anti-human, Clone 124, Code M0887, Dako, Denmark), respectively. For immunohistochemical analysis the 5 μm thick sections of the placental tissue were mounted on a silanized slide, and left to dry for 24 h. The section were than deparaffinized in xylene, rehydrated through a graded series of alcohol and washed in phosphate buffered saline (PBS). PBS was used for all subsequent washes and for antiserum dilution. Tissue sections were treated with Catalyzed Signal Amplification (CSA) System II for use with mouse primary antibodies (Code K1497, Dako, Denmark). The CSA System II was adapted for the detection of rabbit polyclonal antibodies by replacing the anti mouse link provided in the kit with the CSA II Rabbit Link (Code 1501, Dako, Denmark). Immunohistochemical staining intensity was evaluated semiquantitatively as: negative (-), weakly positive (1+) if positive in solitary cells, up to 50% of cells; moderately positive (2+) if strongly positive in up to 50% of cells, moderately positive in > 50% of cells and strongly positive (3+) if strongly positive in > 50% of cells. The immunoreactivity was assessed in different parts of the placenta: extravillous trophoblast (EVT) in the basal decidua, villous cytotrophoblast (CT) and syncitiotrophoblast (ST), the villous stromal cells, the blood vessels of the chorionic plate and in the amnionic

epithelium of the chorionic plate. For the analysis of the results, descriptive methods were used.

RESULTS

The mean gestational age of women in group with IUGR/PE was 36 weeks (range 31 – 39 weeks), and in the control group it was 39 weeks (range 37 – 40 weeks). The mean placental weight in the group with IUGR/PE was 330 g (range 250 – 380 g), and in the control group 438 g (range 390 – 520 g). Because of the fact that pregnancies complicated with PE and IUGR frequently terminate earlier, either spontaneously, or because of the threatening asphyxia of the fetus, there was no point in calculating statistical significance of the difference.

Grossly, one placenta in the control group was succenturiate, but on microscopic examination showed normally developed, term villi. On light microscopy histopathological examination of H-E stained placental tissue, fetal membranes and the umbilical cord showed normal morphology in all 10 placentas from IUGR/PE group as well as in the control group. Out of 10 placenta in the IUGR/PE group, none showed completely normal histological features. In 9/10 placentas the findings were consistent with hypoxic damage, while in 1/10 placentas a diffuse chronic villitis of unknown etiology (VUE) was found. In the control group of placentas, 1/10 showed a solitary, peripherally localized chronic infarct. The histopathological findings of the placental tissue stained with H-E are shown in Table 1.

TABLE 1
Histopathological findings of the placental tissue stained with H-E.

Group/findings	IUGR/PE*	Control
Multiple chronic infarcts (+s.k**)	4	0
Solitary chronic infarct (+s.k.**)	1	1
Chorangiosis	3	0
Loss of vasculosyncytial membranes	1	0
Chronic villitis	1	0
Normal findings	0	9
Total	10	10

*Placentas from pregnancies complicated with IUGR/PE ** Excessive quantity of syncytial knots

Immunoreactivity for Bax in both, group with IUGR/PE and the control group showed the same pattern in all 10 placentas in each group: strong diffuse expression (3+) in amnionic epithelium (Figure 1) blood vessel wall and endothelium in the chorionic plate and the placental villi (Figure 2), and the villous stroma. In the EVT in the basal decidua strong expression (3+) was found in 8/10 placentas in both groups, while the expression was graded as moderate (2+) in 2/10 placentas in both groups (Figure 3). The Bax expression was strong and diffuse (3+) in both CT and ST in the IUGR/PE group in 8/10 placentas, and moderate (2+) in 2/10 placentas in this group (Figure 4). In the control group Bax expression was strong and diffuse (3+) in both CT and ST in all 10/10 placentas (Figure 5). The syncytial knots in both groups showed strong (3+) positivity for Bax (Figure 6). The results of Bax expression are summarized in Table 2.

The Bcl-2 expression in both groups was found only in CT and ST, and estimated as strong (3+) in both analyzed groups (Figure 7). All other elements were negative for Bcl-2 (Table 3).

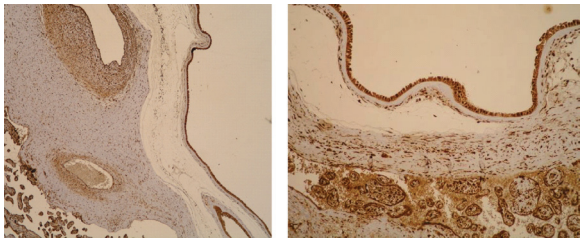

Figure 1. *Strong diffuse (3+) immunoreactivity for Bax in the amnionic epithelium of the placenta in the group with IUGR/PE (left) and in the control group (right). (Bax x 200)*

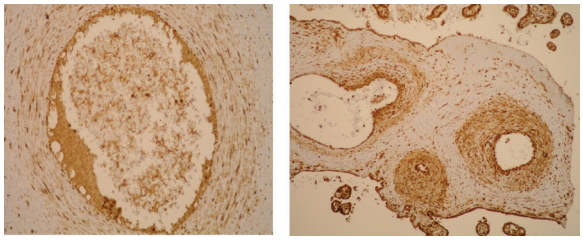

Figure 2. *Strong diffuse (3+) immunoreactivity for Bax in the amnionic epithelium of the placenta in the group with IUGR/PE (left) and in the control group (right). (Bax x 200)*

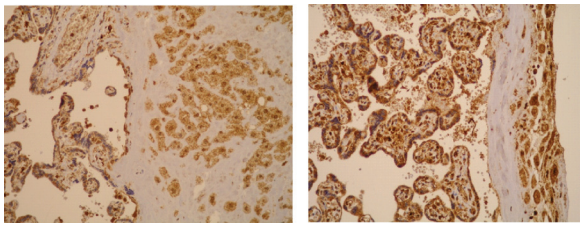

Figure 3. *Immunoreactivity for Bax in the EVT of the placenta in the group with IUGR/PE (left) and in the control group (right). (Bax x 200)*

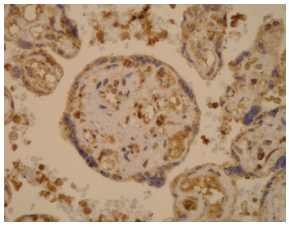

Figure 4. *Moderate immunoreactivity (2+) for Bax in the villous trophoblast in the placenta in the group with IUGR/PE was found in 2/10 placentas. (Bax x 400)*

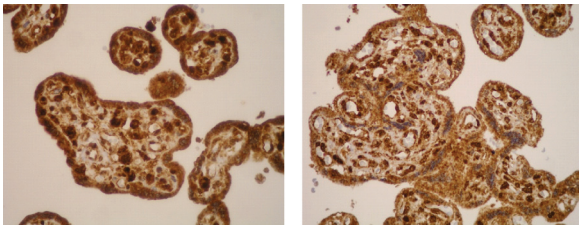

Figure 5. *Strong diffuse immunoreactivity (3+) for Bax in the villous trophoblast in the placenta was found in 8/10 placentas in the group with IUGR/PE (left) and in 10/10 placentas in the control group (right). (Bax x 400)*

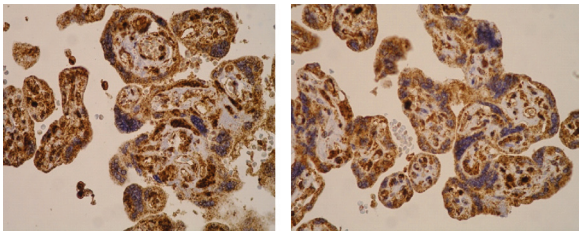

Figure 6. *Strong immunoreactivity for Bax in the syncitial knots in the placenta in the group with IUGR/PE (left) and in the control group (right). (Bax x 400)*

TABLE 2

Bax expression in IUGR/PE placentas and the control group.

Group/localization	IUGR/PE (n=10)	Control (n=10)
EVT in the basal decidua		10
3+	8	8
2+	2	2
CT and ST		
3+	8	10
2+	2	
Villous stromal cells		
3+	10	10
Blood vessels		
3+	10	10
Amnionic epithelium		
3+	10	10

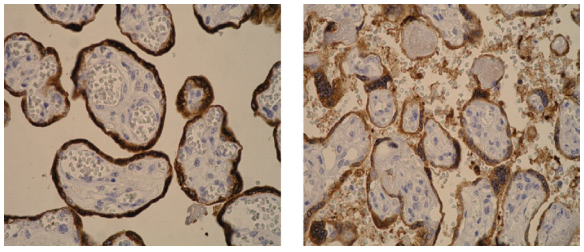

Figure 7. *Strong immunoreactivity (3+) for Bcl-2 in the villous trophoblast in the placenta in the group with IUGR/PE (left) and in the control group (right). (Bax x 400).*

TABLE 3

Bcl-2 expression in IUGR/PE placentas and the control group.

Group/localization	IUGR/PE* (n=10)	Control (n=10)
EVT in the basal decidua	0	0
CT and ST		
3+	10	10
Villous stromal cells	0	0
Blood vessels	0	0
Amnionic epithelium	0	0

DISCUSSION

In PE and IUGR, the placenta is frequently small (weight under the 10th percentile for gestational age, feto-placental ratio innappropriate for gestational age), with gross findings of multiple infarcts, and (especially in PE) 3-5 times more frequent findings of retroplacental hematoma. Histological findings may be very different, but mostly point out towards the inappropriate uteroplacental blood flow, such as the thickened cytotrophoblastic layer, very numerous syncitial knots and chorangiosis *(18).* Due to the development of a high pressure placental blood supply, the developing villous tree can be damaged, showing changes in placental structure *(19, 20).* In placentas from pregnancies complicated by IUGR these sites of injury and repair are covered with fibrin containing deposits, that can be found in every placenta, but are observed with increased frequency in placentas with IUGR. The fibrin matrix serves as a scaffold for trophoblast to re-epithelialize the villous surface. Syncytial knots, that are also observed in much greater quantity in placentas with PE, are clusters of apoptotic syncytial nuclei that bulge from the surface into the intervillous space. The underlying cytotrophoblast population provides a source for new syncytium during normal epithelial turnover and at sites of syncytial re-epithelialization of fibrin on the surface of villi, resulting with thickened cytotrophoblastic layer seen on light microscopy *(18).*

Programmed cell death by apoptosis and its associated regulatory mechanisms are intimately involved in placental homeostasis, growth and remodeling with the apoptotic rates that increase progressively during normal pregnancy as part of normal placental development *(21).* Previous studies showed that Bcl-2 was generally expressed at low levels during the entire gestational period, while Bax was low during the first trimester and increased towards the end of gestation. In accordance with the change of ratio of these two molecules, the increase of apoptotic cells was observable in the third trimester *(22).* In their study of Bcl-2 and Bax expression in preterm, term and postterm placentas Daher et al. found the same pattern of immunostaining for Bcl-2 and Bax in all samples, but reactivity for Bax was higher in preterm and postterm placentas, whether the reactivity for Bcl-2 decreased in preterm placentas. This reactivity pattern resulted in the higher Bax/Bcl-2 ratio in both pre-term and post-term placental samples compared with term placentas *(23).* All these data indicate that Bcl-2 and Bax are temporally regulated during placental development and that the different expression of the above mentioned genes is at least in part responsible for the delicate balance between cell proliferation and programmed cell death in the human placenta during pregnancy.

In the present study, the analysis od Bax showed strong or, in the minority of cases, moderate expression in CT, ST, EVT, stromal cells of the villi, and endothelial cells in both analyzed groups. These findings confirm the findings of Cobellis *et al. (24).*

Although many molecules are associated with the induction and prevention of apoptosis in different models one of them is a proapoptotic factor Bax and the same authors also observed an increase of Bax expression in all placental compartments in preeclampsia and diabetes, compared to term placentas from uncomplicated pregnancies *(24).* Several studies demonstrated that apoptosis increases in pregnancies complicated by some pathologies such as preeclampsia, fetal growth restriction and diabetes *(21, 25-27).* Other authors' observations were similar, not only by means of immunohistochemistry alone, but also with combined methods and TUNEL (terminal deoxyribonucleotidyltransferase-mediated dUTP and-labelling) *(16).* The etiology of IUGR and PE is still unclear, however placental dysfunction is considered a common underlying cause in both conditions. The role of apoptosis in the development of placental pathology of these conditions has yet to be explained. The theory that the placentas in these pregnancy disorders display altered cell kinetics is corroborated by the findings of an increase of Bax expression is observed in all the placental compartments, especially in preeclampsia *(28).* In both IUGR and PE, apoptosis may disrupt the cytotrophoblast turnover that is, especially in PE increased, commencing with increased proliferation of cytotrophoblast, that could produce increased end stages of apoptosis in the syncitioblast *(25).* Han *et al.* demonstrated that caspase-10 and death receptor 3 (DR-3) were upregulated in placenta from preeclamptic patients, suggesting that placental apoptosis and altered gene expression in the trophoblast may influence the pathogenesis of preeclampsia *(27).*

The results of this study did not confirm the above mentioned findings, because the expression of Bax was practically of the same intensity in the group of placentas with IUGR/PE and the control group in all placental compartments. Perhaps the studied group was too small, perhaps the immunoreagents was not properly chosen and perhaps in this group there was just no difference. Although some authors found that the expression of Bax is often associated with fibrinoid deposits and syncitial knots, in this study no difference was found between the expression of Bax in fibrinoid deposits in the investigated placentas and the control group *(16, 17).* As to the expression of Bcl-2, many factors capable of regulating apoptosis are present in the villous trophoblast, but it seems that their expression change with pregnancy progression. The syncitiotrophoblast is protected against unwanted apoptosis by expression of Bcl-2 (and some other anti-apoptotic factors). In this study, the expression of Bcl-2 was restricted to villous trophoblast, where it was found to be mostly strong, and in several cases (in the IUGR/PE group) moderate. It was also strong in the syncytial knots, in both analyzed groups, but the expression of Bax was found to be strong in both analyzed groups as well.

Recently, it has been suggested that vascular remodeling that occurs during placentation may be indirectly controlled by intravascular trophoblast that stimulates endothelial cells to secrete chemokines. These chemokines attract decidual leukocytes, particularly uterine natural killer cells and macrophages, leading to vascular smooth muscle cell apoptosis *(29).* A suggested mechanism for endothelial cell destruction is via the Fas/FasL system, which is present on endothelial and vascular smooth muscle cells of the uterine spiral arteries *(30).* In PE and IUGR, there may be a reduction in the number of trophoblast cells within the spiral arteries, which has been associated with increased apoptosis and a reduced luminal size *(30, 31).* Many authors have found significant increases in placental apoptosis, which may be the underlying cause in the pathophysiology of preeclampsia and IUGR, but this was not confirmed in this study. The reason for this discrepancy cannot be found. However, there is a growing body of evidence that suggests that abnormal apoptosis has important effects at the very beginning of the placental development, namely the physiological conversion of spiral arteries to uteroplacental vessels. In our opinion, this is the real cause of IUGR and/or PE, and the attention has to be shifted from the analysis of apoptosis in the third trimester or term pla-

centas to the apoptosis in the placental bed in cases of spontaneous early miscarriages, or missed abortions.

REFERENCES

1. JONES H N, POWELL T L, JANSSON T 2007 Regulation of placental nutrient transport – a review. *Placenta 28:* 763–74
2. HUPPERTZ B 2008 The anatomy of the normal placenta. *J Clin Pathol 61:* 1296–1302
3. ZEEK P M, ASSALI N S 1950 Vascular changes in the decidua associated with eclamptogenic toxemia of pregnancy. *Am J Clin Patol 20:* 1099-1109
4. MEEKINS J W, PIJNENBORG R 1994 A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre-eclamptic pregnancies. *BJOG 101:* 669–674
5. SMITH S C, BAKER P N, SYMONDS E M 1997 Placental apoptosis in normal human pregnancy. *Am J Obstet Gynecol 177:* 57–65
6. BAE S N, KIM J, LEE Y S, KIM J D, KIM M Y, PARK LO 2007 Cytotoxic effect of zinc-citrate compound on choriocarcinoma cell lines. *Placenta 28:* 22–30
7. QIAO S, NAGASAKA T, HARADA T, NAKASHIMA N 1998 p53, Bax and Bcl-2 expression, and apoptosis in gestational trophoblast of complete hydatidiform mole. *Placenta 19:* 361–369
8. HEAZELL A E, LACEY H A, JONES C J, HUPPERTZ B, BAKER P N, CROCKER I P 2008 Effects of oxygen on cell turnover and expression of regulators of apoptosis in human placental trophoblast. *Placenta 29:* 175–186
9. ATHAPATHU H, JAYAWARDANA M A, SENANAYAKA L 2003 A study of the incidence of apoptosis in the human placental cells in the last weeks of pregnancy. *J Obstet Gynaecol 23:* 515–517
10. HEAZELL A E, CROCKER I P 2008 Live and let die – regulation of villous trophoblast apoptosis in normal and abnormal pregnancies. *Placenta 29:* 772-783
11. CROCKER I P, TANSINDA D M, BAKER P N 2004 Altered cell kinetics in cultured placental villous explants in pregnancies complicated by pre-eclampsia and itrauterine growth restriction. *J Pathol 204:* 11–18
12. SOLEYMANLOU N, JURISICOVA A, WU Y, CHIJIIWA M, RAY J E, DETMAR J, TODROS T, ZAMUDIO S, POST M, CANIGGIA I 2007 Hypoxic switch in mitochondrial myeloid cell leukemia factor-1/Mtd apoptotic rheostat contributes to human trophoblast cell death in preeclampsia. *Am J Pathol 171:* 496–506
13. LEVY R, SMITH S D, CHANDLER K, SADOVSKY Y, NELSON D M 2000 Apoptosis in human cultured trophoblasts is enhanced by hypoxia and diminished by epidermal growth factor. *Am J Physiol Cell Physiol 278:* C982–988
14. HUNG T H, SKEPPER J N, CHARNOCK-JONES D S, BURTON G J 2002 Hypoxia-reoxygenation: a potent inducer of apoptotic changes in the human placenta and possible etiological factor in preeclampsia. *Circ Res 90:* 1274–1281
15. HUNG T H, CHEN S F, LIOU J D, HSU J J, LI M J, YEH Y L, HSIEH T T 2008 Bax, Bak and mitochondrial oxidants are involved in hypoxia-reoxygenation-induced apoptosis in human placenta. *Placenta 29:* 565–583
16. RATTS V S, TAO X J, WEBSTER C B, SWANSON P E, SMITH S D, BROWNBILL P, KRAJEWSKI S, REED J C, TILLY J L, NELSON D M 2000 Expression of BCL-2, BAX and BAK in the trophoblast layer of the term human placenta: a unique model of apoptosis within a syncytium. *Placenta 21:* 361–366
17. YAMADA Z, KITAGAWA M, TAKEMURA T, HIROKAWA K 2001 Effect of maternal age on indices of apoptotic and proliferative cells in trophoblasts of full-term placenta. *Mol Hum Reprod 7:* 1179–1185
18. KOS M, LENIČEK T 2011 Osnove patologije posteljice. Zagreb, Medicinska naklada.
19. BURTON G J, JAUNIAUX E, CHARNOCK-JONES D S 2009 The influence of the intrauterine environment on human placental development. *Int J Dev Biol 54:* 303–312
20. HUTCHINSON E S, BROWNBILL P, JONES N W, ABRAHAMS V M, BAKER P N, SIBLEY C P, CROCKER I P 2009 Utero-placental haemodynamics in the pathogenesis of pre-eclampsia. *Placenta 30:* 634–641
21. SGARBOSA F, BARBISAN L F, BRASIL M A, COSTA E, CALDERON I M, GONÇALVES C R, BEVILACQUA E, RUDGE M V 2006 Changes in apoptosis and Bcl-2 expression in human hyperglucemic, term placental trophoblast. *Diabetes Res Clin Pract 73:* 143-149
22. DE FALCO M, DE LUCA L, ACANFORA F, CAVALLOTTI I, COTTONE G, LAFORGIA V, DE LUCA B, BALDI A, DE LUCA A 2001Alteration of the Bcl-2:Bax ratio in the placenta as pregnancy proceeds. *Histochem J 33:* 421-425
23. DAHER S, GUIMARAES A J, MATTAR R, ISHIQAI M M, BARREIRO E G, BEVILACQUA E 2008 Bcl-2 and Bax Expressions in Pre-Term, Term and Post-Term Placentas. *Am J Reprod Immunol 60:* 172-178
24. COBELLIS L, DE FALCO M, TORELLA M, TRABUCCO E, CAPRIO F, FEDERICO E, MANENTE L, COPPOLA G, LAFORGIA V, CASSANDRO R, COLACURCI N, DE LUCA A 2007 Modulation of Bax expression in physiological and pathological human placentas throughout pregnancy. *in Vivo 21:* 777-784
25. HUPPERTZ B, KADYROV M, KINGDOM J C P 2006 Apoptosis and its role in the trophoblast. *Am J Obstetr Gynecol 195:* 29-39
26. LEUNG D N, SMITH S C, TO K F, SAHOTA D S, BAKER P N 2001 Increased placental apoptosis in pregnancies complicated by preeclampsia. *Am J Obstet Gynecol 184:* 1249-1250
27. HAN J Y, KIM Y S, CHO G J, ROH G S, KIM H J, CHOI W J, PAIK W Y, RHO G J, KANG S S, CHOI W S 2006 Altered gene expression of caspase-10, death receptor-3 and IGFBP-3 in pre-eclamptic placentas. *Mol Cells 22:* 168-174
28. CROCKER I P, COOPER S, ONG S C, BAKER P N 2003 Differences in apoptotic susceptibility of cytotrophoblasts and syncytiotrophoblasts in normal pregnancy to those complicated with preeclampsia and intrauterine growth restriction. *Am J Pathol 162:* 637–643
29. SMITH S D, DUNK C E, APLIN J D, HARRIS L K, JONES R L 2009 Evidence for immune cell involvement in decidual spiral arteriole remodeling in early human pregnancy. *Am J Pathol 174:* 1959–1971
30. ASHTON S V, WHITLEY G S J, DASH P R, WAREING M, CROCKER I P, BAKER P N, CARTWRIGHT J E 2005 Uterine spiral artery remodeling involves endothelial apoptosis induced by extravillous trophoblasts through Fas/FasL interactions. *Arterioscler Thromb Vasc Biol 25:* 102–108
31. DIFEDERICO E, GENBACEV O, FISHER S J 1999 Preeclampsia is associated with widespread apoptosis of placental cytotrophoblasts within the uterine wall. *Am J Pathol 155:* 293–301