Dishevelled family proteins (DVL1-3) expression in IUGR placentas

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ABSTRACT

Dishevelled family proteins (DVL1, DVL2, and DVL3) are cytoplasmic proteins that are involved in canonical and non-canonical Wnt signaling pathway during embryonic development. The role of DVL proteins in the placental tissue remains mostly unknown. In the current study, we explored the role of Dishevelled proteins in naturally invasive tissue, trophoblast. Formalin-fixed paraffin-embedded samples of 15 term placentas from physiologic term pregnancies and 15 term placentas from pregnancies complicated with intrauterine growth restrictions (IUGR) were used for the study. Expression levels of mRNA for DVL1, DVL2, and DVL3 in placentas were analyzed by quantitative real-time PCR (qRT-PCR). DVL1, DVL2, and DVL3 protein expression were semi-quantitatively analyzed using immunohistochemistry. The expression of DVL3 protein was significantly higher in trophoblasts and endothelial cells in placental villi from IUGR pregnancies compared with the control group of term placentas, while DVL2 protein expression was significantly higher in trophoblasts in placental villi from IUGR pregnancies compared with normal term placentas. The observed differences at protein levels between normal and IUGR placentas were not confirmed at the mRNA levels of DVL genes. Our data indicate the active involvement of DVL proteins in IUGR-related placentas. No significant changes were observed in DVL mRNA levels between the two groups of placentas. Further studies are required to explore the clinical relevance of these observations.

KEYWORDS: Intrauterine growth restriction; placenta; Dishevelled family proteins; DVL1; DVL2; DVL3

INTRODUCTION

The invasion into the endometrium and remodeling of the mother’s spiral arteries are essential steps for the proper functions of the placenta during pregnancy [1]. Trophoblast invasion is a spatially and temporally precisely regulated process so that it does not end up too shallow, as is the case in preeclampsia and in intrauterine growth restriction (IUGR), or too deep, as it occurs in placenta percreta and placenta accreta.

IUGR is defined as a condition in which the fetus cannot achieve his full, genetically determined growth potential [2], thus causing long- and short-term morbidity and mortality [3]. De Jesus et al found an almost 4-fold higher risk of neurodevelopmental impairment or neonatal death and 2.6-fold increased risk of cerebral palsy in IUGR neonates born before 27 weeks’ gestation compared with age-matched non-small for gestational age neonates [4].

Wnt signaling pathway is, along with Hippo, Hedgehog, Notch, and TGF-β pathways, one of the evolutionarily preserved pathways that are essential for embryonic development, control of early axis formation and organogenesis [5,6] by enabling cell differentiation, proliferation, migration, polarity, and survival [7].

Wnt intracellular signaling transmission and interpretation are mediated by a family of cytoplasmic proteins called Dishevelled (DVL), human homologs of the Drosophila Dishevelled gene (dsh) – DVL 1, DVL 2, and DVL 3, through secreted frizzled-related proteins (SFRP) at the cell membrane [7,8].

We have previously shown increased activation of the SFRP family members, inhibitors of the Wnt signaling pathway, in pathological placentas from pregnancies with IUGR [9]. In addition, it has been found that mice with hypermethylated Wnt2 gene promoter have a lower birth weight (they are small for gestational age, SGA) [10]. In contrast, another gene,
Wnt/β-catenin, turns out to be necessary for the fusion of chorion and allantois in mice [11].

In the current study, we explored the status of DVL1-3 in a naturally invasive placental tissue using quantitative real-time PCR (qRT-PCR) and immunohistochemistry. Our goal was to understand the patterns of DVL protein expression in IUGR placentas and compare it with placentas from physiological pregnancies.

MATERIALS AND METHODS

Materials

The samples used in the study were a part of a collection of placental tissue samples belonging to the University of Zagreb School of Medicine and had been collected in collaboration with the University Hospital “Merkur” Zagreb, both of which are parts of the Scientific Center of Excellence for Reproductive and Regenerative Medicine. This study was approved by the Ethical Committees of the School of Medicine, University of Zagreb and the University Hospital “Merkur” and was performed according to ethical standards of the Declaration of Helsinki.

In the examination of placentation, a control group consisted of formalin-fixed paraffin-embedded (FFPE) tissue samples of 15 placentas, obtained from complication-free pregnancies, physiological singleton, and delivered at term (between 38 and 42 weeks of gestation) of a newborn with normal body weight (between 10th and 90th percentile for gestational age, newborn sex, and mother’s parity). The experimental group consisted of 15 term placentas from pathological pregnancies with fetal growth restriction (FRG, IUGR) observed on serial ultrasound (at least twice), with the assessment of the body weight below 10th percentile for the duration of pregnancy, fetal sex, mother’s parity, and confirmed at birth by measuring newborn body weight. Only pathological pregnancies with IUGR were included, and exclusion criteria for both pathological pregnancies and controls were as follows: Multiple pregnancies, tobacco and drug use, intrauterine viral infections (TORCH and Parvovirus B19), chorioamnionitis, hypertension, preeclampsia, respiratory distress syndrome (A.S., S.V.). The tissue compartments (trophoblasts, stromal cells, and endothelial cells) were scored as follows: 0 if no staining was observed; 1 if <10% cells were stained; 2 if 10-50% cells were stained; and 3 if >50% cells were stained [12]. All the discordant result was resolved at the double-headed microscope evaluation.

Quantitative analysis of DVL1-3 protein expression

The expression of DVL1, DVL2, and DVL3 in placentas was independently assessed by two board-certified pathologists (A.S., S.V.). The tissue components of trophoblasts, stromal cells, and endothelial cells were scored as follows: 0 if no staining was observed; 1 if <10% cells were stained; 2 if 10-50% cells were stained; and 3 if >50% cells were stained [12]. All the discordant result was resolved at the double-headed microscope evaluation.

RNA extraction, reverse transcription, and qRT-PCR

Total RNA was isolated from 5 × 5 μm sections of IUGR (n = 15) and control placental (n= 15) FFPE tissue blocks. Shortly, all samples were deparaffinized using 1.0 mL xylene (Invitrogen, UK), followed by incubation for 3 min at 50°C and centrifugation for 5 minutes at maximum speed. The supernatant was then discarded, and the pellet was washed twice with 1.0 mL absolute ethanol. The samples were incubated with 300μL of protease K digestion buffer (20 mM Tris-HCl [pH 8.0]; 1 mM CaCl₂; 0.5% sodium dodecyl sulfate and 500 μg/ml protease K) for 3h at 55°C. Subsequently, RNA was isolated with TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer’s instructions. RNA purity and concentration were evaluated by NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, United States). Two micrograms of total RNA from each sample were reverse transcribed using the high capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific, United States), following the manufacturer’s protocol. DVL1, DVL2, and DVL3 gene expression were quantified using the CFX-96 real-time PCR detection system using a C1000 thermal cycler (Bio-Rad Laboratories, United States). All qPCR reactions were performed in triplicates in the presence of the TB Green™ Premix Ex Taq™ II (Tli RNaseH Plus PCR master mix, Takara
Biotechnology Co., Ltd.) under the following thermocycler conditions: Stage 1: 95°C for 30 s, one cycle; stage 2: 95°C for 5 s and 60°C for 30 s, 40 cycles. The CFx96 manager software was used to generate the cycle threshold (CT) values, and the data were analyzed by the 2-ΔΔCT method. The relative expression of targeted DVL1-3 genes was normalized against the β-actin (ACTB) gene that served as an endogenous control [12]. The specificity of qPCR amplification was confirmed using melt curve analysis. The sequence of used oligonucleotide primers were as follows: DVL1 FW: 5’-CCCCTCTTCCACCCAAATG-3’, RW: 5’-GTGACATGCACACGGACTCGGACCG-3’ (Accession: NM_001330311.2); DVL2 FW: 5’-TGAGCACAAGCTGACGCTGG-3’, RW: 5’-GCAGGTCATTGCGCTGGA-3’ (Accession: NM_004423.4); ACTB FW: 5’-GGCATGGGTACCTGGCA-3’, RW: 5’-AGCTCCGATGGGTTATCAGCAC-3’ (Accession: NM_004423.4); DVL3 FW: 5’-ACAATGCAAGCTACCATGCTTC-3’, RW: 5’-AGTTGGTGACGATGCCGTG-3’ (Accession: NM_001101) [12].

Statistical analysis

The Kolmogorov–Smirnov test and Shapiro–Wilk W-test assessed the distribution of the data. Student’s t-test analyzed the clinical data of normal and IUGR pregnancies. A Mann–Whitney test was used for comparison among placental expression of DVL1, DVL2, and DVL3 proteins, with significant differences accepted at a probability value of p < 0.05. The data were analyzed using the GraphPad Prism 5.01 program (GraphPad Software, Inc., San Diego, CA, USA) and IBM SPSS Statistics, Version 21.

RESULTS

Clinical data – normal and IUGR pregnancies

Thirty term placentas were studied, of which 15 were from pregnancies with IUGR and 15 serving as controls, were from healthy pregnancies. The following clinical variables were analyzed: Age, blood pressure, body weight and height of pregnant women, body weight gain, and body mass index before gestation and at time of delivery, fetal body weight and height, placental weight, and fetal/placental weight ratio (Table 1). As expected, a statistically smaller fetal weight and height and placental weight were found in newborns from IUGR pregnancies (p < 0.0001). The mean age of pregnant women with IUGR was 32 years compared with healthy controls (28 years; p = 0.073).

Expression of Dishevelled family proteins in IUGR placentas

Expression of all three Dishevelled proteins was detected in the cytoplasm of both trophoblasts and stromal cells of the placentas. DVL1, DVL2, and DVL3 were expressed in >10% of trophoblast cells in 100% and 66.7% of samples, respectively, while in normal placentas, DVL2 and DVL3 were expressed in <10% of epithelial cells in 26.6% and 66.7% of samples, respectively (Table 1). The qRT-PCR analysis results showed that all three human DVL gene homologs were transcriptionally active in both IUGR and healthy control tissue samples (Figure 1G and H). The relative mRNA expression levels of DVL1 and DVL2 were 1.28 fold and 1.44 fold higher in the IUGR than the control tissue samples. In contrast, DVL3 transcripts were reduced in IUGR samples compared with the control placental tissues (Figure 1G). However, none of the observed differences in mRNA expression levels was statistically significant. Regarding the mRNA expression levels of DVL3 homologs, in IUGR tissue samples analyzed as a separate group, the DVL2 gene showed the highest transcriptional activity. In contrast, the expression levels of DVL2 and DVL3 in the control tissue group were almost identical (Figure 1H).

DISCUSSION

This study aimed to determine the expression of Dishevelled proteins and their mRNA in healthy placentas and placentas with intrauterine growth restriction (IUGR). Since the placenta needs to continually adapt during its development to the demands placed on it by the growing embryo/
**TABLE 1.** Clinical parameters of mothers and children from normal (control) and pathologic (intrauterine growth restriction – IUGR) term pregnancies

| Parameter                                      | Control (n=15) | IUGR (n=15) | p-value |
|------------------------------------------------|----------------|-------------|---------|
| Maternal age (year)                            | 28.6±3.60*     | 32.1±6.41   | 0.073   |
| Gestational age at delivery (week)             | 39±3/7–40±3/7  | 37±2/7–41±5/7 |        |
| Pre-pregnancy body mass index (BMI)            | 20.97±2.25     | 20.67±2.42  | 0.279   |
| Body mass index at delivery (BMI)              | 26.61±2.65     | 25.72±3.10  | 0.407   |
| Systolic blood pressure (mmHg)                 | 118.3±9.19     | 120.9±6.25**| 0.431   |
| Diastolic blood pressure (mmHg)                | 73.3±4.87      | 75±5**      | 0.403   |
| Maternal body height (cm)                      | 167.6±5.65     | 167.4±6.45  | 0.929   |
| Maternal body weight at delivery (kg)          | 74.7±8.12      | 72.6±11.2   | 0.496   |
| Total weight gain during pregnancy (kg)        | 15.86±3.09     | 14.26±5.56  | 0.328   |
| Fetal birth weight (g)                         | 3562±287.75    | 2422±866±216.51 | <0.0001***|
| Fetal height (cm)                              | 51.3±1.44      | 46.4±1.92   | <0.0001***|
| Placental weight (g)                           | 560±75.78      | 384±87.59   | <0.0001***|
| Fetal placental weight ratio                   | 6.46±0.91      | 6.5±1.28    | 0.820   |
| Gender                                         |                |             |         |
| Male                                           | 11             | 6           |         |
| Female                                         | 4              | 9           |         |
| Mode of delivery                               |                |             |         |
| Cesarean section                               | 0              | 6           |         |
| Vaginal delivery                               | 15             | 9           |         |

*Mean±SD, **Eleven IUGR placentas were used in data analysis, ***Significantly different variables

**FIGURE 1.** DVL1, DVL2, and DVL3 protein expression in human term placentas from normal pregnancies – control (A–C) and human term placentas from intrauterine growth restriction pregnancies (D–F). Relative versus normalized expression values of DVL1, DVL2, and DVL3 mRNA in IUGR versus control placental tissue (G) and their expression inside the individual tissue sample group (H).
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fetus, the cells that contribute most to this adaptability, the trophoblast cells, must be under constant control a series of molecular mechanisms [13]. One such mechanism is the Wnt signaling pathway, whose central mediators are the DVL proteins.

One of the critical events during placentation is the epithelial-mesenchymal transition (EMT), which allows interstitial and endovascular invasion of the trophoblast cells. The reverse process, the mesenchymal-epithelial transition, contributes to stopping the invasion [14]. The timely progress of these processes is a prerequisite for successful placentation and, consequently, a healthy child’s birth. It has been previously demonstrated that unsuccessful trophoblast invasion occurs in placentas associated with fetal IUGR and hypertensive disorders [15,16].

Wnt signaling promotes cell motility and invasion [17]. SFRP proteins that represent the Wnt pathway’s essential inhibitors are more pronounced in IUGR placentas than in normal pregnancies, suggesting that the placental invasion regulation is one of the crucial factors during pregnancy [18].

Based on the current understanding, we hypothesized that in tissues with less prominent trophoblast invasiveness, that is, those resulting from a defective invasion of the decidua or placental bed, signal transmission through the Wnt/DVL signaling pathway will be impaired because of the diminished expression of DVL proteins. Surprisingly, we found a significant increase in the expression of DVL2 and DVL3 proteins in trophoblasts in placental villi from IUGR placentas, compared with the control term placentas and higher DVL3 protein expression in endothelial cells of placental villi from IUGR placentas, compared with the normal placentas. Intense staining of DVL3 protein was present in stem villous stroma in the IUGR placentas. These results are not in line with those where the expression of positive regulators of trophoblast invasion

| TABLE 2. Expression of DVL1, DVL2, and DVL3 proteins in term placentas from normal pregnancies and term placentas from IUGR pregnancies |
|---------------------------------------------------------------|
|                                                                 |
|                   | DVL1 expression | DVL2 expression | DVL3 expression |
|                   | >50% | 10-50% | <10% | 0% | >50% | 10-50% | <10% | 0% | >50% | 10-50% | <10% | 0% |
| Normal placentas (15 samples) |
| Trophoblasts | 3 (20) | 5 (33.3) | 3 (20) | 4 (26.7) | 6 (40) | 5 (33.4) | 2 (13.3) | 2 (13.3) | - | 5 (33.3) | - | 10 (66.7) |
| Stromal cells | 6 (40) | 8 (53.3) | 1 (6.7) | 5 (33.3) | 9 (60) | - | 1 (6.7) | - | 2 (13.3) | 12 (80) | 1 (6.7%) | - |
| Endothelial cells | 4 (26.7) | 3 (20) | - | 8 (53.3) | 1 (6.7) | 4 (26.7) | - | 10 (66.6) | - | 3 (20) | - | 12 (80) |
| IUGR placentas (15 samples) |
| Trophoblasts | 5 (33.3) | 3 (20) | 4 (26.7) | 3 (20) | 12 (80) | 3 (20) | - | - | 4 (26.7) | 6 (40) | - | 5 (33.3) |
| Stromal cells | 5 (33.3) | 9 (60) | - | 1 (6.7) | 9 (60) | 5 (33.3) | 1 (6.7) | - | 4 (26.7) | 11 (73.3) | - | - |
| Endothelial cells | 4 (26.7) | 4 (26.7) | 1 (6.6) | 6 (40) | 3 (20) | 5 (33.3) | - | 7 (46.7) | 2 (13.3) | 7 (46.7) | - | 6 (40) |

FIGURE 2. DVL3 protein expression in endothelial cells (arrows) in placental villi from normal (control) and intrauterine growth restriction pregnancies (A and C), and DVL3 protein expression in stem (arrows) and terminal villous stroma in the normal (control) and intrauterine growth restriction placentas (B and D).
(e.g., SNAIL protein) is decreased [19] and those where the expression of negative regulators (e.g., ELF5) is increased [20]. We also observed the discrepancies between protein and mRNA levels of all three DVLs. The discrepancy in expression levels of mRNA for DVL1, DVL2, and DVL3 genes and their protein expression may be due to the whole tissue sections analyzed by qRT-PCR. Simultaneously, immunohistochemical analysis enabled protein expression analysis in specified tissue compartments (trophoblasts vs. endothelial cells of placental villi) of IUGR and normal placental tissues.

Karimu and Burton previously showed that the pressure increased in the intervillous space reduced the fetal perfusion and increased the fetoplacental impedance by reducing fetoplacental capillaries width [21]. This mechanism has been proposed as a significant underlying cause of the IUGR. Several factors may affect the fetoplacental vasculature, including endocrine, metabolic, environmental, and oxygen factors [22]. Since a deficiency of oxygen in the tissue can be present in IUGR placentas, a lack of oxygen can stimulate capillaries’ growth to improve tissue oxygenation. Wnt signaling pathway induces vascular endothelial growth factor (VEGF) activation, one of the growth factors that drive angiogenesis [23]. This is in concordance with our results, which showed that increased expression of DVL3 protein in endothelial cells of placental villi from IUGR placentas might be associated with induced angiogenesis. During placental development, chorionic villi undergo developmental, transformative, regenerative, and reparative changes to establish a normal fetal blood flow. These changes can be observed in trophoblast cells and chorionic villi stroma because it is involved in the respective processes [24]. In IUGR placentas, these processes may be induced by unfavorable conditions (e.g., hypoxia), resulting in increased trophoblast cells and stroma in the chorionic villi. This is in line with our study that revealed a significant increase in DVL2 and DVL3 proteins’ expression in the trophoblast in placental villi from IUGR placentas and intense staining of DVL3 protein in stem villous stroma from IUGR placentas.

Steroids (estrogens and androgens) are actively involved in placental functions and pathologies [25,26]. It has been shown that DVL1 and DVL3 proteins could regulate placental aromatase expression and the aromatase gene transcription [27] that regulate human trophoblast differentiation [28]. Aromatase is an enzyme that converts androgens into estrogens, while estrogens promote cell proliferation and growth [24]. Besides, estrogens formed by placental aromatase may enhance placental angiogenesis [29]. These findings further support our view that overexpression of DVL3 protein in IUGR placentas might be associated with induced angiogenesis and increased activity of trophoblasts and stroma in the chorionic villi.

In conclusion, our study demonstrated the active and potentially complex roles that DVL proteins may play in IUGR-related pregnancies’ pathogenesis. Further studies should elucidate the clinical relevance of the observed DVL alterations.

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REFERENCES

[1] Serman A, Serman L. Development of placenta in a rodent--model for human placentation. Front Biosci 2011;3:233-9. https://doi.org/10.2741/e238.
[2] Figuera F, Gratacos E. Update on the diagnosis and classification of fetal growth restriction and proposal of a stage-based management protocol. Fetal Diagn Ther 2014;36(2):86-98. https://doi.org/10.1159/000357592.
[3] Resnik R. Intrauterine growth restriction. Obstetr Gynecol 2002;99(1):390-6. https://doi.org/10.1016/s0029-7844(01)01780-x.
[4] De Jesus LC, Pappas A, Shankaran S, Li L, Das A, Bell EF, et al. Outcomes of small for gestational age infants born at <27 weeks' gestation. J Pediatr 2013;163(1):55-60.e1-3. https://doi.org/10.1016/j.jpeds.2012.12.097.
[5] Logan CY, Nourse R. The Wnt signaling pathway in development and disease. Ann Rev Cell Dev Biol 2004;20:781-810. https://doi.org/10.1146/annurev.cellbio.20.010403.111216.
[6] Clevers H. Wnt/beta-catenin signaling in development and disease. Cell 2006;127(3):469-80. https://doi.org/10.1016/j.cell.2006.10.018.
[7] Sharma M, Castro-Piedras I, Simmons GE Jr, Pruitt K. Dishevelled: A masterful conductor of complex Wnt signals. Cell Sign 2018;47:52-64. https://doi.org/10.1016/j.cellsig.2018.03.004.
[8] Mlozdziak M. The dishevelled protein family: Still rather a mystery after over 20 years of molecular studies. Curr Top Dev Biol 2016;117:75-91. https://doi.org/10.1016/bs.ctdb.2015.11.027.
[9] Zmijanac Partl J, Karin V, Skrtic A, Nikuseva-Martic T, Serman A, Milnaric J, et al. Negative regulators of Wnt signaling pathway SFRPs and SFRP3 expression in preterm and term pathologic placentas. J Mater Fet Neonat Med 2018;31(22):2971-9. https://doi.org/10.1080/14767058.2017.1359830.
[10] Ferreira JC, Choufani S, Grafodatskaya D, Butcher DT, Zhao C, Chitayat D, et al. WNT2 promoter methylation in human placenta is associated with low birthweight percentile in the neonate. Epigenetics 2011;6(4):440-9. https://doi.org/10.4161/epi.6.4.14554.
[11] Parr BA, Cornish VA, Cybulsky MI, McMahon AP. Wnt/β-catenin regulates placentental development in mice. Dev Biol 2001;237(2):324-32. https://doi.org/10.1006/dbio.2001.0373.
[12] Karin-Kujundzic V, Kardum V, Sola IM, Paic F, Skrtic A, Skenderi F, et al. Dishevelled family proteins in normal and pathological placentas: A clinicopathologic and molecular study. APMIS 2021;129(3):201-10. https://doi.org/10.1111/apm.13012.

[13] Vukasovic A, Grbasa D, Nikuseva Martic T, Kusic V, Miskovic B, Serman A, et al. Glycosylation pattern and αααα expression in normal and IUGR placentae. J Mater Fetal Neonat Med 2015;28(5):558-63. https://doi.org/10.3109/14767058.2014.926216.

[14] Kokkinos M, Murtti P, Wafi R, Thompson EW, Newgreen DE. Caderhins in the human placenta–epithelial-mesenchymal transition (EMT) and placental development. Placenta 2010;31(9):747-55. https://doi.org/10.1016/j.placenta.2010.06.017.

[15] Pijnenborg R, Anthony J, Davey DA, Rees A, Tiltman A, Vercruysse L, et al. Placental bed spiral arteries in the hypertensive disorders of pregnancy. Br J Obstetr Gynaecol 1994;101(7):648-55. https://doi.org/10.1111/j.1471-0528.1994.tb13450.x.

[16] Zhou Y, Damsky CH, Fisher SJ. Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome? J Clin Investig 1997;99(9):2152-64. https://doi.org/10.1172/jci119388.

[17] Sedgwick AE, D'Souza-Schorey C. Wnt signaling in cell motility and invasion: Drawing parallels between development and cancer. Cancers 2016;8(9):80. https://doi.org/10.3390/cancers8090080.

[18] Partl JZ, Fabijanovic D, Skrtic A, Vranic S, Martic TN, Serman A, et al. Immunohistochemical expression of SFRP1 and SFRP3 proteins in normal and malignant reproductive tissues of rats and humans. Appl Immunohistochem Mol Morphol 2014;22(6):681-7. https://doi.org/10.1097/PAI.000000000000019.

[19] Fan M, Xu Y, Hong F, Gao X, Xin G, Hong H, et al. Rac1/β-catenin signalling pathway contributes to trophoblast cell invasion by targeting snail and MMP9. Cell Physiol Biochem 2016;38(4):1319-32. https://doi.org/10.1007/pai.000000000000019.

[20] Jarkovíc I, Gecské I, Skrtic A, Zmijanac Partl J, Nikuseva Martic T, Serman A, et al. Elf3 transcription factor expression during gestation in humans and rats—an immunohistochemical analysis. J Matern Fetal Neonat Med 2017;30(11):1261-6. https://doi.org/10.1080/14767058.2016.1210596.

[21] Karimu AL, Burton GI. The effects of maternal vascular pressure on the dimensions of the placental capillaries. Br J Obstetr Gynaecol 1994;101(1):57-63. https://doi.org/10.1111/j.1471-0528.1994.tb3011.x.

[22] Burton GI, Jauiaux E. Pathophysiology of placental-derived fetal growth restriction. Am J Obstetr Gynecol 2008;208(5):S745-6. https://doi.org/10.1016/j.ajog.2007.11.577.

[23] Zhang C, Tanous E, Zheng J. Oxidative stress upregulates Wnt signaling in human retinal microvascular endothelial cells through activation of disheveled. J Cell Biochem 2019;120(8):14044-54. https://doi.org/10.1002/jcb.28679.

[24] Benirschke KB, Baergen RN. Pathology of the Human Placenta. 6th ed. Berlin, Germany: Springer-Verlag; 2012.

[25] Kumar S, Gordon GI, Abbott DH, Mishra JS. Androgens in maternal vascular and placental function: Implications for preeclampsia pathogenesis. Reproduction 2018;156(6):R155-67. https://doi.org/10.1530/rp-18-0278.

[26] Modh Kamal DA, Ibrahim SF, Mokhtar MH. Androgen effect on connexin expression in the mammalian female reproductive system: A systematic review. Rosn J Basic Med Sci 2020;20(3):293-302. https://doi.org/10.17705/ijbms.2019.4501.

[27] Castro-Piedras I, Sharma M, den Bakker M, Molehin D, Martinez EG, Vartak D, et al. DVL1 and DVL3 differentially localize to CYP19A1 promoters and regulate aromatase mRNA in breast cancer cells. Oncotarget 2018;9(86):35699-35714. https://doi.org/10.18632/oncotarget.26257.

[28] Muralimohanaran S, Kwak YT, Mendelson CR. Redox-sensitive transcription factor NRF2 enhances trophoblast differentiation via induction of miR-1246 and aromatase. Endocrinology 2018;159(5):2022-33. https://doi.org/10.1210/en.2017-03024.

[29] Albrecht ED, Pepe GJ. Estrogen regulation of placental angiogenesis and fetal ovarian development during primate pregnancy. Intl J Dev Biol 2010;54(2-3):397-408. https://doi.org/10.1387/ijdb.082758ea.