Expression of 52-kDa FK506-Binding Protein (FKBP52) in Human Placenta Complicated by Preeclampsia and Intrauterine Growth Restriction

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OBJECTIVE: To investigate the expression of 52-kDa FK506-binding protein (FKBP52) in human placentas complicated by preeclampsia (PE) and intrauterine growth restriction (IUGR).
STUDY DESIGN: Case-control study including placentas from 6 PE pregnancies, 6 IUGR pregnancies, and 6 controls. FKBP52 expression was determined by immunohistochemistry and Western blot techniques.
RESULTS: FKBP52 expression was downregulated in PE group placentas compared to control and IUGR group placentas. In IUGR group placentas FKBP52 expression was upregulated compared to control and PE group placentas. FKBP52 expression differences between PE and IUGR group placentas ($p=0.008$) and control and IUGR group placentas ($p=0.042$) were statistically significant. There was FKBP52 immunoreactivity in decidua, syncytiotrophoblast, villous stromal cells, and vascular endothelium in all groups. Unlike control and PE group placentas, FKBP52 expression was continuous in syncytiotrophoblast of IUGR group placentas.
CONCLUSION: FKBP52 seems to be disrupted in PE and IUGR pregnancies. Decrease of FKBP52 protein levels in PE and increase in IUGR group placentas might have an importance and be involved in the pathogenesis of PE and IUGR. (Anal Quant Cytopathol Histopathol 2015;37:000–000)

Keywords: FK506-binding protein 52, FKBP52, heat shock-binding immunophilin, heat-shock protein, human placenta, intrauterine growth restriction, placenta, preeclampsia.

The placenta is a critical organ affecting the outcome of pregnancy. It regulates many metabolic activities between mother and fetus. Fetal growth is directly related to the placental development. Abnormal placental development, which is among the most important causes of early embryonic death, lies at the core of many common complications of pregnancy, such as preeclampsia (PE) and intrauterine growth restriction (IUGR).[1]

PE is a complex multisystem disorder and complicates 7–10% of all gestations.[2] PE is characterized by high blood pressure and proteinuria in the second half of pregnancy.[3] It presents serious risk of both maternal and fetal morbidity and mortality.[4]

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Preterm delivery, low birth weight, fetal death, and neonatal death due to complications of preterm delivery are common perinatal outcomes associated with PE.[5] Whereas the pathophysiology of PE in particular remains incompletely understood, it appears to result from poor development of placental blood vessels,[6] which may disrupt the normal pattern of blood flow into the intervillous space, potentially risking ischemia/reperfusion injury.[7]

IUGR is defined as fetal weight below the 10th percentile of a given population at the same gestational age.[8,9] Vascular development of the placenta is inadequate in IUGR, resulting in impaired uteroplacental blood flow and poor nutrient and oxygen supply to the fetus.[10] Sustained hypoxemia and undernutrition of the developing fetus can affect fetal programming of vital organs and an increased risk for disease later in life. IUGR due to placental insufficiency occurs in 5–10% of gestations and is a major determinant of perinatal morbidity and mortality.[8,10-12]

52-kDa FK506-binding protein (FKBP52) belongs to a subclass of immunophilin proteins—FK506-binding proteins (FKBPs)—based on its ability to bind the immunosuppressive drug FK506 (Tacrolimus), which is clinically used after organ transplantation. FKBP52 is composed of 4 distinct domains. Domain I facilitates binding to the immunosuppressive drug FK506. The first 2 domains include a functional site for peptidyl-prolyl *cis/trans* isomerase (PPIase) activity and a PPIase-like region. PPIase (also known as rotamase activity) is a chaperoning function that catalyzes the conversion of prolyl-peptide bonds from *trans*- to *cis*-proline, often a rate-limiting step in protein folding.[13] The first 138 amino acids from the N-terminus constitute the PPIase domain of FKBP52. Three tetratricopeptide repeat (TPR) domains occupy the third structural domain, while the fourth C-terminal domain contains a putative binding site for calmodulin. Beyond the TPR domains are 2 putative calmodulin binding sites occupying the C-terminus of FKBP52 (Domain IV). FKBP52 is heat-shock protein 90 (Hsp90) co-chaperone that modifies steroid hormone receptor activity. Despite its PPIase activity, co-chaperone activity by FKBP52 seems to be independent of the protein's PPIase domain[14] and dependent instead on the TPR domains via a mechanism that requires Hsp90 binding.[15] FKBP52 associates with receptor-Hsp90 complexes and has roles in steroid hormone receptor signaling, including receptor maturation, hormone binding, and nuclear translocation.

FKBP52 has been characterized as an important positive regulator of androgen,[16] glucocorticoid,[17] and progesterone[18] receptor signaling pathways, but not the estrogen or mineralocorticoid receptor.[17] In the absence of ligand, steroid receptors remain sequestered in the cytoplasm and/or nucleus in complex with chaperone and co-chaperone proteins including, but not limited to, Hsp90, heat-shock protein 70 (Hsp70), a 23-kDa co-chaperone (p23), and one of a family of proteins characterized by the presence of an Hsp90-binding TPR domain. FKBP52 association with receptor-chaperone complexes results in an enhancement of receptor hormone binding[17,19,20] and influences receptor localization within the cell.[21]

A role for FKBP52 in mammalian reproductive development and success emerged from the development and study of two independently derived *FKBP52*-deficient mouse lines.[16,22] *FKBP52*-deficient male mice display phenotypes consistent with partial androgen insensitivity syndrome including dysgenic seminal vesicles and prostate, ambiguous external genitalia, hypospadias, and nipples retained into adulthood.[16,22] In addition to alterations in primary and accessory sex organs, the epididymis of *FKBP52*-deficient male mice have significantly depressed sperm counts, and the sperm display abnormal morphology.[23]

The loss of FKBP52 in female *FKBP52*-deficient mice results in infertility in both the C57BL6/129 and CD1 backgrounds.[18,24] The mice appear morphologically normal; moreover, ovulation and fertilization are not overtly hindered. Infertility appears to be due to progesterone (P_4) resistance and uterine defects resulting in embryonic implantation and decidualization failure.[24,25] Implantation failure as a result of reduced P_4 signaling may be due to increased uterine oxidative stress (OS) in *FKBP52*-deficient female mice as a result of reduced levels of the antioxidant Peroxiredoxin-6 (Prdx6).[26] Implantation failure was observed in *FKBP52*-deficient, but not wild-type female mice with paraquat-induced oxidative stress.[24] Exogenous P_4 alone did not rescue implantation, but it did with the addition of an antioxidant.

FKBP52 may promote endometriosis because women with endometriosis show reduced FKBP52 expression, and the P_4 resistance observed in *FKBP52*-deficient mice results in increased cell proliferation, inflammation, and angiogenesis, which lead to

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endometriotic lesions.[27] Thus, evidence suggests a crucial role for FKBP52 in female reproduction and uterine signaling.

In addition to the actions of FKBP52, this protein has been shown to regulate microtubule dynamics and transient receptor potential channels. The roles of FKBP52 in control of amyloid beta toxicity and copper homeostasis,[28] modulation of synuclein aggregation,[29] and control of protooncogene RET[30] have been shown.

P_4 binds to progesterone receptor (PR) to activate gene transcripts involved in ovulation, endometrial receptivity, implantation, decidual reaction, and maintenance of pregnancy.[31] Since the success of transcriptional activation depends on the binding of PR to the chaperone FKBP52 in this study, we aimed to detect the immunolocalization and expression levels of FKBP52 in human control, PE, and IUGR term placentas.

Materials and Methods

Human Tissues

Term placentas from healthy women were obtained immediately after cesarean section and were used as the control group (n=6). Control women had no history of pregnancies with PE, recurrent spontaneous abortion (RSA), or IUGR. Placentas from women with PE (n=6) and IUGR (n=6) were obtained immediately after cesarean deliveries. The gestational age of control group placentas ranged from 36–40 weeks. The gestational age of PE and IUGR specimens ranged from 35–40 weeks. The samples were supplied from the Department of Obstetrics and Gynecology, Akdeniz University, Medical Faculty, after informed consent of patients. The Ethical Committees of Medical Faculty of Akdeniz University approved the consent forms and protocols. Preeclamptic cases were defined as persistent blood pressure above 140/90 mmHg and proteinuria of >0.3 g/24 hours or ≥2+ according to a dipstick test, developing after 20 weeks of pregnancy.[32] IUGR was defined as birth weight below the 10th percentile of customized birth weight for gestational age.[8] Exclusion criteria of the study were preexisting hypertension treated with antihypertensive drugs, diabetes mellitus, gestational diabetes, renal disease, heart disease, and fetal anomalies. Only singletons were included in the study.

Tissue Preparation

Healthy preeclamptic and intrauterine growth retarded human placentas were dissected and either fixed for immunohistochemistry or stored in liquid nitrogen for Western blotting. The placentas were fixed in 10% formalin (100 mL 37% formaldehyde, 900 mL distilled water, pH~7) at room temperature for 12–18 hours. Formalin was removed by several washings with tap water. This was followed by dehydration; immersion in 70%, 80%, and 90% ethanol for 24 hours each and 100% ethanol for 4 hours. After dehydration, tissues were cleared in xylene and embedded in paraffin wax.

Immunohistochemistry

Formalin-fixed paraffin-embedded samples were cut into 5-µm sections and placed on slides coated with poly-l-lysine. After deparaffinization, slides were boiled in citrate buffer (pH 6.0) for 4 minutes, 3 times, for antigen retrieval and cooled for 20 minutes at room temperature. Then sections were immersed in 3% hydrogen peroxide for 20 minutes to block endogenous peroxidase. Slides were then incubated in a humidified chamber with UltraV block (Lab Vision, Fremont, California, U.S.A.) for 7 minutes at room temperature. Excess serum was drained and sections were incubated with mouse monoclonal anti-FKBP52 antibody (NB110-96874, Novus Biologicals, Colorado, U.S.A.) at 1:500 dilution for overnight at 4°C in a humidified chamber. Isotype controls were performed by replacing the primary antibody with the nonimmune IgG (sc-2025, Santa Cruz Biotechnologies, Santa Cruz, California, U.S.A.) in the same dilutions as the specific antibodies. The next day the sections were washed for 5 minutes, 3 times with phosphate-buffered saline (PBS) and then incubated with biotinylated secondary antibody (Vector Laboratories, BA-9200, California, U.S.A.) at 1:500 dilution for 40 minutes then with peroxidase labeled streptavidin (TP-125-HL, Thermo Scientific, Waltham, Massachusetts, U.S.A.) for 20 minutes. Then slides were washed for 5 minutes, 3 times, with PBS and the resulting signal was developed with diaminobenzidine (DAB) (K3466, DakoCytomation, Glostrup, Denmark). All samples were treated with the same protocol. Sections were counterstained with hematoxylin, dehydrated, mounted in Kaiser's glycerin gelatin (OB514196, Merck, Whitehouse Station, New Jersey, U.S.A.) and examined by light microscopy (Zeiss, Oberkochen, Germany).

Semi-quantitative Evaluations

The distribution of FKBP52-positive immunoreac-

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tive cells in healthy preeclamptic and intrauterine growth retarded human placentas were determined semiquantitatively: 0=negative, (+)=weak positive, +=positive, ++=strong positive, and +++=very strong positive. After evaluations were done, photographs of sections were taken with a digital camera (Diagnostic Instruments Inc. Spot Insight Qe, Sterling Heights, Michigan, U.S.A.) using a Zeiss Axioplan 100 light microscope (Zeiss, Oberkochen, Germany).

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis and Western Blotting

Protein extraction and immunoblot analysis were performed as described previously.[33] Healthy and pathologic human and rat placenta samples were weighed and put into a homogenization buffer supplemented with Complete R protease inhibitor cocktail (Boehringer, Mannheim, Germany). After homogenization, samples were centrifuged at 10,000 g for 10 minutes. Supernatants were collected and stored at –80°C.

The protein concentration was determined by Lowry assay[34] and 50 mg of protein was applied per lane. Samples were subjected to SDS polyacrylamide gel electrophoresis (30% acrylamide in 7.5% gel) at 100 V for approximately 1.5 hours and were then transferred onto nitrocellulose membranes (Amersham Pharmacia, Piscataway, New Jersey, U.S.A.) in a buffer containing 0.2 mol/L glycine, 25 mmol/L Tris and 20% methanol overnight at +4°C, under 32 V and 65 A. The membranes were blocked for 1 hour with 5% nonfat dry milk (BioRad Laboratories, Hercules, California, U.S.A.) and 0.1% Tween 20 (TA 125 TW, Lab Vision) in 0.14 mol/L Tris-buffered saline (TBS; pH 7.2–7.4) at +4° C. Membranes were incubated with a 1:2,000 dilution of mouse monoclonal anti-FKBP52 antibody (NB110-96874, Novus Biologicals, Colorado, U.S.A.) overnight. After the washing steps the membranes were further incubated with horse anti-mouse IgG horseradish peroxidase conjugate (PI-2000, Vector Laboratories) diluted 1:2,000.

Immunolabeling was visualized by using the chemiluminescence-based Super Signal CL HRP Substrate System (34080, Thermo Scientific) prepared according to the manufacturer's instructions, and the membranes were exposed to Hyperfilm (28906837, Amersham, Bucks, U.K.). Membranes were also labeled by an identical protocol for binding of a 1:2,000 dilution of a mouse monoclonal anti–beta actin antibody (ab 6276, Abcam, Cambridge, U.K.) as an internal control to confirm the equal loading of the samples. The bands were quantified using NIH image analysis software (Image J Version 1.36b, National Institutes of Health, Maryland, U.S.A.).

Statistical Analysis

FKBP52 Western blot band values of control, PE, and IUGR group placentas were done by one-way analysis of variance (ANOVA) tests followed by post hoc Tukey tests. Probability values of <0.05 were considered significant. All statistical analyses were performed using Sigma Stat 3.5 (Statcon, Witzenhausen, Germany).

Results

Immunohistochemical Findings on Human Placentas

Semiquantitative immunolabeling density distribution patterns of FKBP52 on human control, PE, and IUGR group placentas are shown in Table I.

FKBP52 Immunolocalization on Human Control, PE, and IUGR Group Placentas

FKBP52 immunolocalization was both cytoplasmic and nuclear. In PE group placentas, syncytiotrophoblasts, villous stromal cells, and vascular endothelium had weak positive FKBP52 immunoreactivity (Figure 1a). FKBP52 immunoreactivity was not continuous in syncytiotrophoblasts (Figure 1a). There was positive FKBP52 immunostaining in decidua (Figure 1b). There was no reaction in isotype control group (Figure 1c). In control group placentas FKBP52 immunoreactivity was stronger as compared to that of the PE group. Positive FKBP52

Table I *Semi-quantitative Scoring of FKBP52 Immunolabeling Intensities in Human Control, PE, and IUGR Group Placentas*

	Decidua	Syncytiotrophoblast	Villous stromal cells	Vascular endothelium
PE	+	(+)	(+)	(+)
Control	++	+	+	+
IUGR	++	+++	++	++

0 = negative, (+) = weak positive, + = positive, ++ = strong positive, +++ = very strong positive.

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immunoreactivity was seen on syncytiotrophoblasts (Figure 1d); immunoreactivity was not continuous as in the PE group. Villous stromal cells and vascular endothelium also had positive FKBP52 immunostaining (Figure 1d). Decidua had strong positive FKBP52 immunoreactivity (Figure 1e). No reaction was seen in isotype control group (Figure 1f). In comparison to control group placentas there was stronger FKBP52 immunoreactivity in IUGR group placentas. Very strong positive FKBP52 immunostaining was seen on syncytiotrophoblast. Different from control and PE groups, FKBP52 immunostaining was continuous in IUGR group placentas. Villous stromal cells of vascular endothelium had strong positive (Figure 1g), syncytiotrophoblast had very strong positive (Figure 1h), and decidua had strong positive (Figure 1h) FKBP52 immunoreactivity. Since there was no reaction in isotype control, we were sure that FKBP52 immunoreactivity was specific (Figure 1i).

FKBP52 Western Blotting on Human Control, PE, and IUGR Group Placentas

FKBP52 Western blot results were compatible with FKBP52 immunohistochemical results. FKBP52 protein levels were lower in PE group placentas as compared to control group placentas, but the difference was not statistically significant

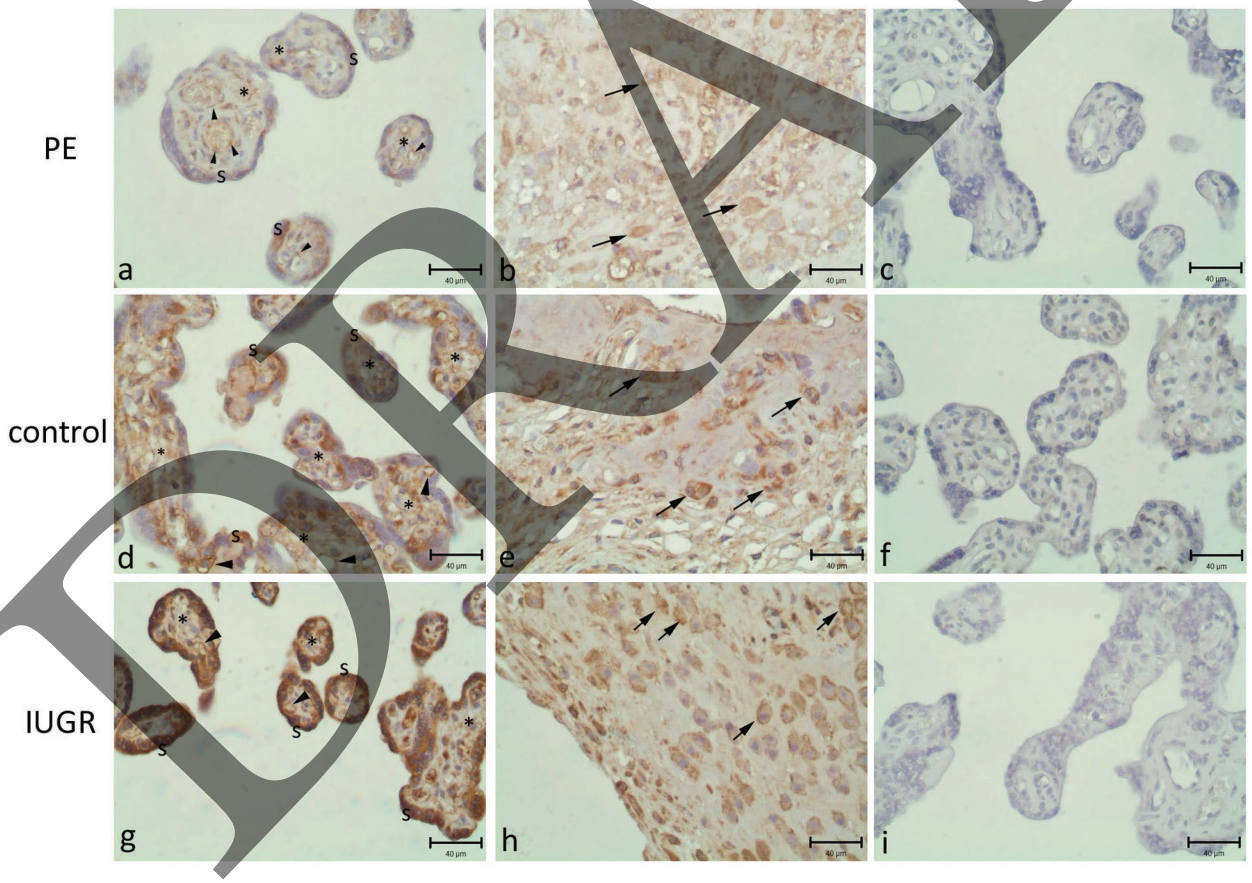


Figure 1 FKBP52 immunostaining in human PE (a, b, c), control (d, e, f), and IUGR (g, h, i) group placentas. In PE group placentas FKBP52 immunoreaction was seen in vascular syncytiotrophoblast (s), endothelium (arrow head), villous stromal cells (asterisks) (a), and decidua (arrows) (b). Isotype control is seen in (c). FKBP52 immunoreaction was stronger in syncytiotrophoblast (s), vascular endothelium (arrow head), villous stromal cells (asterisks) (d), and decidua (arrow) (e) in control group placentas as compared to PE group placentas. Isotype control is seen in (f). In the IUGR group FKBP52 immunoreactivity was stronger as compared to both control and PE group placentas: syncytiotrophoblast (s), vascular endothelium (arrow head), villous stromal cells (asterisks) (g), and decidua (arrows) (h). Isotype control is seen in (i).

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(p=0.089). FKBP52 expression was increased in IUGR group placentas as compared to control group placentas, and it was statistically significant (p=0.042). FKBP52 protein level difference between PE and IUGR group placentas was also statistically significant (p=0.008) (Figure 2).

Discussion

Adequate placental growth and function are fundamental to the well-being, growth, and development of the fetus throughout gestation.[35] PE refers to vascular alterations characterized by maternal hypertension and proteinuria.[36] PE is a cause of maternal mortality and one of the main causes of perinatal mortality and neurological sequelae as well as prematurity.[37-39] PE is characterized by poor perfusion of maternal and fetal circulations of the placenta, which thus affects placental growth and development.[6,38,39]

IUGR is defined as the inability of the fetus to reach its potential intrauterine growth and clinically defined as the estimated fetal weight under 10th percentile.[37] Epidemiological data show that diabetes, hyperlipidemia, hypertensive disease, and coronary vascular disease are more common among adults who were smaller than normal at birth and experienced IUGR.[40] An elevated oxidative stress level and reduced antioxidant activity has been reported in placentas from pregnancies with IUGR as compared with normal pregnancies.[37] An oxidative stress stage is closely associated with placental dysfunction in IUGR by still not very well characterized mechanisms involving reactive oxygen species (ROS).[37,39]

FKBP52 gene deletion in both male and female mice causes infertility,[16] which indicates the importance of FKBP52 immunophilin in reproduction. FKBP52 associates with steroid-hormone receptor–chaperone complexes to regulate hormone binding and is critical in receptor translocation to the nucleus and regulates nuclear receptors.[41]

As far as we know, no study has examined the expression of FKBP52 in human PE and IUGR group placentas. This study demonstrates the expression of immunophilin FKBP52 protein in human term placentas that were healthy but complicated by PE and IUGR. We found that in the PE group placentas FKBP52 expression level was lower as compared to control and IUGR group placentas. Since PE appears to result from poor development of placental blood vessels,[6] downregulation of FKBP52 in PE placentas might have led to impaired proliferation and invasion of cytotrophoblasts. According to our results FKBP52 expression was upregulated in IUGR group placentas as compared to control and PE group placentas. Increase of FKBP52 expression in IUGR group placentas might be an adaptation to maintain pregnancy. In our control and PE group placenta samples there was FKBP52 expression on some regions of syncytiotrophoblast, but in IUGR group placentas FKBP52 expression was continuous on syncytiotrophoblast. A different expression pattern of FKBP52 on syncytiotrophoblastin IUGR group placentas might have importance.

P_4 has a key function in female pregnancy. P_4 is involved in maintenance of pregnancy. The success of transcriptional activation depends on the binding of PR to the chaperone FKBP52. The role of the placenta in progestogen production varies by species. In the sheep, horse, and human, the placenta takes over the majority of progestogen production, whereas in other species the corpus luteum remains

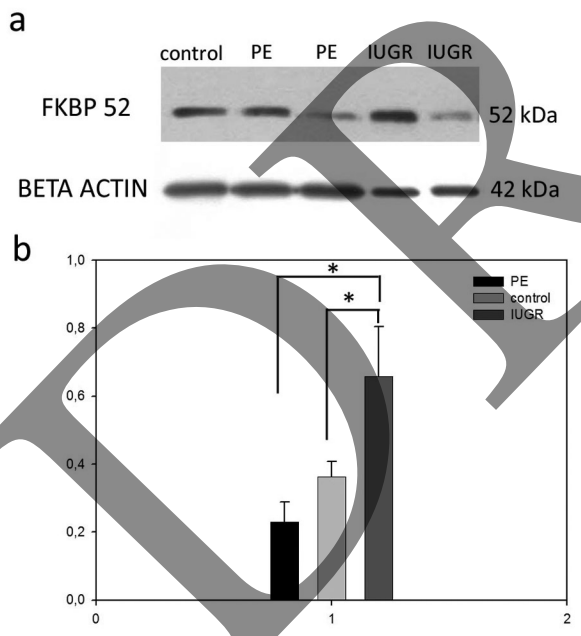


Figure 2 (a) Western blotting results of FKBP52 in human control, PE, and IUGR group placentas. Beta-actin was used as an internal control. (b) Quantitative analysis of FKBP52 Western blotting data. The values in the bar graphs were obtained by dividing the protein intensity value by its corresponding beta-actin intensity value. The differences seen between control and IUGR group placentas and PE and IUGR placentas were statistically significant (*). The image is representative of 3 independent experiments.

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the primary source of progestogens. In the sheep and human, P_4 is the major placental progestogen. Zamudio et al[42] searched for P_4 serum levels in PE women and they found that PE women had higher P_4 concentrations throughout pregnancy. Similarly, Salas et al[43] studied serum concentrations of P_4 in women with PE and IUGR. P_4 concentrations were higher in PE than in either control or IUGR from weeks 18–21 until term. In contrast, IUGR pregnancies had reduced P_4 and E_2 concentrations after week 34. They speculated that the early rise in P_4 may have a pathogenic role in the development of PE.[43] According to these studies women with PE have higher and women with IUGR have lower P_4 levels in their serum. According to our study women with PE have lower and women with IUGR have higher FKBP52 levels in their placenta. This contrast between P_4 and FKBP52 levels might be an adaptation of PE or IUGR placentas to compensate for increase or decrease of P_4, respectively.

When we searched the literature, there was only one study done on placenta/chorionic villi that searched for FKBP52 expression. Chen et al[44] searched for FKBP52 expression in chorionic villi samples of recurrent spontaneous abortion (RSA) cases via semiquantitative reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry. They showed decrease of FKBP52 expression in chorionic villi of RSA samples as compared to the control ones with a statistically significant difference. They concluded that low FKBP52 expression in pregnant women may lead to abortion by affecting P_4 and PR functions. Hence, the expression of FKBP52 may play a key role in early pregnancy maintenance; the low expression of FKBP52 may be involved in the pathogenesis of RSA.

In another study Wang et al[45] determined the relationship between expression of FKBP52 and ultrasonic evaluation of endometrial receptivity. They investigated the mRNA and protein expression of FKBP52 in different pattern endometria classified according to ultrasonic evaluation. The mRNA and protein expression of FKBP52 differed significantly between Patterns A and C endometrium. The protein expression of FKBP52 was higher in Pattern A endometrium than in Pattern C endometrium. This finding indicated that FKBP52 may play an important role in classifying the endometrium of various ultrasonic patterns. Increase of FKBP52 expression in Pattern SC endometrium, which is generated by local injury, suggests that FKBP52 protein may play a role in improving the receptivity of the endometrium. According to this study FKBP52 expression differs between endometria of women and it determines whether the uterus is receptive. We found that FKBP52 expression shows the difference between healthy, PE, and IUGR placentas. In this manner the question comes to mind: could determination of FKBP52 expression in endometria of women be used as a marker before PE or IUGR develops?

In conclusion, our study analyzed the expression levels of FKBP52 in placentas of women with PE and IUGR, which may provide additional information regarding the causes of PE and IUGR. We consider that the abnormal expression of FKBP52 in placentas of women with PE and IUGR might have a role in the pathophysiology of these syndromes via affecting FKBP52-mediated P_4 functions. However, further studies are required to characterize the various functions of FKBP52 during pregnancy.

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