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3Ds: decorin, discordance, and diamniotic dichorionic twins

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Summary

Investigation: The cause of discordance in dichorionic diamniotic (DD) twins is still unknown. The authors aimed to compare decorin (DCN) and oxidative/antioxidative state levels between the placentas of discordant and concordant twins. *Materials and Methods:* Prospective study of 43 spontaneous DD twin pregnancies included and placentas samples taken from each twin and prepared for homogenization. Total oxidant/antioxidant status levels in placental tissue were determined by automated colorimetric method. Decorin levels were detected by using ELISA method; 23 of these were discordant and 20 of them were concordant. *Results:* DCN levels in the placentas of the low birth-weight twins were significantly lower than the levels of the placentas of appropriate gestational age twins ($p = 0.006$). There were no statistically significant differences in total antioxidant status (TAS), total oxidant status (TOS), or arylesterase (ARES) levels in discordant ($p = 0.631$, $p = 0.370$, and $p = 0.079$, respectively) and in the placental DCN, TAS, TOS, or ARES levels of the concordant twins ($p = 0.407$, $p = 0.035$, $p = 0.194$, and $p = 0.979$, respectively). When the authors compared the twins of similar birth weight, the DCN, TAS, and TOS levels were significantly lower in the discordant twins ($p < 0.001$, $p < 0.001$, and $p = 0.002$, respectively). *Conclusions:* Decreased levels of DCN in discordant twin fetuses compared to the same birth weight-concordant twins shows that it contributes to disease pathogenesis.

Key words: Dichorionic diamniotic twin; Discordant twin; DCN; Oxidative/antioxidative status.

Introduction

Rates of twin pregnancies are rising, as assisted reproductive techniques have become ever more advanced [1]. Approximately 25% of twin deliveries are affected by a discordance in at least 15%, and nearly 5% of twins experience severe discordance (≥ 30%) [2]. Twin pregnancies have significantly higher rates of perinatal morbidity and mortality than singleton pregnancies [3]. İndependent of gestational age at delivery, twins with significant birthweight discordance have poorer perinatal outcomes [4, 5].

Growth retardation in dichorionic diamniotic (DD) pregnancies probably occurs due to genetic causes and uteroplacental insufficiency, as in the case of singleton pregnancies [6]. The etiology of birthweight discordance in twins was extensively investigated, but the mechanisms leading to placental function deterioration are unknown.

Small-leucine-rich proteoglycans (SLRPs) are the most abundant proteoglycans expressed in human fetal membranes [7-9]. Extracellular matrix components are organized with SLRPs and collagen, and this interaction plays an important role in fetal growth and development [10, 11].

The family of SLRPs is divided into three subgroups, named class 1, 2, and 3. Decorin (DCN) and biglycan belong to class 1 and contain only propeptides [11]. Fetal membranes contain, predominantly, smaller proteoglycans, such as biglycan and DCN [12].

DCN is expressed and secreted by the placenta in humans and interacts with various factors to control cell proliferation, cell migration, and vasculogenesis, and therefore is important in tissue assembly [13].

DCN was identified as a decidua-derived inhibitor of normal EVT cell proliferation [14]; this is why we hypothesized that it should have a role in the pathogenesis of discordance in DD twins. Swan *et al.* investigated DCN expression in the placentas of 26 pregnancies with IUGR and 27 with normal babies, by the RT-PCR method, and found it decreased in the IUGRs [15].

The present authors aimed to evaluate the level of DCN and oxidative/antioxidative states of the placentas of concordant and discordant fetuses of twin pregnancies, which is novel; additionally, they attempted to determine their role in the pathogenesis of discordance in DD twins.

Materials and Methods

The present study was performed in the Zekai Tahir Burak Women's Health Education and Research Hospital. Forty-three spontaneous DD twin pregnancies, in which labor started by itself, were included in the study; all women had C-sections. Twenty-three of the pregnancies, having no other complication, ended with newborns having weight differences between 15-25%, called discordant DD twins. The remaining twins, having less than 15%

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difference in their weights, were grouped as concordant DD twins. Following labor, each placenta was sampled separately.

The exclusion criteria were as follows: any fetal abnormalities, gestational diabetes (GDM), preeclampsia, chorioamnionitis, maternal drug use, twins of IVF, monochorionic-monoamniotic or monochorionic diamniotic twins, abnormal Doppler findings as increased resistance, loss or reverse of end-diastolic velocity in umbilical artery, or notch in the uterine artery.

Pregnant women included in the study were homogenized in terms of age, gravida, and body mass index (BMI). BMI was obtained by dividing body weight in kilograms by the square of height in meters. Gestational age was confirmed in all pregnancies by a routine ultrasonographic examination performed during the first trimester of gestation. Informed consent was obtained from all patients participating in the study. The babies were weighed when they were born. Small for gestational age (SGA) twins were defined as those with birth weights below the 5th percentile, adjusted for gestational age and twins. Inter-twin birth weight discordance was calculated as [{(weight larger twin-weight smaller twin)/ weight larger twin}×100].

The placental areas belonging to each of the twins were identified by chorionic plate surface fetal vessel distribution, avoiding decidual contaminations. Using a sterile scalpel, a quadrangular segment along the placental thickness, from the basal towards the chorionic surface, was excised, and localized at the central region of the placenta. Placental tissue samples from 43 women were stored without treatment in sterile tubes at -80 degrees until completion of participation. The remaining placenta was sent to pathology for routine examination to rule out placental pathologies and chorioamnionitis.

Placental tissue was washed with cold saline, weighed, and placed in 10 mM Tris buffer, pH 7.4 (1/10 w/v). Placental tissue was homogenized on ice for three minutes. After homogenization, it was centrifuged at 10,000 g for 30 minutes and the supernatant was removed. The protein concentration of the supernatant was measured by the Lowry method [16]. Results are given in mg/ml of protein.

Total antioxidant status (TAS), total oxidant status (TOS), and arylesterase (ARES) levels in placental tissue were determined by the automated colorimetric method [17]. Results were presented as µmol Trolox Eq/mg for TAS, nmol H_2O_2 Eq/mg for TOS, and U/mg for ARES in placental tissue. DCN levels in placental tissue were analyzed using an enzyme-linked immunosorbent assay (ELISA) kit, and the results are presented in pg/mg.

Statistical analysis

The authors used Statistical Package for the Social Sciences (SPSS) version 21.0, PASTE programs, and the independent-sample *t*-test. A Pearson's correlation was used to examine the correlations of variables. Categorical data were shown with number (n) and the percentage (%) and compared with Pearson's Chi-square (χ^2) among groups. Data were examined at the 95% confidence level, and a two-tailed *p*-value < 0.05 was considered significant.

Results

Clinical characteristics of growth-discordant and -concordant DD twin pairs are presented in Table 1. Age, number of gravidity, and BMI were homogeneous in all groups. Birth week in the labor occurred was 36 (33- 37) for discordant twins and 36 (32- 37) for concordant twins; birth weeks were significantly lower in the discordant group ($p = 0.651$).

The differences between the birth weights of newborns were 495.65 ± 132.96 grams in discordant twins and 136.80 ± 77.80 grams in concordant twins. The difference of birth weights in the discordant group was significantly higher than in the concordant group ($p < 0.001$) (Table 1). The mean percentage of the difference of birth weights, calculated by [(weight of larger twin-weight of smaller twin) /weight of larger twin]×100 formula was 19.22 ± 4.94 in the discordant group and 6.20 ± 3.32 in the concordant group. The differences of birth weight percent in the discordant group were significantly higher than in the concordant group ($p < 0.001$) (Table 1).

Table 1. — *Clinical characteristics of growth in discordant and concordant twin pairs.*

	Concordant (n=20)	Discordant (n=23)	*p* value*
Age (years)	31.1±5.8	28.5±4.9	0.115
BMI (kg/m^2)	30.6±4.2	31.1±4.0	0.703
Gravida	2 (1-6)	1 (1-5)	0.073
Birth age (weeks)	36 (33-37)	36 (32-37)	0.651
Birth weight difference (g)	136.80±77.80	495.65±132.96	<0.001
Birth weight difference (%)	6.20±3.32	19.22±4.94	<0.001

Pearson Chi-Square Test (Exact). * between two groups.
BMI: body mass index, g: gram, %: percent.

Table 2. — *Comparison of placental DCN, TAS, TOS, and ARES levels for concordant and discordant twin pairs.*

	Infant-1	Infant-2	*p* value*
Concordant DD twin			
Birth weight (g)	2559.5±310.3	2578.5±312.4	0.600
DCN (pg/mg)	2963.1±781.4	2748.3±735.5	0.407
TAS (µmol Trolox Eq/mg)	0.18±0.08	0.13±0.04	0.055
TOS (H_2O_2 Eq/mg)	3.2±1.0	3.9±1.8	0.194
ARES (U/mg)	303.0±87.7	302.1±96.1	0.979
Discordant DD twin			
Birth weight (g)	2537.4±459.9	2236.1±525.5	0.003
DCN (pg/mg)	1707.1±574.4	1343.8±293.4	0.006
TAS (µmol Trolox Eq/mg)	0.09±0.03	0.09±0.03	0.631
TOS (H_2O_2 Eq/mg)	2.2±0.6	2.4±0.7	0.370
ARES (U/mg)	289.6±82.9	246.4±78.7	0.079

Independent-sample *t*-test. * between two groups.
DD: dichorionic-diamniotic, TAS: total antioxidant status, TOS: total oxidant status, ARES: arylesterase, g: gram.

Placental DCN, TAS, TOS, and ARES levels of the discordant and concordant groups

The level of DCN was 1343.8 ± 293.4 pg/mg in the placentas of babies who were lighter than their twin companion in the discordant group. This was 1707.1 ± 574.4 pg/mg in the placentas of the babies who were appropriate for their gestational ages in weight; the difference between these two was statistically significant ($p = 0.006$) (Table 2, Figure 1).

The levels of TAS, TOS, and ARES of the babies who

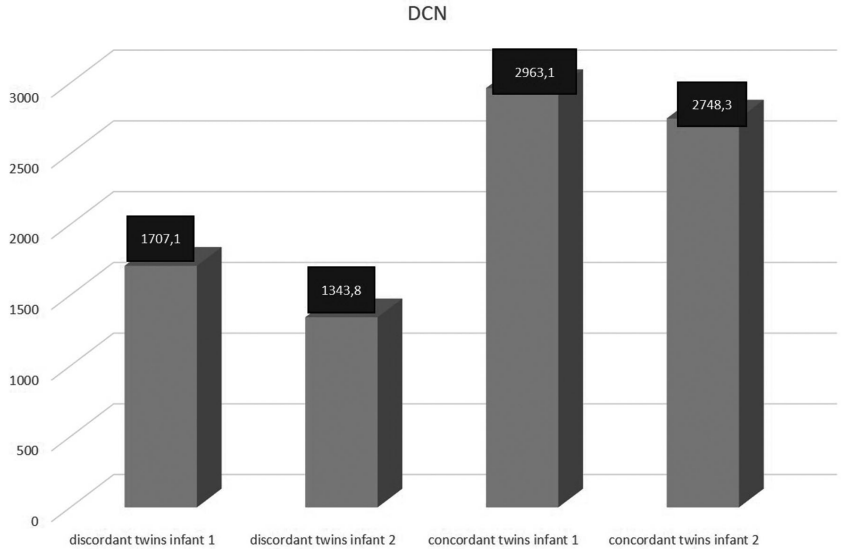

Figure 1. — The level of DCN in the placentas of concordant and discordant DD twins.

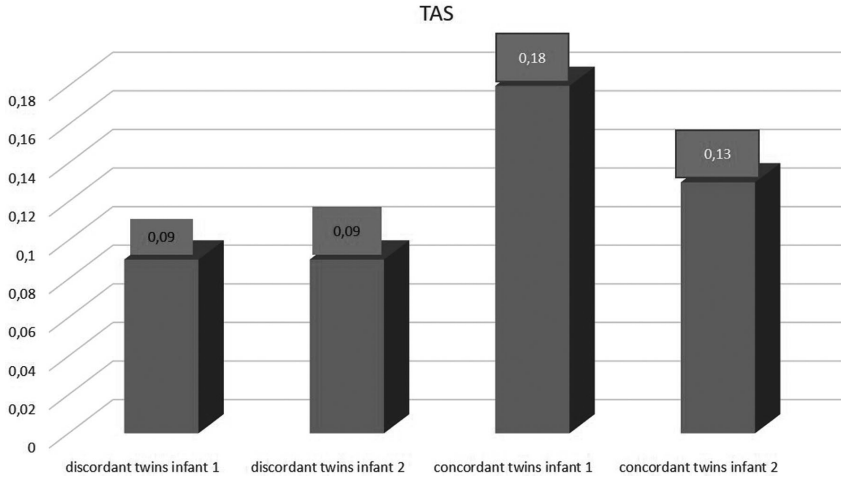

Figure 2. — The level of TAS in the placentas of concordant and discordant DD twins.

were 15% lighter than their twin companions in the discordant group were 0.09 ± 0.03 µmol Trolox Eq/mg, 2.4 ± 0.7 H_2O_2 Eq/mg, and 246.4±78,7 U/mg, respectively. Babies with weights appropriate for their age had TAS, TOS, and ARES values of 0.09 ± 0.03 µmol Trolox Eq/mg, 2.2 ± 0.6 H_2O_2 Eq/mg, and 289.6 ± 82.9 U/mg, respectively. No significant difference was observed for TAS, TOS, and ARES levels between the babies of appropriate weight and the ones weighing 15% less (p = 0.631, p = 0.370, and p = 0.079, respectively) (Table 2).

The levels of DCN, TAS, TOS, and ARES were 2963.1 ± 781.4 pg/mg, 0.18 ± 0.08 µmol Trolox Eq/mg, 3.2 ± 1.0 H_2O_2 Eq/mg, and 303.0 ± 87.7 U/mg, respectively, for the first infant, and 2748.3 ± 735.5 pg/mg, 0.13 ± 0.04 µmol Trolox Eq/mg, 3.9 ± 1.8 H_2O_2 Eq/mg, and 302.1 ± 96.1 U/mg, respectively, for the second infant in the concordant group. There was no significant difference between levels of placental DCN, TAS, TOS, or ARES in the concordant group (p = 0.407, p = 0.035, p = 0.194, and p = 0.979, respectively) (Table 2, Figures 1-3).

Placental DCN, TAS, TOS, and ARES levels of concordant and discordant infant groups which have no difference between birth weights

The birth weights of concordant infant-1 and discordant infant-1 were 2559.5 ± 310.3 g and 2537.4 ± 459.9 grams, respectively; there was no significant difference between these two groups (p = 0.635). Placental DCN, TAS, TOS, and ARES levels of concordant infant-1 and discordant infant-1 are compared in Table 3. The DCN, TOS, and TAS levels in discordant infant-1 were significantly lower than concordant infant-1 ($p < 0.001$ and p = 0.002, respectively), but the ARES levels in the discordant infant-1 were not significantly lower than concordant infant-1 (p = 0.688) (Table 3).

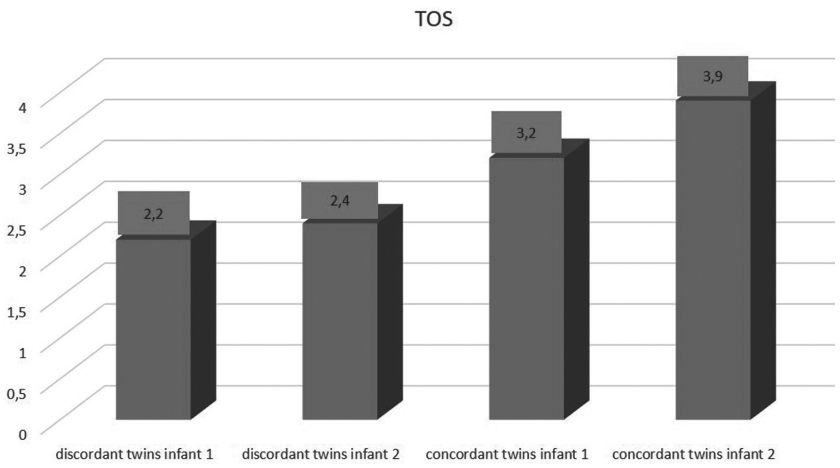

Figure 3. — The level of TOS in the placentas of concordant and discordant DD twins.

Table 3. — *Comparison of placental DCN, TAS, TOS, and ARES levels of concordant and discordant infant groups which had no difference in birth weights.*

	Concordant Infant-1 (n=20)	Discordant Infant-1 (n=23)	*p* value*
DCN (pg/mg)	2963.1±820.46	1707.1±593.93	< 0.001
TAS (µmol Trolox Eq/mg)	0.18±0.08	0.09±0.03	< 0.001
TOS (H_2O_2 Eq/mg)	3.2±1.0	2.2±0.6	0.002
ARES (U/mg)	303.0±87.7	289.6±82.9	0.688
Birth weight (g)	2559.5±310.3	2537.4±459.9	0.635

Indipendent-sample *t*-test. * between two groups.
TAS: total antioxidant status, TOS: total oxidant status,
ARES: arylesterase, g: gram.

Discussion

The present study was the first to examine the oxidative and antioxidative states of the placentas and their DCN levels at the same time in concordant and discordant DD twins; DCN was the target in the development of discordance.

The cause of birth weight discordance in twin gestations is often unclear. While birth weight differences in monochorionic twins have been largely attributed to hemodynamic factors, the etiology of discordance in dichorionic twins remains elusive. Differences in genetic potential, fetal sex, environmental factors, absence of endovascular trophoblast invasion of myometrial segments of the spiral arterioles, persistent smooth muscle histology in the maternal spiral arterioles causing hypoperfusion, hypoxia, reperfusion injury, and oxidative stress were considered to contribute to disparate fetal growth [18].

Pregnancy is a period in which oxidative and antioxidative processes must be in balance [19]. Structural and functional changes may be observed in the organs and fetoplacental circulation when there is oxidative stress [20]. Meinert *et al.* suggested that changes in the relative proportions of these extracellular molecules are crucial for the proposed fetal membrane maturation process during the last weeks of pregnancy [12]. Lysiak *et al.* observed that ectopic expression of DCN leads to marked growth retardation and change in the morphology and adhesion properties of TGF-β-dependent cells [21]. Swan *et al.* demonstrated that DCN is localised to the stroma surrounding fetal blood vessels of placental villi [15].

The present authors observed a significant decrease in the placental DCN level of the smaller baby in discordant DD twin pairs. No difference was observed in placental TAS, TOS, or ARES levels. DCN, TAS, TOS, and ARES levels were similar in the concordant DD twin group. These results led the present authors to believe that decreases in the amounts of DCN of the smaller twins in the discordant twin pair might cause growth retardation, although the oxidative/antioxidative state of the media did not change. They also observed that TAS and TOS levels were decreased in discordant twins compared to concordant ones, in synergy with DCN. This further led the present authors to believe that deterioration of the oxidative/antioxidative states in the discordant DD twins permit their protective effect to disappear and the decrease in DCN levels accompanies dysfunction in the fetoplacental unit; this may be a reason for IUGR and the worse morbidity outcomes observed in discordant twins than the concordant twins and singleton IUGR pregnancies.

Discordant twins had lower levels of DCN, TAS, TOS, and ARES, when discordant and concordant twin pairs with suitable birth weights for their gestational ages were compared. This led the present authors to believe that lower levels of DCN and antioxidative media might be the reason for the poor perinatal outcomes, even though the birth weights were similar and adjusted to the gestational ages.

In this study, the authors defined DD twins as discordant when the difference between their birth weights was equal to or higher than 15%. In order to determinate the DCN levels and oxidative/antioxidative status, they calculated the proportions of each twin and then compared discordant and con-

cordant twin groups regarding DCN, TAS, TOS, and ARES levels and found no difference between these two groups; however, there was a linear and proportional difference of DCN levels in discordant DD twins, higher than concordant DD twins.

Emeline *et al.* studied venous blood samples of 32 DD twins, one of which had severe IUGR (birth weight less than 5% for the adjusted gestational week, impaired Doppler ultrasound parameters, early onset IUGR), and observed higher oxidative stress in the fetuses having severe IUGR [22]. Yamaguchi *et al.* observed that expression of high levels of DCN in hamster ovary cells had a dramatic effect on their morphology and growth properties. They showed the growth effect of DCN worked by binding to transforming growth factor-beta receptors in hamster ovaries. Additionally, they suggested that DCN may be a component of a feedback system regulating cell growth [23]. Grant *et al.* showed that DCN inhibited angiogenesis in vitro by blocking the migratory function of endothelial cells and tumor-induced angiogenesis in vivo by antagonizing endogenous VEGF cells [24]. Mete *et al.* found significantly decreased maternal blood DCN levels in the 14 IUGR singleton pregnancies, when compared to 13 healthy controls; they also observed a negative correlation of DCN with the birth weights [25].

The present authors observed that DCN levels of discordant DD twins were lower than those of concordant DD twins. When the discordant twin group was evaluated, the babies lighter for their gestational week had less placental DCN, in synergism with TAS, TOS, and ARES levels.

This study may guide those searching for the effects of DCN on IUGR and discordance in twin pregnancies, and must be improved by additional samples and the use of advanced molecular techniques.

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References

[1] Blondel B., Kaminski M.: "Trends in the occurrence, determinants, and consequences of multiple births". *Semin. Perinatol*., 2002, *26*, 239.

[2] Jacobs A.R., Demissie K., Jain N.J., Kinzler W.L.: "Birth weight discordance and adverse fetal and neonatal outcomes among triplets in the United States". *Obstet. Gynecol*., 2003, *101*, 909.

[3] Spellacy W.N., Handler A., Ferre C.D.: "A case-control study of 1253 twin pregnancies from a 1982-1987 perinatal data base". *Obstet. Gynecol*., 1990, *75*, 168.

[4] Hollier L.M., McIntire D.D., Leveno K.J.: "Outcome of twin pregnancies according to intrapair birth weight differences". *Obstet. Gynecol.,* 1999, *94*, 1006.

[5] Breathnach F.M., McAuliffe F.M., Geary M., Daly S., Higgins J.R., Dornan J., *et al.*: "Definition of intertwin birth weight discordance". *Obstet. Gynecol.,* 2011, *118*, 94.

[6] Fick A.L., Feldstein V.A., Norton M.E., Wassel Fyr C., Caughey A.B., Machin G.A.: "Unequal placental sharing and birth weight discordance in monochorionic diamniotic twins". *Am. J. Obstet. Gynecol.,* 2006, *195*, 178.

[7] Wu Z., Horgan C.E., Carr O., Owens R.T., Iozzo R.V., Lechner B.E.: "Biglycan and decorin differentially regulate signaling in the fetal membranes". *Matrix Biol.*, 2014, *35*, 266.

[8] Gogiel T., Bankowski E., Jaworski S.: "Proteoglycans of Wharton's jelly". *Int. J. Biochem. Cell. Biol.,* 2003, *35*, 1461.

[9] Valiyaveettil M., Achur R.N., Muthusamy A., Gowda D.C.: "Characterization of chondroitin sulfate and dermatan sulfate proteoglycans of extracellular matrices of human umbilical cord blood vessels and Wharton's jelly". *Glycoconj. J.,* 2004, *21*, 361.

[10] Iozzo R.V.: "The family of the small leucine-rich proteoglycans: key regulators of matrix assembly and cellular growth". *Crit. Rev. Biochem. Mol. Biol.,* 1997,32(2):141-74.

[11] Iozzo R.V.: "The biology of the small leucine-rich proteoglycans. Functional network of interactive proteins". *J. Biol. Chem*., 1999, *274* 18843.

[12] Meinert M., Eriksen G.V., Petersen A.C., Helmig R.B., Laurent C., Uldbjerg N., *et al.*: "Proteoglycans and hyaluronan in human fetal membranes". *Am. J. Obstet. Gynecol.,* 2001, *184*, 679.

[13] Zhou Q.Y., Li C.C., Huo J.H., Zhao S.H.: "Expression and genomic imprinting of DCN, PON2 and PEG3 genes in porcine placenta". *Anim. Reprod. Sci.,* 2011, *123*, 70.

[14] Graham C.H., Lysiak J.J., McCrae K.R., Lala P.K.: "Localization of transforming growth factor-beta at the human fetal-maternal interface: role in trophoblast growth and differentiation". *Biol. Reprod.,* 1992, *46* 561.

[15] Swan B.C., Murthi P., Rajaraman G., Pathirage N.A., Said J.M., Ignjatovic V., et al.: "Decorin expression is decreased in human idiopathic fetal growth restriction". *Reprod. Fertil. Dev.,* 2010, *22*, 949.

[16] Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J.: "Protein measurement with the Folin phenol reagent". *J. Biol. Chem.,* 1951, *193*, 265.

[17] Erel O.: "A new automated colorimetric method for measuring total oxidant status". *Clin. Biochem.* 2005, *38*, 1103.

[18] Eberle A.M., Levesque D., Vintzileos A.M., Egan J.F., Tsapanos V., Salafia C.M.: "Placental pathology in discordant twins". *Am. J. Obstet. Gynecol.,* 1993, *169*, 931.

[19] Zein S., Rachidi S., Hininger-Favier I.: "Is oxidative stress induced by iron status associated with gestational diabetes mellitus?" *J. Trace Elem. Med. Biol.,* 2014, *28*, 65.

[20] Leach L.: "Placental vascular dysfunction in diabetic pregnancies: intimations of fetal cardiovascular disease?" *Microcirculation,* 2011, *18*, 263.

[21] Lysiak J.J., Hunt J., Pringle G.A., Lala P.K.: "Localization of transforming growth factor beta and its natural inhibitor decorin in the human placenta and decidua throughout gestation". *Placenta,* 1995, *16*, 221.

[22] Maisonneuve E., Delvin E., Ouellet A., Morin L., Dube J., Boucoiran I., *et al.*: "Oxidative conditions prevail in severe IUGR with vascular disease and Doppler anomalies". *J. Matern. Fetal Neonatal Med.,* 2015, *28*, 1471.

[23] Yamaguchi ., Mann D.M., Ruoslahti E.: "Negative regulation of transforming growth factor-beta by the proteoglycan decorin". *Nature,* 1990, *346*, 281.

[24] Grant D.S., Yenisey C., Rose R.W., Tootell M., Santra M., Iozzo R.V.: "Decorin suppresses tumor cell-mediated angiogenesis". *Oncogene*, 2002, *21*, 4765-77.

[25] Caglar M., Yavuzcan A., Goksu M., Alkan B. G., Sitki I. B., Ustun Y., *et al.*: "Decorin: a possible marker for fetal growth restriction". *Gynecol. Endocrinol.,* 2014, *30*, 141.

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