Villous Vascular Tree 3D Morphology of Ex Vivo Perfused Human Placental Cotyledon

TEXAS TECH UNIVERSITY HEALTH SCIENCES CENTER at the Permian Basin

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Introduction

In human pregnancy, the first half of gestation is associated with the prevalence of branching angiogenesis, and the second half of gestation is marked by prevalence of non-branching angiogenesis. Some adverse maternal conditions, e.g pre-eclampsia are associated with excessive branching and decreased flow-mediated vasodilation. Mathematical models of placental oxygen exchange and consumption used different approaches to connect placental vascular structure and placental function, however, the physiological data is sparse.

Objective

The aim of this study was to evaluate 3D vascular structure of ex-vivo perfused human placental cotyledon and compare vascular tree morphology with physiological parameters.

Materials and Methods

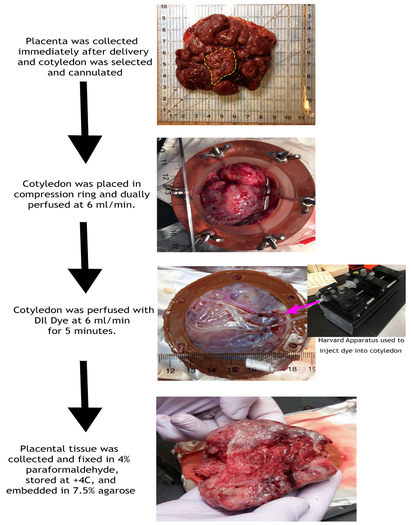

Figure 1. Diagram of placenta perfusion methodology.

At the end of perfusion experiment, each cotyledon was infused with 12 ml of 1,1'-Dioctadecyl-3,3,3',3'-tetramethylindocarbocaninperchlorate (DiI, Cat No. 42364, Sigma-Aldrich; St. Louis, MO, USA) at the rate 6 ml/min, and subsequently with 12 ml of 4% paraformaldehyde at the same flow rate, using modified published protocol. Subsequently a portion of the cotyledon was fixed for 1-7 days in 4% paraformaldehyde (4.0ºC). All placental specimens were embedded in 4% agarose and sections with 300-400 µm thickness were cut with vibratome (The Vibratome Co., St. Louis, MO, USA). All fluorescent images were taken using the T1-E microscope with A1 confocal and STORM super-resolution modules (Nikon Instruments Melville NY, USA. Images were quantified using Image-Pro Premier software (Media Cybernetics, Inc, Rockville, MD. USA) and Imaris 9 (Bitplane, USA). Number of branching points was calculated using Imaris 9 algorithm. Details regarding methodology of estimation of placental oxygen consumption are in poster # F-162.

Results

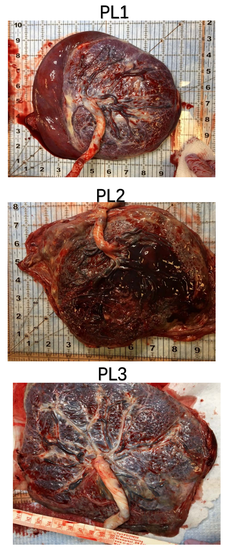

Figure 2. Pictures of placentas used for perfusion collected after delivery.

Table 1. Patient characteristics.

Patient	Gravidity	Parity	Pre-preg weight (kg)	Height (cm)	Gestational Age (wks.days)	Maternal Age (Years)	Race	Prenatal medication other	Drugs-Alcohol	GD	Beta Strept status	Anaesthesia	Delivery	Apgar (1 minute)	Apgar (5 minute)
PT1	4	3	72.6	152	39.8	21	Caucasian	Keflex. Zafran	Yes	No	-	Epidural	C-section	7	9
PT2	3	2	83.9	165	39	37	Caucasian	Glyburide. Fenofibrate	No	yes	+	Epidural	C-section	7	8
PT3	1	0	89.5	162	40	20	N/A	N/A	No	No	+	Epidural	Vaginal	9	10

Table 2. Placenta characteristics.

Placenta	Placenta Weight (g)	Placenta Thickness (cm)	Placenta Diameter (cm)	Number of Cotyledons	Cotyledon Weight (grams) (after perfusion)	Fetal weight (grams)	Fetal sex
PL1	575	2.6	22 x 20	29	18.06	3540	F
PL2	555	2.5	18 X 16.5	19	64.15	3460	F
PL3	655	3	22 X 21	17	67.64	3470	M

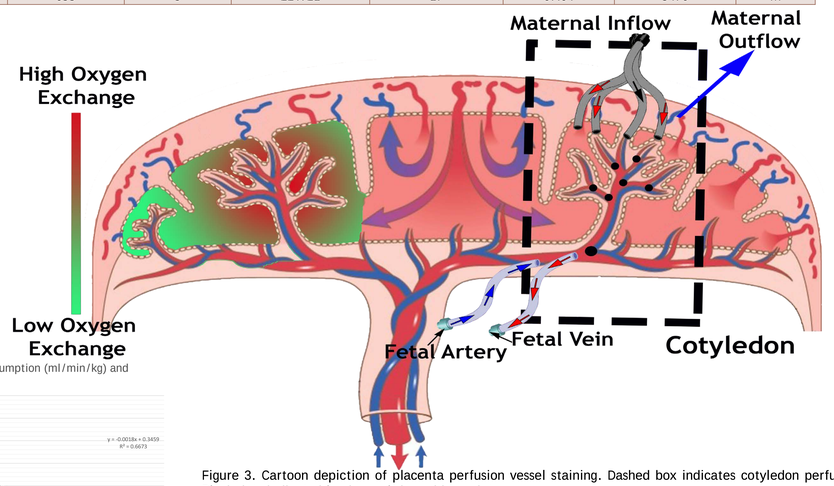

Figure 3. Cartoon depiction of placenta perfusion vessel staining. Dashed box indicates cotyledon perfused. Black dots indicate blood vessels branching points.

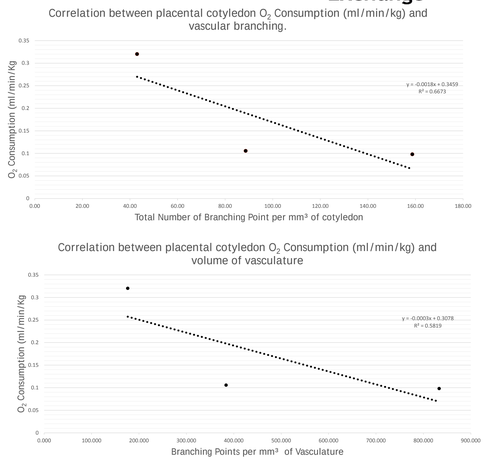

Table 3. Perfusion parameters and morphology of villous tree

Placenta	Fetal Input Pressure (mmHg)	Maternal Input Pressure (mmHg)	Fetal Flow (ml/min)	Maternal Rate (ml/min)	Fetal Leakage	Absolute number of Branching Points (BP)	Volume of Tissue (mm³)	BP/Volume of tissue (mm³)	Volume of Vasculature (mm³) - mean ± SEM	BP/Volume of Vasculature (mm³)	Volume of stem Villi Vasculature (mm³)*	BP/Volume of Stem Villi Vasculature (mm³)
PL1	35.15	33.88	4.8	15	11.12%	52 ± 20	1.21±0.18	42.98	0.295 ± 0.105	176.271	0.0177 ± 0.00361	2.938E+03
PL2	26.81	62.11	4.8	15	8.30%	165 ± 53	1.04±.015	158.65	0.198 ± 0.060	833.333	0.0119 ± 0.00180	1.387E+04
PL3	33.19	32.4	4.5	15	13%	109 ± 26	1.23±0.14	88.62	0.284 ± 0.039	383.803	0.0170 ± 0.00469	6.412E+03

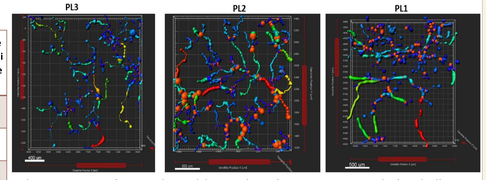

Figure 5. Vascular tracing with Imaris software. Orange circles indicate points of vascular branching.

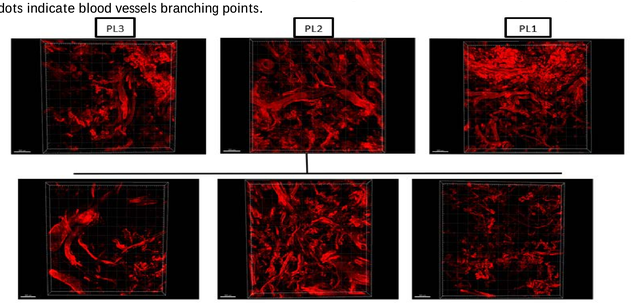

Figure 4. Variation in vascular tree morphology between cotyledons (upper panel) and within cotyledon (lower panel) of perfused human placenta (endothelium- specific DiI staining). Magnification 10X. Scale bar for all images: 300um.

Discussion and Conclusion

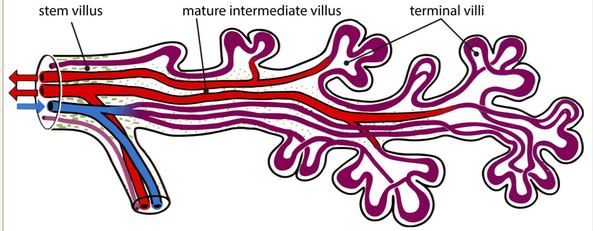

Fig.6 Villous tree structure with the emphasis on villous branching (Benirschke et al., 2006)

The differences in the methodologies, applied for calculation of villous number and vascular branching points (Table 4) makes comparison between studies difficult. Our data is similar to published by *Haeussner et al., 2016* with Neuro-Lucida analyses and 3D microvillous reconstruction. Despite differences in the absolute numbers, our data is in line with published by *Jirkovska et al. 2012,* regarding increased number of branching points in patients with diabetes. Mathematical models of the placental oxygen exchange, summarized by *Serov (2015),* include flow rate, membranes permeability and thickness, porosity, etc. Recent model, published by *Lin et al. (2016)* (Figure 7), demonstrated correlation between angle of villous tree/type of villi and oxygen uptake, demonstrating weak influence of number of branching points on the placental oxygen uptake. Our functional study showed association between number of branching points with the oxygen uptake. SGI Poster#F162 describes relationship between flow rate and oxygen uptake in the same model.

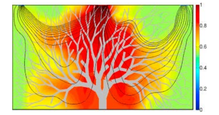

Fig. 7. Flow streamlines (black lines) and normalised oxygen concentration field (colours) predicted for a placentome containing a villous tree with random branching angles correspond with the asymmetrical branching of the villous tree. (Lin et al., 2016)

Ex vivo placental model could be used for testing mathematical computational algorithms linking placental structure and function.

Table 4. Published data and methods of estimation of placental villous branching.

Authors	Method	Vasculature's and villous branching	Study's endpoints
Haeussner, E. et al. 2014.	Sholl-analyses based Neuro-Lucida software and 3D reconstruction.	11.1 ± 5.140 7.1 ± 3.824 1.74 ± 1.019	Terminal distance ordering of branches
Haeussner, E. et al. 2016.	Sholl-analyses based Neuro-Lucida software and 3D reconstruction.	CTR:1 x 10^5 um^{3*} EXP: 8 x 10^4 um^{3*} *Estimated from graphs.	Intrauterine Growth Restriction
Jirkovská, M. et al. 2012	Confocal microscopy, 3D reconstruction and immunohistochemistry.	CTR: 0.027 ± 0.109 BP per villus EXP: 0.410 ± 0.202 BP per villus	Diabetes Mellitus 1
Kato, Y. et al. 2016.	Computational Model	2.22 – 6.02 bifurcation ratio	Contraction of stem villi
Mayhew, T. M. et al. 2004.	Stereological analyses	Branching Index: 0.28 ± 0.03	Intrauterine Growth Restriction
Thunbo, MØ. et al. 2018.	Computed topography angiography and 3D image segmentation.	1.02 ± 1.73 vessel junctions per convex	Fetal Growth Restriction
Serov, A.S. et al. 2014	Mathematical Model	0.47 ± 0.06 villous density	Oxygen Uptake

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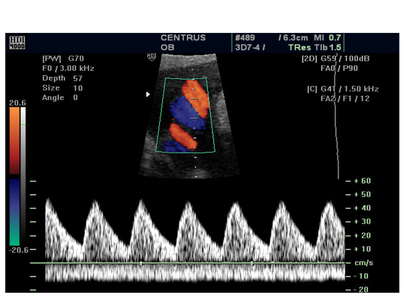

Table 1. Structural variability (number of branching points) in ex vivo perfused human placenta.

	Number of branching points				mean ± SEM
Sections of cotyledon	A	B	C	D	
PL1	91	34	31	N.A	52 ± 20
PL2	285	205	135	36	165 ± 53
PL3	183	74	105	75	109 ± 26