Int J Clin Exp Pathol 2021;14(6):734-740
www.ijcep.com /ISSN:1936-2625/IJCEP0131450

Original Article

In vitro fertilization is associated with placental accelerated villous maturation

Ambrogio P Londero[1*], Maria Orsaria[2*], Nadia Parisi[1], Alice Tassi[1], Carla Pittini[3], Lorenza Driul[1], Laura Mariuzzi[2]

[1]Clinic of Obstetrics and Gynecology, DAME, University of Udine, University Hospital of Udine, Italy; [2]Institute of Pathology, DAME, University of Udine, University Hospital of Udine, Italy; [3]Unit of Neonatology, University Hospital of Udine, Italy. [*]Equal contributors.

Received February 5, 2021; Accepted March 31, 2021; Epub June 15, 2021; Published June 30, 2021

Abstract: Objective: Accelerated placental maturation is regarded as a sign of vascular malperfusion and is often interpreted as a compensatory response by the placenta. In vitro embryo culture affects placental development. This study assessed placental maturation in spontaneous conceived and in vitro conceived pregnancies. Methods: Retrospective cohort study on a single center between 2014 and 2017. For this study, preterm placentas of singleton pregnancies between 24 and 36 weeks were considered. Routine placental examinations were retrospectively reviewed. Results: During the considered period, 423 placentas of singleton pregnancies were assessed. Three hundred ninety-six placentas were from spontaneous conception and 20 from in vitro fertilization and embryo transfer (IVF/ET). IVF/ET was significantly associated with accelerated villous maturation (AVM) and distal villous hypoplasia (DVH) ($P<0.05$). Conclusions: Placental AVM and DVH were significantly associated with in vitro fertilization in singleton pregnancies. This result supports the hypothesis that AVM is a compensatory response by the placenta to improve its transport capacity in specific settings such as in vitro fertilization.

Keywords: Hypermaturation, accelerated villous maturation, in vitro fertilization, placenta, hyporamification, distal villous hypoplasia, placental maturation, hypobranching

Introduction

The placenta must continuously adapt to the environment. Maternal vascular malperfusion and tissue hypoxia seem to cause aging, as a compensatory response, and placental accelerated villous maturation (AVM), associated with placental distal villous hypoplasia (DVH) [1, 2].

In several studies, it has been seen how in vitro fertilization and embryo culture affect fetal and placental development in utero, causing a lower birth weight in infants conceived in vitro [3]. This finding suggests that suboptimal placental development may cause this reduced function [3, 4]. It should be noted that the fetal and placental weight change following manipulation in vitro, causing a rise of the placental index (placental weight/fetal weight ratio) that is increased in in vitro conceived pregnancies and is considered a marker of intrauterine stress [3, 5-7] and a sign of placental plasticity [6].

An increased placental index and increased placental maturation rate are considered an adaptive response of the placenta [1, 8, 9]. Accelerated maturation also includes decreased villous branching and aspects of age-related parenchymal damage [10, 11].

The present study aimed to assess placental maturation and villous branching in spontaneous and in vitro-conceived pregnancies.

Materials and methods

This was a retrospective cohort study on a single-center between 2014-2017 and included all consecutive placentas from singleton pregnancies, conceived spontaneously or by IVF/ET, delivered between 24 and 36 weeks' gestation, and routinely examined by the pathologist. Exclusion criteria were: deliveries before 24 weeks' gestation or after 36 weeks' gestation, pregnancies conceived by in utero insemination or by ovulation induction alone,

fetuses affected by aneuploidies, and multiple pregnancies. The present retrospective study was approved by the internal review board of the department of medical area of the University of Udine and was accompanied according to Helsinki's declaration.

The following information was gathered from the clinical files: mode of conception (spontaneous vs. IVF/ET), maternal age, parity, gestational age at delivery, macro-region of origin, the presence of pregnancy-related hypertensive disorders (PRHDs), mode of labor, mode of delivery, neonatal sex, Apgar score at the first and fifth minute, neonatal weight (expressed as grams, as multiple of the median (MoM) value, as small for gestational age (SGA), or as large for gestational age (LGA)), the presence of intrauterine growth restriction (IUGR), placental index, fetal length, fetal head circumference, the presence of congenital malformations, neonatal cardiopulmonary resuscitation, neonatal intensive care unit (NICU) hospitalization, and neonatal status (alive, intrauterine fetal demise (IUFD), or post-natal death). Also, routine placental examinations were retrospectively reviewed, and the following information was recorded: presence of placental AVM and DVH.

The following definitions were adopted in data collection and analysis. In this manuscript, spontaneous conceptions and cases conceived by assisted reproductive technology were regarded. Among cases conceived by assisted reproductive technology, only cases conceived by IVF/ET were considered. Parity was classified into two groups, nulliparous and parous, and maternal age into equal or less and more than 40 years. Gestational age was calculated starting with the last menstrual period's date and confirmed by first-trimester ultrasound [12, 13]. Macro-region of origin was categorized into six groups as previously stated (Italy and Western Europe, Eastern Europe, Asia, Arabian countries, Sub-Saharan Africa, and other countries) [6, 14]. Hypertension was defined as a diastolic blood pressure ≥90 mmHg or systolic blood pressure ≥ of 140 mmHg [12, 13, 15]. Gestational hypertension, preeclampsia, preeclampsia superimposed on chronic hypertension, and eclampsia were grouped as PRHDs [13, 16]. Pre-eclampsia was defined as presence of hypertension (as defined above) with proteinuria or end-organ dysfunction detected after 20 weeks' gestation [15]. Proteinuria was defined as previously stated (urinary protein more than 0.3 g in a 24-h period) [12, 15]. Eclampsia was classified in the same way as preeclampsia, but with seizures. Gestational hypertension was classified in the same way as preeclampsia, but without proteinuria [13, 15]. Chronic hypertension was diagnosed in women who were confirmed to have hypertension before the 20th week of pregnancy [12, 13]. Apgar score at the first and fifth minutes was categorized into two groups equal to seven and less than seven. Birth weight was defined as the first measurement of neonatal body weight, generally within the first hour of life, measured to the nearest gram. Moreover, birth weight MoM was determined as the ratio between neonatal weight and the 50th percentile of neonatal weight at the same gestational age-adjusted per neonatal sex [7, 14]. SGA fetuses were defined as neonatal weight under the 3rd or 10th percentiles for gestational age [6, 17, 18]. LGA was defined as neonatal weight over the 90th or 97th percentiles for gestational age [6, 17, 18]. IUGR fetuses were defined as fetuses whose estimated fetal weight (Hadlock formula) was below the 10th or third percentile for gestational age with or without increased pulsatility index of the umbilical artery more significant than two standard deviations, respectively [18-20]. The placental index was calculated as placental weight in grams (placentas were weighed shortly after delivery with membranes and umbilical cord attached) divided by birth weight in grams [2]. Postnatal death was defined as death at less than 28 days of age and IUFD at a gestational age of 20 weeks, or more [21]. Placental AVM was defined as preterm placenta with the morphologic appearance of term placenta (hypermaturation) [1, 10]. DVH was defined as poorly developed or filiform terminal villi with minimal branching and unbranched capillary loops (hyporamification) [10].

Statistical analysis was performed using the R language and environment for statistical computing (version 3.6.2), and a *P*-value <0.05 was considered significant. The Kolmogorov-Smirnov test assessed the normality of variable distribution. Continuous data were presented as mean ± standard deviation or median and interquartile range (IQR) as appropriate and unless otherwise stated. Categorical variables were presented as a percentage and absolute value. Data were also presented as odds ratio (OR) and corresponding 95% confidence inter-

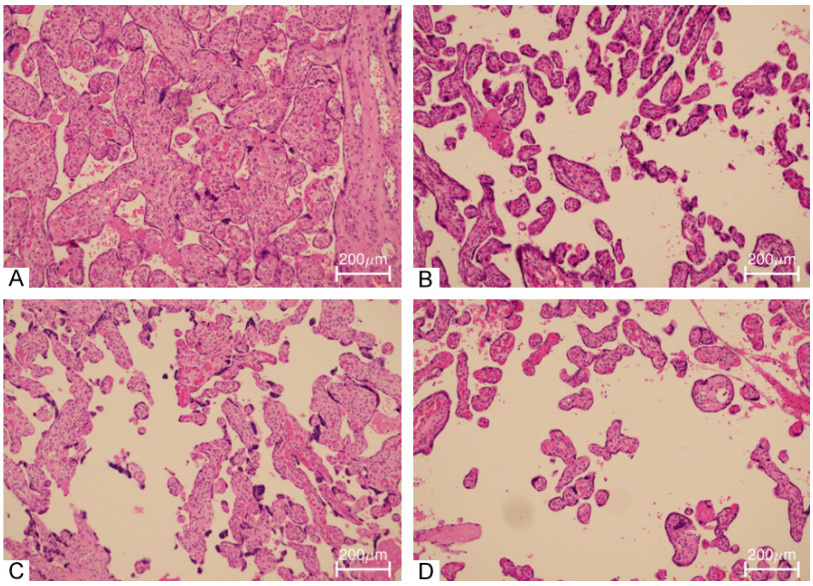

Figure 1. Placental villi stained by H&E. A. Mature intermediate villi at 27 weeks' gestation (H&E, 100×). B. Terminal villi at 30 weeks' gestation (accelerated villous maturation) (H&E, 100×). C. Non-branching/minimal branching villi at 34 weeks' gestation (distal villous hypoplasia) (H&E, 40×). D. Mature intermediate villi and non-branching/minimal branching villi at 30 weeks' gestation (accelerated villous maturation and distal villous hypoplasia) (H&E, 100×).

vals (CI.95). The following statistical tests were used: t-tests or Wilcoxon test to compare means or medians, respectively, in two samples, and the chi-square test or Fisher's exact test to compare proportions. Also, univariate and multivariate logistic regression were performed.

Results

In total, 416 placentas of singleton pregnancies were included. The mean maternal age was 32.68 years (±5.43), and 11.3% of women (47/416) were over 40 years of age. The 60.34% of women (251/416) were nulliparous. In total, 95.19% of pregnancies (396/416) were conceived spontaneously, while 4.81% (20/416) were conceived by IVF/ET. The mean gestational age at delivery was 32.58 weeks (±3.44), and 51.68% of neonates (215/416) were males. Mean neonatal weight was 1976.96 grams (±753.42). The AVM was present in 5.05% of cases (21/416), and DVH was present in 2.4% (10/416) (**Figure 1**).

In **Tables 1** and **2**, spontaneous conceptions were compared to IVF/ET. Women that conceived by IVF/ET were significantly older and were all nulliparous (**Table 1**). Also, among IVF/ET, there was a significantly higher prevalence of PR-HDs and SGA <10th percentile than in spontaneous conceptions (**Tables 1** and **2**).

AVM was significantly associated with IVF-ET pregnancies, OR 4.09 (CI.95 1.15-14.5) (P<0.05) (**Table 3**). Adjusting in the multivariate analysis for maternal age, IVF-ET was still a significant risk factor for AVM OR 4.59 (CI.95 1.19-17.75) (P<0.05) (**Table 3**). IVF/ET was significantly associated with DVH, OR 6.18 (CI.95 1.36-28.09) (**Table 3**).

Discussion

In our study, in vitro fertilization/embryo transfer (IVF/ET) was significantly associated with placental AVM and distal villous hypoplasia (DVH) in singleton pregnancies.

The present result adds evidence to support the hypothesis that AVM is a compensatory response by the placenta to improve its transport capacity in specific settings such as in vitro fertilization. These singleton pregnancies conceived by IVF/ET could be correlated with a hypoxia pattern, causing an increase of placental maturation that would reduce diffusion distance to increase transport and diffusion. Accelerated maturation is considered a response to an adverse environment, as occurs in preeclampsia [8]. Animal experiments have shown how placental transport depends on vascular and trophoblast transport, with different patterns in IUGR, preeclampsia, or diabetic pregnancies [22, 23]. The trophoblast has a role in transport function, hormone production, and substrate metabolism [22, 23]. In our study, the AVM was significantly associated with IVF-ET pregnancies, and it was associated with decreased villous branching, confirming previously published data [24]. Placental AVM is associated with improved neonatal outcome [1]. Following previously published data, a high pla-

Table 1. Population description subdivided between spontaneous and IVF/ET conception

	Spontaneous (396)	IVF/ET (20)	P
Maternal age (years)	32.42 (±5.37)	37.90 (±3.70)	<0.05
Maternal age ≥40 years	10.35% (41/396)	30.00% (6/20)	<0.05
Nulliparity	58.33% (231/396)	100.00% (20/20)	<0.05
Gestational age at delivery (weeks)	32.57 (±3.45)	32.80 (±3.17)	0.751
Gestational age at delivery <32 weeks	28.28% (112/396)	25.00% (5/20)	0.750
Gestational age at delivery <34 weeks	49.75% (197/396)	50.00% (10/20)	0.982
Macro-region of origin			
Italy and Western Europe	70.45% (279/396)	90.00% (18/20)	0.059
Eastern Europe	15.66% (62/396)	10.00% (2/20)	0.494
Sub-Saharan Africa	6.31% (25/396)	0.00% (0/20)	0.246
Arabian countries	1.52% (6/396)	0.00% (0/20)	0.579
Asia	4.04% (16/396)	0.00% (0/20)	0.359
Other	2.02% (8/396)	0.00% (0/20)	0.521
PRHDs	15.66% (62/396)	35.00% (7/20)	<0.05
Mode of labour			
Spontaneous	35.10% (139/396)	25.00% (5/20)	0.354
Induction/augmentation	15.91% (63/396)	30.00% (6/20)	0.098
Without labour	48.99% (194/396)	45.00% (9/20)	0.728
Mode of delivery			
Operative vaginal delivery	1.01% (4/396)	15.00% (3/20)	<0.05
Spontaneous delivery	37.37% (148/396)	25.00% (5/20)	0.263
Cesarean section	61.62% (244/396)	60.00% (12/20)	0.885

Acronyms: PRHD = pregnancy related hypertensive disorder; IVF/ET: in vitro fertilization and embryo transfer.

cental index was significantly associated with poor maternal-fetal outcome (PRHDs, impaired fetal growth, complicated postnatal infant care) and pregnancies conceived by IVF-ET [3, 6]. This study confirms that IVF-ET pregnancies were associated with a higher prevalence of PRHDs and SGA <10th percentile than spontaneous conceptions.

Placental AVM is more common in preeclamptic pregnancies and pregnancies characterized by IUGR fetuses or pregnancies with a hypertensive disease, and it is probably a sign of hypoxia and placental plasticity. According to Christians et al., placental AVM is related to a better neonatal outcome than nonhypermaturated villi [1]. The term "accelerated maturation of the placenta" describes several features of the placenta, one of which is vascular accelerated maturation, a preterm placenta with the morphologic aspect of a term placenta. DVH and AVM are characteristics that are closely linked, both signs of an adaptive, compensatory response to hypoxia and placental plasticity [1]. Little has been written about the association between AVM of the placenta and in vitro fertilization. However, some studies show that assisted reproduction techniques can affect placental function [4, 6, 7]. Zhang and coworkers' study shows that the placentas of patients undergoing IVF/ET were characterized by alterations of the placental barrier, including thickening of the placental barrier and decreased density of syncytiotrophoblast apical microvilli and consequent DVH (or hyporamification). In animal models, in vitro fertilization has been shown to result in reduced efficacy of the placenta and placental exchanges, and it can lead to structural abnormalities of the placenta [25, 26].

This study's strengths include collecting a large cohort of preterm delivery placentas and an absence of other similar manuscripts in the existing literature. Another strength is the histologic evaluation of the placentas by a single pathologist team according to standardized protocols. The limitations of this study include retrospective design and the low number of subjects undergoing IVF/ET. Furthermore, in a reference structure for tertiary care, it is diffi-

Table 2. Neonatal characteristics and outcomes subdivided between spontaneous and IVF/ET conception

	Spontaneous (396)	IVF/ET (20)	P
Neonatal male sex	51.77% (205/396)	50.00% (10/20)	0.877
Apgar score 1st minute <7	41.16% (163/396)	25.00% (5/20)	0.151
Apgar score 5th minute <7	10.86% (43/396)	0.00% (0/20)	0.247
Neonatal weight (grams)	1983.91 (±757.27)	1839.25 (±674.84)	0.363
Neonatal weight (MoM)	1.00 (±0.19)	0.90 (±0.17)	<0.05
Placental weight (grams)	429.75 (±153.59)	378.09 (±113.51)	0.171
Placental index <0.20	47.98% (190/396)	50.00% (10/20)	0.860
Fetal length (cm)	42.49 (±5.66)	39.75 (±10.74)	0.271
Fetal head circumference (mm)	296.71 (±44.78)	282.40 (±74.03)	0.402
SGA <3rd	2.78% (11/396)	0.00% (0/20)	0.450
SGA <10th	11.62% (46/396)	35.00% (7/20)	<0.05
LGA >90th	10.86% (43/396)	0.00% (0/20)	0.120
LGA >97th	3.54% (14/396)	0.00% (0/20)	0.392
IUGR	7.58% (30/396)	15.00% (3/20)	0.231
Congenital malformations	3.03% (12/396)	0.00% (0/20)	0.430
Neonatal cardiopulmonary resuscitation	36.96% (146/395)	35.00% (7/20)	0.859
NICU hospitalization	64.39% (255/396)	75.00% (15/20)	0.332
Neonatal outcome			
Alive	94.67% (373/394)	100.00% (20/20)	0.289
IUFD	2.28% (9/394)	0.00% (0/20)	0.494
Post-natal death	3.05% (12/394)	0.00% (0/20)	0.428

Acronyms: IVF/ET: in vitro fertilization and embryo transfer; MoM = multiple of the median; SGA = small for gestational age; LGA = large for gestational age; IUGR = intrauterine growth restriction; NICU = neonatal intensive care unit; IUFD = intrauterine fetal demise.

Table 3. Uni-/multi-variate logistic regression (dependent variables placental AVM or DVH)

	OR (CI.95)	P	OR (CI.95) (*)	P (*)
AVM				
IVF/ET	4.09 (1.15-14.5)	<0.05	4.59 (1.19-17.75)	<0.05
DVH				
IVF/ET	6.18 (1.36-28.09)	<0.05		

Acronyms: AVM = accelerated villous maturation; DVH = distal villous hypoplasia. *Multivariate logistic regression (correction for maternal age.

cult to find a control group with purely low-risk pregnancies.

This study can be applied to pregnant women's general population, even if data were collected in a structure for tertiary care because these data are essential to better approach the IVF/ET pregnancy population; in fact, couples increasingly use this method to procreate. These results help with identifying those pregnancies with placental AVM that probably if they came to full term, could be associated with premature placental aging, and could benefit from a different approach in obstetric management.

Conclusion

Placental AVM and DVH were significantly associated with in vitro fertilization in singleton pregnancies. This result supports the hypothesis that AVM is a compensatory response to an adverse environment by the placenta to improve its transport capacity in specific settings such as in vitro fertilization and other obstetric pathologies such as preeclampsia.

Acknowledgements

The authors would like to thank the whole staff collaborating in article collection, selection, reading and in paper writing and reviewing.

Disclosure of conflict of interest

None.

Abbreviations

AVM, accelerated villous maturation; CI.95, 95% confidence interval; DVH, distal villous hypoplasia; H&E, hematoxylin and eosin; IQR, interquartile range; IUFD, intrauterine fetal demise; IUGR, intrauterine growth restriction; IVF/ET, in vitro fertilization and embryo transfer; LGA, large for gestational age; MoM, multiple of the median; NICU, neonatal intensive care unit; OR, odds ratio; PRHD, pregnancy related hypertensive disorder; SGA, small for gestational age.

Address correspondence to: Dr. Ambrogio P Londero, Clinic of Obstetrics and Gynecology, Academic Hospital of Udine, Piazza Santa Maria della Misericordia, 15, 33100 Udine, Italy. Tel: 390432-559635; E-mail: ambrogio.londero@gmail.com

References

[1] Christians JK and Grynspan D. Placental villous hypermaturation is associated with improved neonatal outcomes. Placenta 2019; 76: 1-5.

[2] Londero AP, Orsaria M, Marzinotto S, Grassi T, Fruscalzo A, Calcagno A, Bertozzi S, Nardini N, Stella E, Lellé RJ, Driul L, Tell G and Mariuzzi L. Placental aging and oxidation damage in a tissue micro-array model: an immunohistochemistry study. Histochem Cell Biol 2016; 146: 191-204.

[3] Haavaldsen C, Tanbo T and Eskild A. Placental weight in singleton pregnancies with and without assisted reproductive technology: a population study of 536,567 pregnancies. Hum Reprod 2012; 27: 576-582.

[4] Delle Piane L, Lin W, Liu X, Donjacour A, Minasi P, Revelli A, Maltepe E and Rinaudo PF. Effect of the method of conception and embryo transfer procedure on mid-gestation placenta and fetal development in an IVF mouse model. Hum Reprod 2010; 25: 2039-2046.

[5] Barker DJ, Bull AR, Osmond C and Simmonds SJ. Fetal and placental size and risk of hypertension in adult life. BMJ 1990; 301: 259-262.

[6] Londero AP, Bertozzi S, Visentin S, Fruscalzo A, Driul L and Marchesoni D. High placental index and poor pregnancy outcomes: a retrospective study of 18,386 pregnancies. Gynecol Endocrinol 2013; 29: 666-669.

[7] Londero AP, Salvador S, Fruscalzo A, Bertozzi S, Biasioli A, Ceraudo M, Visentin S, Driul L and Marchesoni D. First Trimester PAPP-A MoM values predictive for breech presentation at term of pregnancy. Gynecol Endocrinol 2013; 29: 503-507.

[8] Moldenhauer JS, Stanek J, Warshak C, Khoury J and Sibai B. The frequency and severity of placental findings in women with preeclampsia are gestational age dependent. Am J Obstet Gynecol 2003; 189: 1173-1177.

[9] Stanek J. Hypoxic patterns of placental injury: a review. Arch Pathol Lab Med 2013; 137: 706-720.

[10] Khong TY, Mooney EE, Ariel I, Balmus NCM, Boyd TK, Brundler MA, Derricott H, Evans MJ, Faye-Petersen OM, Gillan JE, Heazell AEP, Heller DS, Jacques SM, Keating S, Kelehan P, Maes A, McKay EM, Morgan TK, Nikkels PGJ, Parks WT, Redline RW, Scheimberg I, Schoots MH, Sebire NJ, Timmer A, Turowski G, van der Voorn JP, van Lijnschoten I and Gordijn SJ. Sampling and definitions of placental lesions: Amsterdam placental workshop group consensus statement. Arch Pathol Lab Med 2016; 140: 698-713.

[11] Redline RW. Classification of placental lesions. Am J Obstet Gynecol 2015; 213 Suppl: S21-28.

[12] Visentin S, Londero AP, Camerin M, Grisan E and Cosmi E. A possible new approach in the prediction of late gestational hypertension: the role of the fetal aortic intima-media thickness. Medicine (Baltimore) 2017; 96: e5515.

[13] Londero AP, Rossetti E, Pittini C, Cagnacci A and Driul L. Maternal Age and the risk of adverse pregnancy outcomes: a retrospective cohort study. BMC Pregnancy Childbirth 2019; 19: 261.

[14] Londero A, Visentin S, Marin L, Bongiorno C, Visentin D, Bertozzi S, Cosmi E, Cagnacci A and Driul L. Second trimester prediction of small for gestational age and intrauterine growth restriction. Clin Exp Obstet Gynecol 2020; XLVII: 78-82.

[15] Gestational hypertension and preeclampsia: acog practice bulletin summary, number 222. Obstet Gynecol 2020; 135: 1492-1495.

[16] Fruscalzo A, Bertozzi S, Londero AP, Biasioli A, Driul L, Kiesel L and Marchesoni D. Menstrual abnormalities and predisposition to pregnancy-related hypertensive disorders: a retrospective study. Gynecol Endocrinol 2010; 26: 445-450.

[17] Visentin S, Lapolla A, Londero AP, Cosma C, Dalfrà M, Camerin M, Faggian D, Plebani M and Cosmi E. Adiponectin levels are reduced while markers of systemic inflammation and aortic remodelling are increased in intrauterine growth restricted mother-child couple. Biomed Res Int 2014; 2014: 401595.

[18] Fruscalzo A, Londero AP, Orsaria M, Marzinotto S, Driul L, Di Loreto C and Mariuzzi L. Placental fibronectin staining is unaffected in pregnancies complicated by late-onset intrauterine growth restriction and small for gestational age fetuses. Int J Gynaecol Obstet 2020; 148: 253-260.
[19] Visentin S, Londero AP, Calanducci M, Grisan E, Bongiorno MC, Marin L and Cosmi E. Fetal abdominal aorta: doppler and structural evaluation of endothelial function in intrauterine growth restriction and controls. Ultraschall Med 2019; 40: 55-63.
[20] Gordijn SJ, Beune IM, Thilaganathan B, Papageorghiou A, Baschat AA, Baker PN, Silver RM, Wynia K and Ganzevoort W. Consensus definition of fetal growth restriction: a delphi procedure. Ultrasound Obstet Gynecol 2016; 48: 333-339.
[21] Barfield WD; Committee on fetus and newborn. Standard terminology for fetal, infant, and perinatal deaths. Pediatrics 2016; 137: e20160551.
[22] Jones HN, Powell TL and Jansson T. Regulation of placental nutrient transport-a review. Placenta 2007; 28: 763-774.
[23] Fowden AL, Ward JW, Wooding FP, Forhead AJ and Constancia M. Programming placental nutrient transport capacity. J Physiol 2006; 572: 5-15.
[24] Morgan TK, Tolosa JE, Mele L, Wapner RJ, Spong CY, Sorokin Y, Dudley DJ, Peaceman AM, Mercer BM, Thorp JM, O'Sullivan MJ, Ramin SM, Rouse DJ and Sibai B; Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Placental villous hypermaturation is associated with idiopathic preterm birth. J Matern Fetal Neonatal Med 2013; 26: 647-653.
[25] Zhang Y, Zhao W, Jiang Y, Zhang R, Wang J, Li C, Zhao H, Gao L, Cui Y, Zhou Z, Sha J, Liu J and Wang L. Ultrastructural study on human placentae from women subjected to assisted reproductive technology treatments. Biol Reprod 2011; 85: 635-642.
[26] Chen S, Sun Fz, Huang X, Wang X, Tang N, Zhu B and Li B. Assisted reproduction causes placental maldevelopment and dysfunction linked to reduced fetal weight in mice. Sci Rep 2015; 5: 10596.