1 **Pharmacological Profile of Vascular Activity of Human Stem Villous**

2 **Arteries**

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17

Abstract

Introduction

The function of the placental vasculature differs considerably from other systemic vascular beds of the human body. A detailed understanding of the normal placental vascular physiology is the foundation to understand perturbed conditions potentially leading to placental dysfunction.

Methods

Behaviour of human stem villous arteries isolated from placentae at term pregnancy was assessed using wire myography. Effects of a selection of known vasoconstrictors and vasodilators of the systemic vasculature were assessed. The morphology of stem villous arteries was examined using IHC and TEM.

Results

Contractile effects in stem villous arteries were caused by U46619, 5-HT, angiotensin II and endothelin-1 ($p \leq 0.05$), whereas noradrenaline and AVP failed to result in a contraction. Dilating effects were seen for histamine, riluzole, nifedipine, papaverine, SNP and SQ29548 ($p \leq 0.05$) but not for acetylcholine, bradykinin and substance P.

Discussion

Stem villous arteries behave differently to vessels of the systemic vasculature and results indicate that the placenta is cut off from the systemic maternal vascular regulation. Particularly, endothelium-dependent processes were attenuated in the placental vasculature, creating a need to determine the role of the endothelium in the placenta in future studies.

Introduction

46

47 Placental vessels are of low resistance and their control is mainly driven by local humoral factors [1].

48 Due to the lack autonomic innervation, many vasoactive substances of the systemic vasculature

49 exhibit no effects in placental vessels [2]. This 'failsafe' function of placental vessels ensures

50 sufficient blood flow to the fetus at any time, independent from factors affecting the maternal

51 organism.

52 There are two types of vessels in the placenta that exhibit characteristics of resistance arteries with

53 normalised internal diameters of 100-400μm [3] and muscular walls [4]: chorionic plate arteries and

54 stem villous arteries. Stem villous arteries are situated at the site of nutrient transfer and are also

55 present in much higher numbers than chorionic plate arteries. Stem villous arteries are therefore

56 thought to be the most significant structure for the regulation of the placental circulation [5].

57 Although chorionic plate arteries may be less important for direct regulation of the fetoplacental

58 flow, they might affect the downstream vasculature by release of mediators [6].

59 Several publications report the effect of various pharmacological compounds on placental vessels [7-

60 12]. However, most of the recent literature on placental vessels focusses on chorionic plate arteries

61 whereas knowledge about stem villous arteries dates back to research conducted in the 1990s. This

62 early work was mostly performed using perfusion of whole placentae or isolated vessels, often

63 under nonphysiological conditions using high oxygen pressures and high resting tensions, potentially

64 leading to distorted findings. Therefore, the present study undertook to test the effect of a selection

65 of known vasoconstrictors and vasodilators on stem villous arteries under physiological experimental

66 conditions.

Methods

Chemicals and Solutions

Two types of buffers were used for wire myography experiments, physiological salt solution (PSS) and high potassium physiological salt solution (KPSS). The composition of PSS (in mM) is sodium chloride 119, potassium chloride 4.7, magnesium sulfate heptahydrate 1.17, sodium bicarabonate 25, potassium dihydrogen orthophosphate 1.18, EDTA 0.027, D-(+)-glucose 5.5, calcium chloride dehydrate 2.5. For KPSS, sodium chloride was replaced with 123.7 mM potassium chloride. Both were prepared according to protocols developed by Mulvany [3]. (R)-(-)-Phenylephrine hydrochloride (P6126), [Arg^8]-Vasopressin acetate salt (V9879), acetylcholine chloride (A6625), angiotensin II human (A9525), bradykinin acetate salt (B3259), histamine (H7125), indomethacin (I7378), L-norepinephrine hydrochloride (74480), nifedipine (N7634), NΩ-nitro-L-arginine methyl ester hydrochloride (L-NAME) (N5751), papaverine hydrochloride (P3510), riluzole (R116), sodium nitroprusside dehydrate (S0501) and Substance P acetate salt hydrate (S6883) were bought from Sigma, UK. U46619 (1932) was bought from Tocris, UK. Endothelin-1 (human, porcine) (ab120471) was purchased from Abcam, UK. Serotonin (hydrochloride) (14332) was bought from Cayman, US. SQ29548 (BML-RA103) was purchased from Enzo, UK.

Tissue collection

Placentae were collected from healthy pregnant women after obtaining fully informed written consent. Ethics approval was granted by Derby Research Ethics Committee (REC Reference No. 09/H0401/90). Patient demographics for collected placentae are shown in Table 1. All subjects of the study delivered via caesarean section.

Wire myography

Stem villous arteries were dissected within one hour after collection and placed in physiological salt solution (PSS). In order to accurately identify arteries in stem villi for the purposes of myography, the umbilical artery from the point of cord insertion was followed to first excise a full cotyledon. The cotyledon was cleaned from excess blood using PSS and the artery was then followed directly while

93   continuously removing surrounding villous tissue using blunt forceps and a fine pair of scissors,

94   taking extreme care not to damage the vessel wall. From the third to fourth order of the branch,

95   dissection needed to be continued under the dissecting microscope in order to distinguish the artery

96   from the vein that usually runs in close proximity within a villus. The arteries were cleaned from

97   surrounding tissue and cut into 2mm segments. Vessel segments were mounted onto 40μm wires of

98   a DMT 620 myograph system (Aarhus, Denmark) and normalised. For the present study, all vessels

99   were normalised to a target pressure of 5.1kPa in order to simulate physiological placental

100   conditions [10]. An internal circumference of $0.9*IC_{5.1kPa}$ was used as optimal working diameter for

101   stem villous arteries. Experiments were performed at 37°C in PSS gassed with 2% oxygen, 5% carbon

102   dioxide in nitrogen (BOC special gas, British Oxygen Company, UK) to reproduce placental conditions

103   at term [13].

104   Experiments were started with an initial contraction to 10^{-6}M U46619 that served as a viability

105   control. Effects of subsequently tested contractile or relaxant agents were expressed in % of this

106   contraction. For the assessment of relaxant effects, test compounds were added in increasing

107   concentrations directly following the initial U46619 contraction. U44619 was chosen as its

108   vasoconstrictive effects have been shown to be consistent and reproducible in placental vessels [10].

109   For assessment of contractile effects, the initial U46619 dose was washed out and the vessels left to

110   equilibrate to baseline tension before adding increasing concentrations of a test compound. Every

111   experiment was completed with a final contraction by changing the buffer from PSS to KPSS to

112   confirm viability.

113   **Immunohistochemistry (IHC)**

114   1mm long stem villous arteries post myography were placed immediately placed in Bouin's solution

115   overnight at 4°C. Following fixation, samples were mounted in Optimum Cutting Temperature (OCT)

116   embedding medium (Tissue Tek) and rapidly frozen in liquid N_2 cooled isopentane. The freshly frozen

117   samples were then transferred to a cryostat maintained at -18°C (Leica CM1900) and sectioned to

118   give 5μm thick slices. The sections were then adhered to a gelatine-coated slide (76 x 26 x 1.2mm,

119   VWR) then left to air dry. Haematoxylin and eosin (H&E) staining was used to stain vessels by

120   washing the slides in running tap water for 5mins before placing them in Mayer's Haematoxylin for

121   10mins. The samples were then washed again in running tap water before washing them in Scott's

122   tap water for 2mins to stain the nuclear chromatin and nuclear membranes blue. The samples were

123   washed again in running water for 5mins before placing them in 1% eosin for 3mins. For

124   immunohistochemistry, additional 5μm thick sections were blocked firstly with 0.3% H_2O_2 for

125   20mins. After a 5min PBS wash, 20% horse serum in PBS was applied for 30mins to block non specific

126   antibody binding. Slides were then washed again for 5mins in PBS followed by incubation of 1:50

127   dilution of primary α-actin antibody (DakoCytomation) of each sample for 2hrs at room temperature

128   without shaking. Slides were again washed in PBS for 5mins, before the staining was developed using

129   the avidin-biotin Vectorstain Elite Kit (Universal, Vector laboratories) and antigen localised using 3,3'

130   diaminobenzidine following the manufacturer's instructions. After a brief wash in running water,

131   slides were dehydrated through an ascending series (70%, 90% and 100%) of alcohol concentrations

132   for 2mins each. The samples were then cleared using xylene for 5mins before mounting the slides

133   with glass coverslips coated in DPX mounting medium before viewing under light microscopy (Zeiss

134   Axiovert 25).

135

136   **Transmission electron microscopy (TEM)**

137   Vessel segments from wire myography were fixed overnight with 3% glutaraldehyde. Following this

138   incubation the tissue specimens were washed in 0.1M cacodylate buffer. The tissue was then post

139   fixed in 1% osmium tetroxide in 0.1M cacodylate buffer. Following five 1min washes in distilled

140   water the samples were dehydrated with graded alcohol treatments (50%, 70%, 90% and 100% for

141   15mins each) before finally being treated with 100% propylene oxide for a final 15mins. The samples

142   were then infiltrated with resin (mixed with propylene oxide at ratio of 3:1) for 4h at RT. Finally, the

143   tissue was embedded in a plastic mould which was left to polymerise overnight. For TEM, ultrathin

144   (70nm) sections were cut using a diamond knife (Diatome) and collected on a copper mesh grid

145   ready for viewing using a FEI Tecnai 12 BioTWIN microscope. Images were captured with a

146   Megaview III camera using Soft Imaging System software.

Data analysis

148   Recorded wire myography data was converted from tension to active effective pressure (AEP) to

149   take varying vessel sizes into consideration. AEP was calculated using Laplace's equation, dividing

150   recorded active tension (mN / mm) measurements by the internal vessel radius (mm). Effects are

151   expressed in AEP as a percentage of the maximal AEP achieved with a preceding reference

152   contraction to 10^{-6}M U46619. Where possible, a nonlinear curve fit was performed using Prism 6

153   (GraphPad Software, La Jolla, USA) to determine the EC_{50} or IC_{50} of a concentration-response curve.

154   The three-parameter logistic equation was used to fit all curves, as recommended for data sets with

155   low numbers of data points. Curve fitting was not performed on concentration-response curves

156   lacking recognisable bottom or top plateaus. These incomplete concentration-response curves were

157   caused by limited availability of drugs. To enable the analysis of incomplete concentration-response

158   curves, responses to drug and vehicle were compared using a mixed two-way ANOVA reporting p-

159   values for the treatment factor. The null hypothesis was rejected at $p < 0.05$. Graphs show mean and

160   SEM unless stated otherwise.

161

Results

Wire myography

164   U46619, as a well-known vasoconstrictor in placental vessels, caused the strongest contraction of all

165   tested agents with an EC_{50} of $1.2*10^{-7}$M. The contraction gave stable plateaus at each concentration

166   point and reversed to baseline within 1h of starting PSS washes. Phenylephrine and noradrenaline

167   were tested as well-known vasoconstrictors of the systemic vasculature. Only phenylephrine caused

168   a small statistically significant contraction at high concentrations ($3.3*10^{-5}$M). Arginine vasopressin

169   (AVP) did not show any effect in stem villous arteries. Results for U46619, phenylephrine,

170   noradrenaline and AVP are shown in Figure 1.

171   Figure 2 shows results for 5-HT, angiotensin II and endothelin-1. 5-HT resulted in a contraction of

172   stem villous arteries with an EC_{50} of $1.1*10^{-7}$M, but the maximum AEP was only about a fifth of

173   U46619's effect. Angiotensin II caused small initial contractions that were not sustained and not

174   consistent across tested vessels. The concentration-response curve therefore did not depict any

175   significant effect of this compound. Endothelin-1 caused the second strongest contraction of tested

176   compounds in stem villous arteries with about 60% of U46619's AEP at 10^{-6}M. Due to limited

177   availability of drug, it was not possible to test higher endothelin-1 concentrations for the

178   determination of relative R_{max} to U46619. The contractions to endothelin-1 resulted in stable

179   plateaus that were difficult to wash out. Endothelin-1 induced contractions did not return to the

180   initial baseline within 2h, even after numerous washes using PSS.

181

182   Results for test compounds examined for relaxant properties are shown in Figure 3 and Figure 4.

183   Acetylcholine, bradykinin and substance P did not cause any effects in stem villous arteries.

184   Histamine and sodium nitroprusside (SNP) relaxed vessels to about 50% of the preconstricted AEP

185   with IC_{50} of $1.7*10^{-6}$M and $7*10^{-6}$M respectively. SQ20548 was the most potent relaxant amongst

186   the test compounds, relaxing the vessel back to baseline tensions with an IC_{50} of $2.3*10^{-7}$M. Other

187   substances that showed significant effects were riluzole, nifedipine and papaverine. All vessels

188   relaxed back to baseline levels when drugs were washed out using SPSS.

189   **IHC and TEM**

190   IHC and TEM imaging of the vessel segments enabled a detailed examination of the cell layers within

191   stem villous arteries after wire myography experiments. It was of special interest to verify the

192   integrity of the endothelial layer in order to interpret the effects seen with wire myography. Figure 5

193   depicts a subsection of a stem villus showing the three important portions of a stem villous artery;

194   namely the lumen, EC and SMC are present. A stem villous artery and vein typically run in close

195 proximity to each other within one stem villous branch. In Figure 6, the single cell layer of

196 endothelial cells (EC) can be distinguished by the elastic lamina (EL) which separates the EC layer

197 from smooth muscle cells (SMC). Figure 5 and Figure 6 show that the EC layer does appear to remain

198 intact following vessel isolation and myography.

199

Discussion

201 The placental circulation facilitates adequate supply of nutrients and gases to the fetus. Still little is

202 known about the physiological behaviour of placental resistance vessels and their role in pregnancy

203 complications. For this reason, the present study aimed to evaluate the effect of a range of

204 pharmacological compounds and endogenous lipids on human placental arteries.

205

Effects of various pharmacological compound on stem villus arteries

207 Given the potential significance of stem villous arteries to placental dysfunction, a selection of

208 pharmacological compounds was assessed for their potential contractile or relaxant effects. Two

209 well-known constrictors of the placental vasculature, thromboxane agonist U46619 and endothelin-

210 1 caused reliable and strong contractions in stem villous arteries as previously demonstrated [7-9,

211 14]. The stable thromboxane A_2 receptor agonist U46619 is a strong and reliable vasoconstrictor.

212 This property makes it a commonly used tool to assess vascular function in uteroplacental vessels. It

213 has been shown by a number of groups that maximum tension development in response to U46619

214 is significantly lower in pre-eclampsia, whereas there is no difference in the sensitivity. This has been

215 shown for stem villous arteries [14], chorionic plate arteries [15, 16] and in a perfusion model of

216 placental lobules[17].

217 AVP as a reliable vasoconstrictor of the systemic vasculature did not cause any contraction in stem

218 villous arteries. Vasoconstriction in response to AVP was reported in chorionic plate arteries, but

219 stem villous arteries seem to be inert against this substance [10, 11, 15, 16]. This may be explained

220   by a low placental expression of the AVP receptor, 1A (AVPR1A), which is the main subtype involved

221   in AVP's contractile effect [18].

222   5-HT concentration-response curves showed mild contractions in stem villous arteries, which is in

223   line with previous findings [7, 11] supporting observations for the presence of 5-HT receptors in the

224   placenta [19].

225   Angiotensin II caused transient contractions in stem villous arteries that were prone to tachyphylaxis

226   as previously noted by others [11, 20, 21]. There are also reports of sustained angiotensin-II

227   contractions, but exclusively in chorionic plate arteries or perfused placental lobule preparations [1,

228   22, 23]. Tachyphylaxis to angiotensin II is documented for many tissues other than placentae and is

229   thought to be caused by internalisation or allosteric conformational change of the angiotensin II

230   receptor [24].

231   As the placenta lacks autonomic innervation, it was not unexpected that substances of the

232   autonomic nervous system showed little or no effect [2]. While noradrenaline did not affect vessel

233   tension at all, phenylephrine caused a weak contraction at high concentrations ($3.3*10^{-5}$M). Despite

234   its importance in the systemic vasculature, previous reports indicate that noradrenaline has reduced

235   effects on placental vessels. No effects of noradrenaline could be observed in stem villous arteries,

236   chorionic plate arteries or placental lobules [10, 11, 25]. In the case of phenylephrine, transient and

237   unreliable contractions of chorionic plate veins were reported, which is similar to the effects seen in

238   stem villous arteries in the present study [10, 26].

239   Similarly, as for previously discussed contractile agents, the lack of autonomic innervation can be

240   observed in the ineffectiveness of several known relaxant agents. Acetylcholine (ACh) did not cause

241   any relaxation of preimposed tone although it is a strong vasodilator in the systemic vasculature. The

242   findings of the present study are supported by the observation that the cholinergic agonist,

243   carbachol, did not show any effects in preconstricted chorionic plate arteries [10]. ACh was

244   previously demonstrated to be endogenously released from single placental cotyledons and whole

245   placentae [27]. Protein and mRNA expression of the nicotinic ACh receptor were demonstrated in

246 the human placental vasculature, while the muscarinic ACh receptor could only be detected in

247 syncytiotrophoblasts but not in placental vessels [28, 29]. ACh could therefore potentially act on the

248 placental vasculature via these ACh receptors and currently there is no evidence to explain its lack of

249 impact on vascular tone.

250 Two other endothelium-dependent vasodilators, bradykinin and substance P, similarly did not cause

251 any alteration of the preimposed tone in stem villous arteries. This is again in line with findings of a

252 range of authors who worked with chorionic plate arteries and stem villous arteries [10, 12, 16, 30].

253 Bradykinin has frequently been used as endothelium-dependent vasodilator in studies on

254 uteroplacental blood vessels, via release of NO, prostacyclin and EDHF. The endothelium-dependent

255 vasodilator substance P is a peptide that plays an important role as a neurotransmitter [31].

256 Of all endothelium-dependent dilators, only histamine gave reliable relaxation to preimposed tone.

257 Previous work observed a relaxation to histamine in stem villous arteries, which could only be seen

258 in vessels that were not denuded of endothelium [7]. This supports that the endothelium in

259 examined stem villous arteries of this study was intact, as also shown by TEM and IHC imaging. In

260 contrast to this, a number of authors reported contractile instead of relaxant effects of histamine in

261 chorionic plate arteries [32-34]. It was later found in chorionic plate arteries that part of the

262 histamine induced relaxation is regulated via the H1-receptor mediated endothelium-dependent

263 pathway and part by a direct H2-receptor mediated VSMC relaxation [35]. An initial contractile

264 element at low concentrations of the histamine dose response was achieved over a direct H2-

265 receptor mediated VSMC activation. In the present study, no contractile element was noted in the

266 histamine dose response, which could indicate a different mechanism of action of histamine in stem

267 villous arteries compared to chorionic plate arteries. However, the preconstruction of vessels in this

268 study might have masked a contractile element of the histamine effect, hence a more detailed

269 investigation is needed to confirm the behaviour of stem villous arteries to histamine.

270 The strongest relaxing effect of all tested substances was observed for SQ29548. The thromboxane

271 A_2 receptor antagonist was shown to reduce the sensitivity to U-46619 and 8-isoPGE$_2$ induced

272   contractions in chorionic plate arteries and a placental lobule perfusion model [36, 37]. The

273   relaxation back to baseline levels is not unexpected, as vessels were precontracted using

274   thromboxane receptor agonist U46619. SQ29548 is most probably acting as a competitive receptor

275   antagonist to U46619.

276   Another strong vasodilator of stem villous arteries was sodium nitroprusside (SNP), which

277   emphasises the important role of NO for the control of the placental vasculature. This is consistent

278   with previous reports in stem villous arteries [7, 9] and chorionic plate arteries [10, 38].

279   Other tested endothelium-independent dilators were riluzole and papaverine. Both caused

280   relaxation of the preimposed tone. Papaverine was previously shown to relax chorionic plate arteries

281   [10] and riluzole was shown to relax stem villous arteries and chorionic plate arteries . The

282   endothelium-independent blood vessel relaxant papaverine was first isolated from opium and acts

283   as a PDE inhibitor and calcium channel modulator. The compound riluzole is a glutamate antagonist,

284   sodium channel blocker and potassium channel opener, used for treatment of amyotrophic lateral

285   sclerosis [39]. It acts on TREK-1 (a two-pore-domain potassium channel), which is expressed in

286   placental vessels.

287   As important drug for the treatment of non-gestational and gestational hypertension, the vascular

288   effects of calcium channel antagonist nifedipine were examined. The compound caused relaxation of

289   the preimposed tone in stem villous arteries. Relaxant effects of nifedipine or nitrendipine were

290   previously demonstrated in chorionic plate arteries [40-42] and stem villous arteries [21]. This

291   indicates the presence of L-type calcium channels in stem villous arteries, which were previously

292   only demonstrated to be expressed in trophoblasts [43].

293   In summary, stem villous arteries responded to a wide profile of pharmacological compounds.

294   Contractile effects in stem villous arteries were caused by U46619, 5-HT, angiotensin II and

295   endothelin-1, whereas noradrenaline and AVP failed to result in a contraction. Dilating effects were

296   seen for histamine, riluzole, nifedipine, papaverine, SNP and SQ29548 but not for acetylcholine,

297   bradykinin and substance P. These findings were mostly consistent with research conducted in

298   placental vessels as reviewed above.

299   In general, it is observed that commonly used vasoactive substances of the systemic vasculature

300   such as noradrenaline, AVP and acetylcholine seem to hardly affect stem villous arteries. This is a

301   common finding in all placental vessels and attributable to the missing innervation in the placenta

302   [2].

303   Placental vessels clearly behave differently to vessels of the systemic vasculature. Chorionic plate

304   arteries and stem villous arteries show similar behaviour in many cases but there are several

305   exemptions as well: AVP did not affect stem villous arteries whereas a contraction in chorionic plate

306   arteries was reported by several authors [10, 15, 44, 45]. Furthermore, no contractile effect of

307   histamine could be observed, as reported in chorionic plate arteries [32-34]. Given their importance

308   in the placental circulation, it is therefore important to consider stem villous arteries as a distinct

309   vascular bed in future research.

310

311   **The endothelium in the placental vasculature**

312   The integrity of the endothelium in the experimental setup is of particular interest, as effects various

313   compounds are dependent on its presence. Therefore, experimental protocols typically involve

314   checking endothelial function using acetylcholine [3]. However, acetylcholine and other

315   endothelium-dependent vasodilators as bradykinin and substance P did not affect vascular tension

316   in stem villous arteries as previously shown by a number of authors [6, 7, 10, 12, 16, 30]. An

317   evaluation of endothelial integrity in the present study using the conventional acetylcholine

318   relaxation was therefore not possible. Only one endothelium-dependent dilator, histamine, caused

319   vasorelaxation whereby part of the dilating effect is, at least in chorionic plate arteries, attributed to

320   an endothelium-independent process [35]. Assessment of the endothelial function in stem villous

321   arteries revealed that histamine induced relaxations only in presence of the endothelium [7, 8].

322   These relaxations to histamine were also observed in the present study, which is an indicator that

323 the endothelium of stem villous arteries used in this study was intact. However, the role of the

324 endothelium in stem villous arteries is poorly characterised. It is also doubtful that knowledge from

325 other vascular beds such as chorionic plate arteries can be transferred and applied to stem villous

326 arteries as they show considerably different vascular behaviour. For this reason, an in depth

327 investigation is required to evaluate the effect of endothelium removal on vascular function in stem

328 villous arteries. TEM imaging showed that the endothelium of stem villous arteries is present after

329 the mounting procedure. The discrepancy of the endothelial function when comparing to other

330 vascular beds can therefore only be explained on cellular level. The absence of effects caused by

331 bradykinin or acetylcholine could also be explained by elevated intrinsic NO levels in the pregnancy

332 [6]. Permanent basal NO production is thought to be key for the physiological maintenance of low

333 vascular resistance in the placenta [1, 5, 6]. At the same time, NO inhibits CYP enzymes and with that

334 the release of EDHF [46]. There are various compounds produced by CYP that are thought to

335 contribute to the EDHF effect [47]. In general, it was suggested that the EDHF pathway might act as

336 backup mechanism in vessels with impaired NO availability possibility due to endothelial dysfunction

337 [48]. In the experimental setup of the present study, NO release by bradykinin or acetylcholine might

338 not considerably add to the already increased NO availability. Furthermore, the NO-independent,

339 CYP dependent component of the bradykinin/acetylcholine relaxation might be attenuated as CYP

340 enzymes are blocked by high NO levels. However, this hypothesis needs to be tested and confirmed.

341

342 In conclusion, the assessment of various pharmacological compounds provided a valuable overview

343 of the physiological behaviour of stem villous arteries. This work will also be useful knowledge for

344 future studies, where pharmacological tools are required to assess vascular function. Substances

345 that are part of the autonomous system such as noradrenaline or acetylcholine showed no effects in

346 stem villous arteries, which cuts the placenta off from the systemic maternal vascular regulation.

347 The fact that stem villous arteries responded to a range of mediators that were previously reported

348 to elicit altered vascular effects in pre-eclampsia, creates the base for future research on stem

349 villous arteries in the context of hypertensive gestational diseases. In this context, use of more

350 specific blockers targeting individual pathways would enable a detailed understanding of the

351 placental physiology.

352 Our observation that particularly endothelium-dependent processes were attenuated in the

353 placental vasculature indicates that there is an urgent need to determine the role of the

354 endothelium in the placenta in future studies.

355

Acknowledgements

361

Conflict of interest

363 None.

364

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488 **Figures**
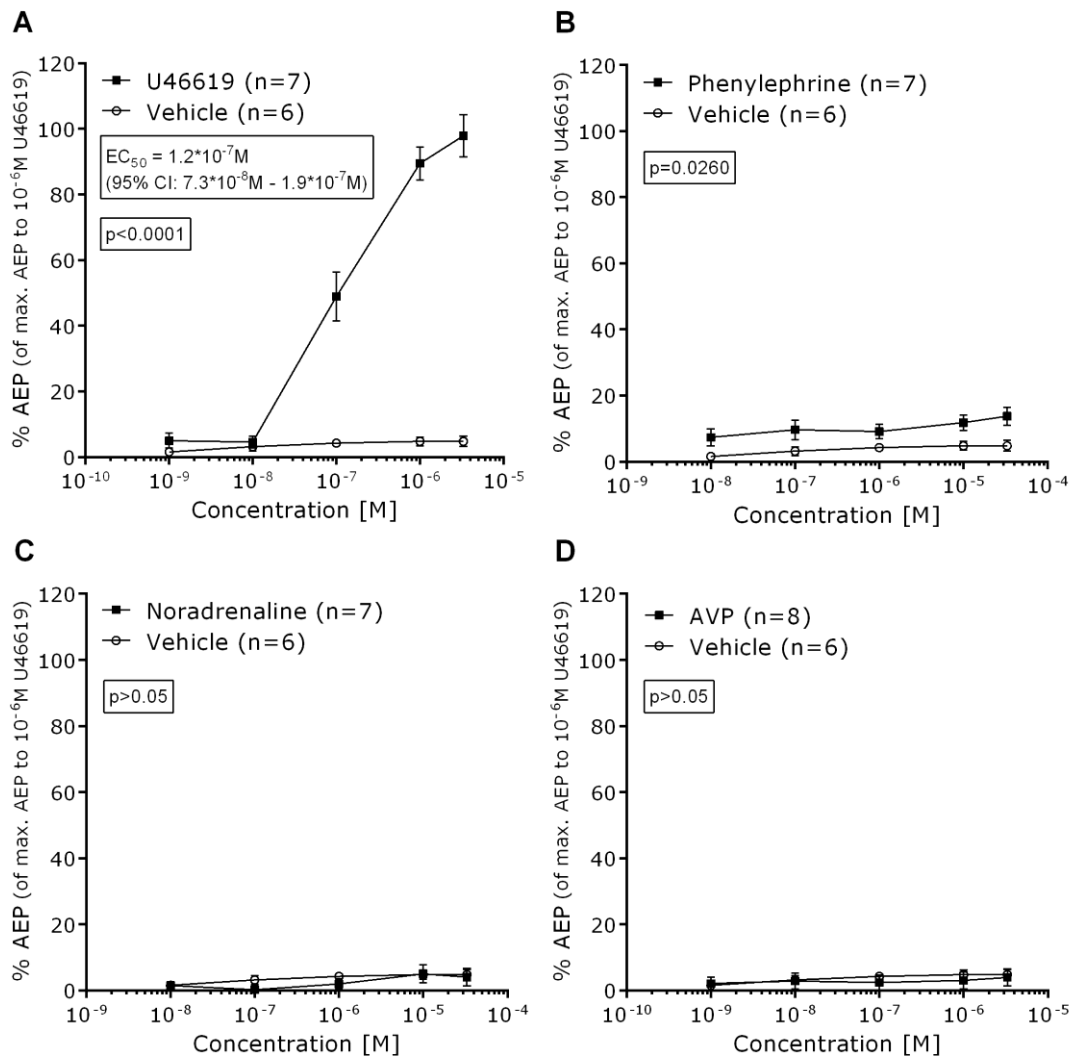
489

490 Figure 1: Effect of (A) U46619, (B) phenylephrine, (C) noradrenaline and (D) AVP on stem villous arteries. Bars show

491 mean and SEM with solid squares representing the tested substance and open circles representing the vehicle control.

492 Effects are expressed as AEP in percent of the maximal AEP achieved with a preceding reference contraction to 10^{-6}M of

493 U46619. All vessels were normalised to $0.9*IC_{5.1}$kPa. Significance was tested using a mixed two-way ANOVA.

A

B
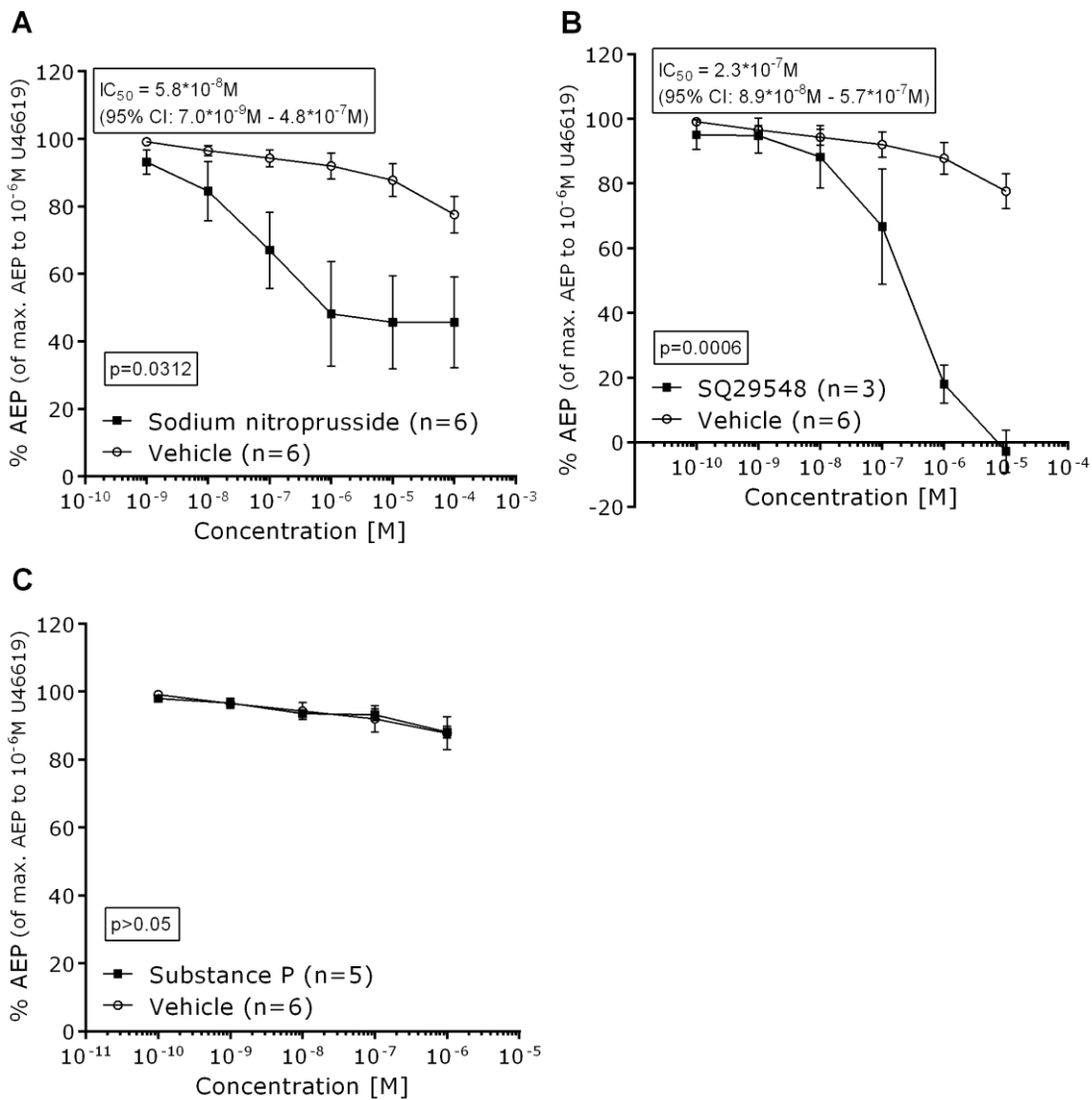
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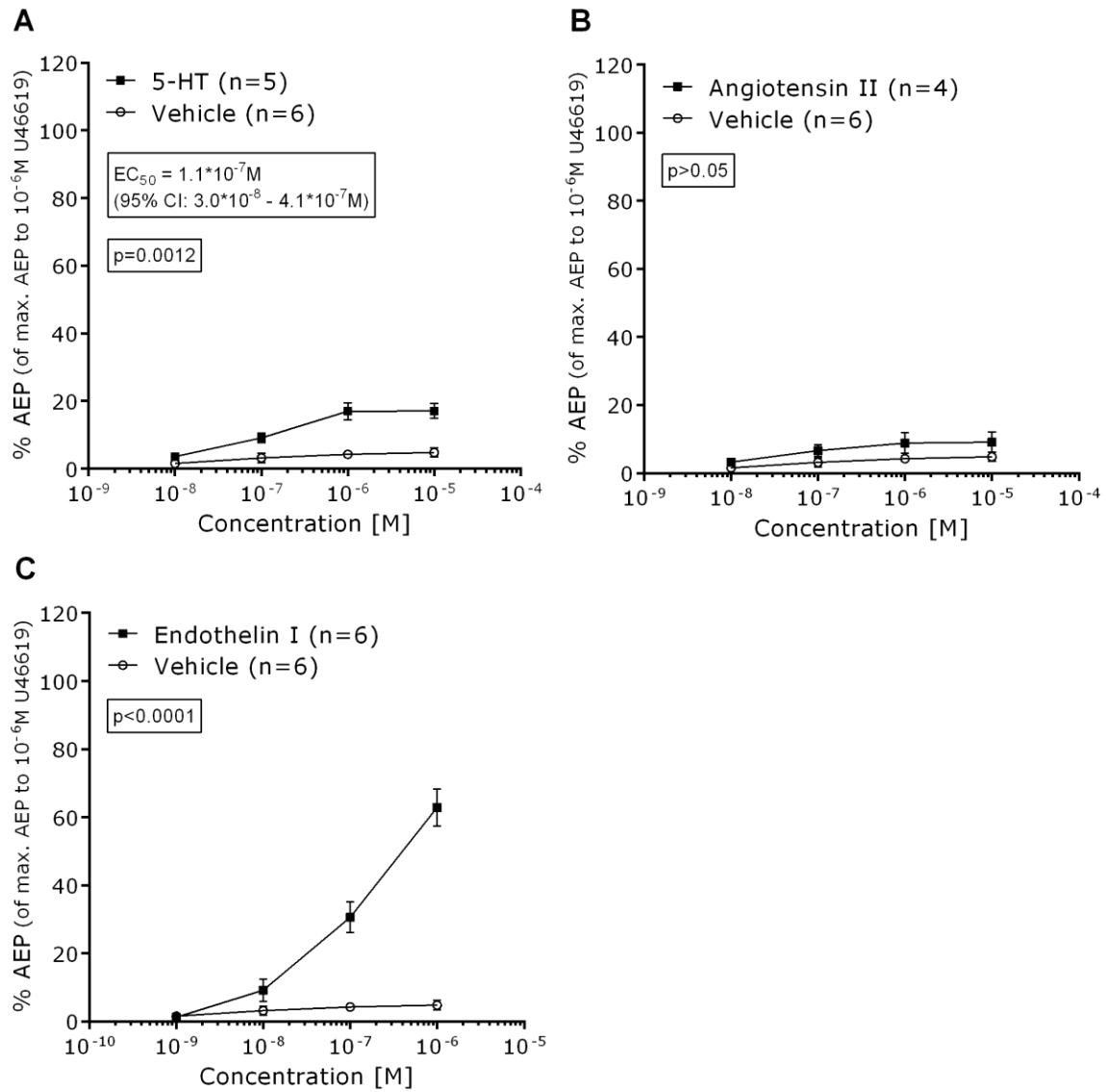
Figure 2: Effect of (A) 5-HT, (B) angiotensin II and (C) endothelin-1 on stem villous arteries. Bars show mean and SEM with solid squares representing the tested substance and open circles representing the vehicle control. Effects are expressed as AEP in percent of the maximal AEP achieved with a preceding reference contraction to 10^{-6}M of U46619. All vessels were normalised to $0.9*IC_{5.1}$kPa. Significance was tested using a mixed two-way ANOVA.
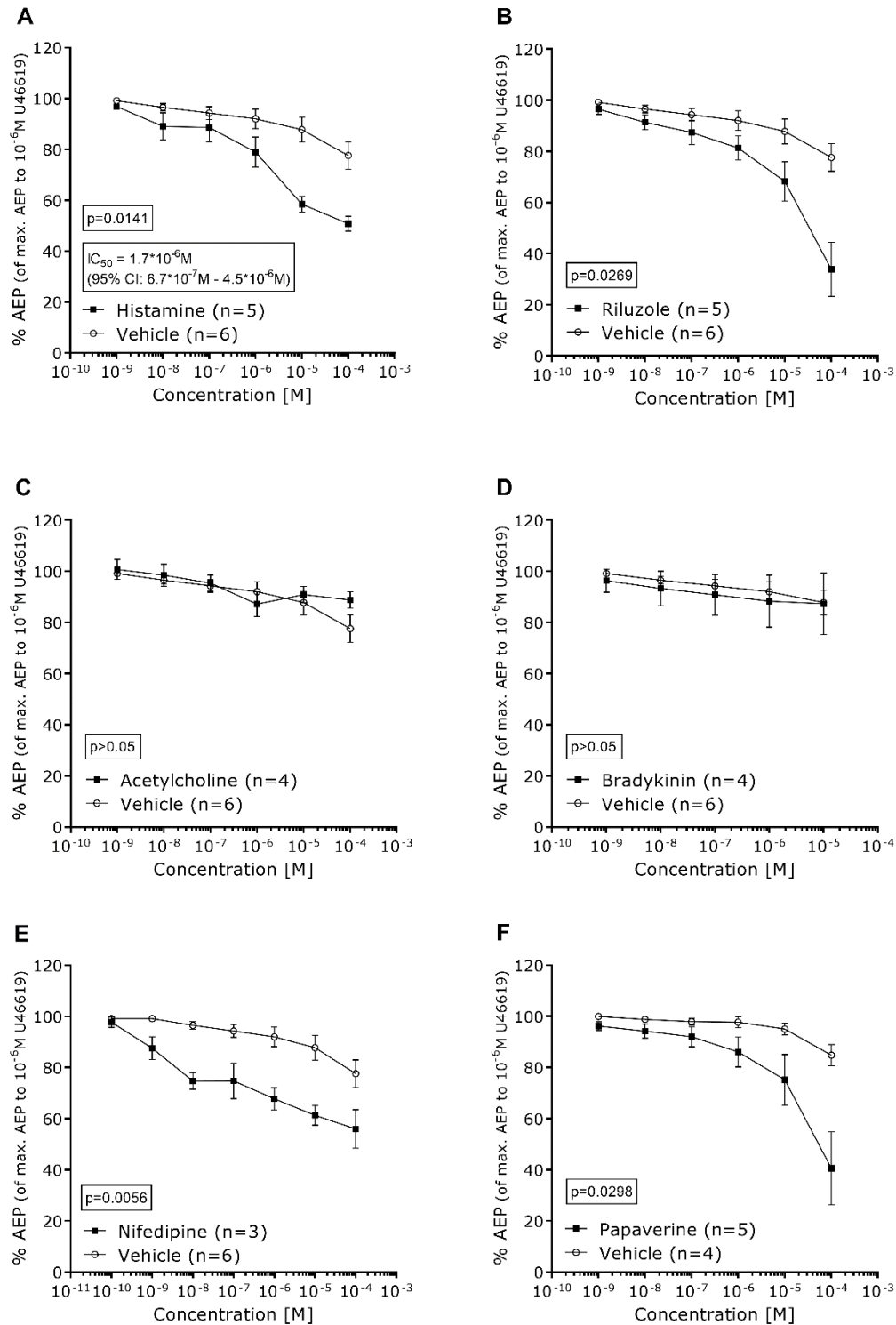
Figure 3: Effect of (A) histamine, (B) riluzole, (C) acetylcholine, (D) bradykinin, (E) nifedipine and (F) papaverine on stem villous arteries. Bars show mean and SEM, solid squares representing the tested substance and open circles representing the vehicle control. Effects are expressed as AEP in percent of the maximal AEP achieved with a preceding reference contraction to 10^{-6}M of U46619. All vessels were normalised to $0.9*IC_{5.1}$kPa. Significance was tested using a mixed two-way ANOVA.

506

Figure 4: Effect of (A) sodium nitroprusside, (B) SQ29548 and (C) substance P on stem villous arteries. Bars show mean and SEM with solid squares representing the tested substance and open circles representing the vehicle control. Effects are expressed as AEP in percent of the maximal AEP achieved with a preceding reference contraction to 10^{-6}M of U46619. All vessels were normalised to $0.9*IC_{5.1}$kPa. Significance was tested using a mixed two-way ANOVA.

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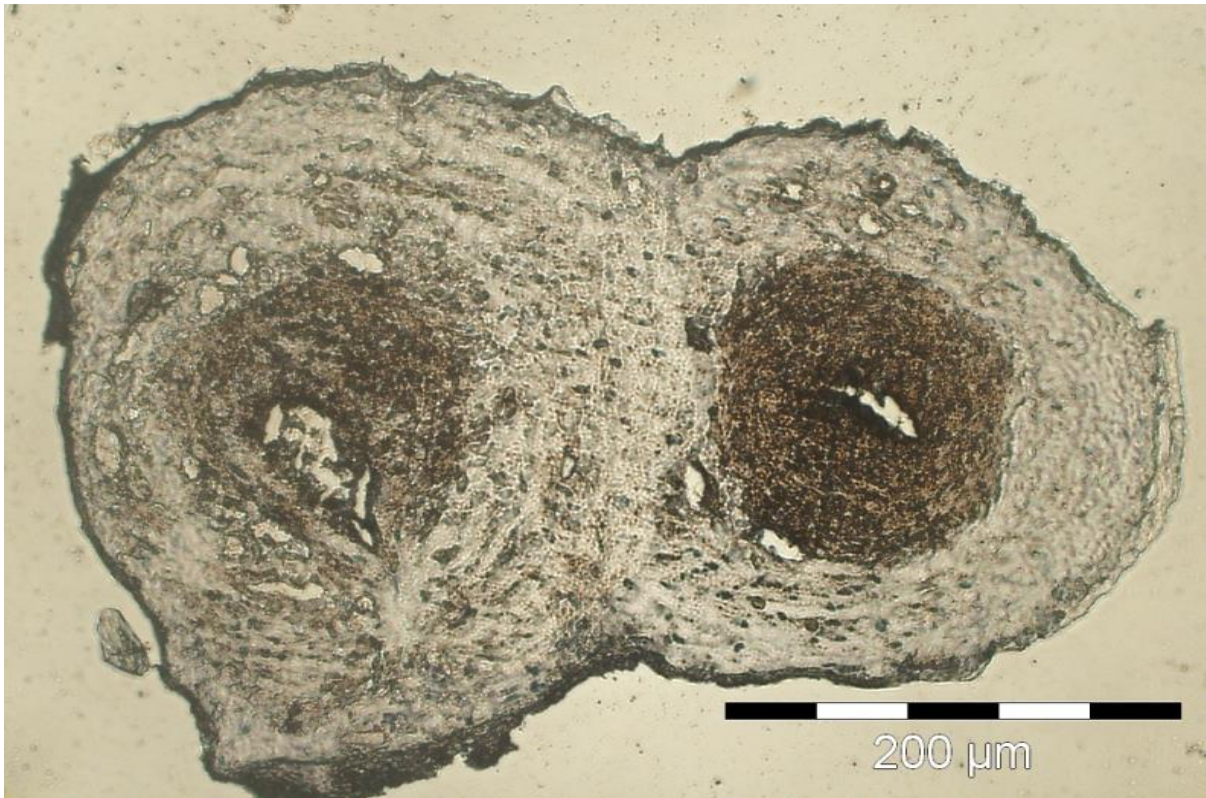
513

514 **Figure 5: IHC showing a subsection of a stem villus. The SMC layer around the artery (right) detected with α-actin is**

515 **thicker and more prominent compared to the vein (left). The endothelium can be seen as a dense stain around the**

516 **lumen of the stem villous artery.**
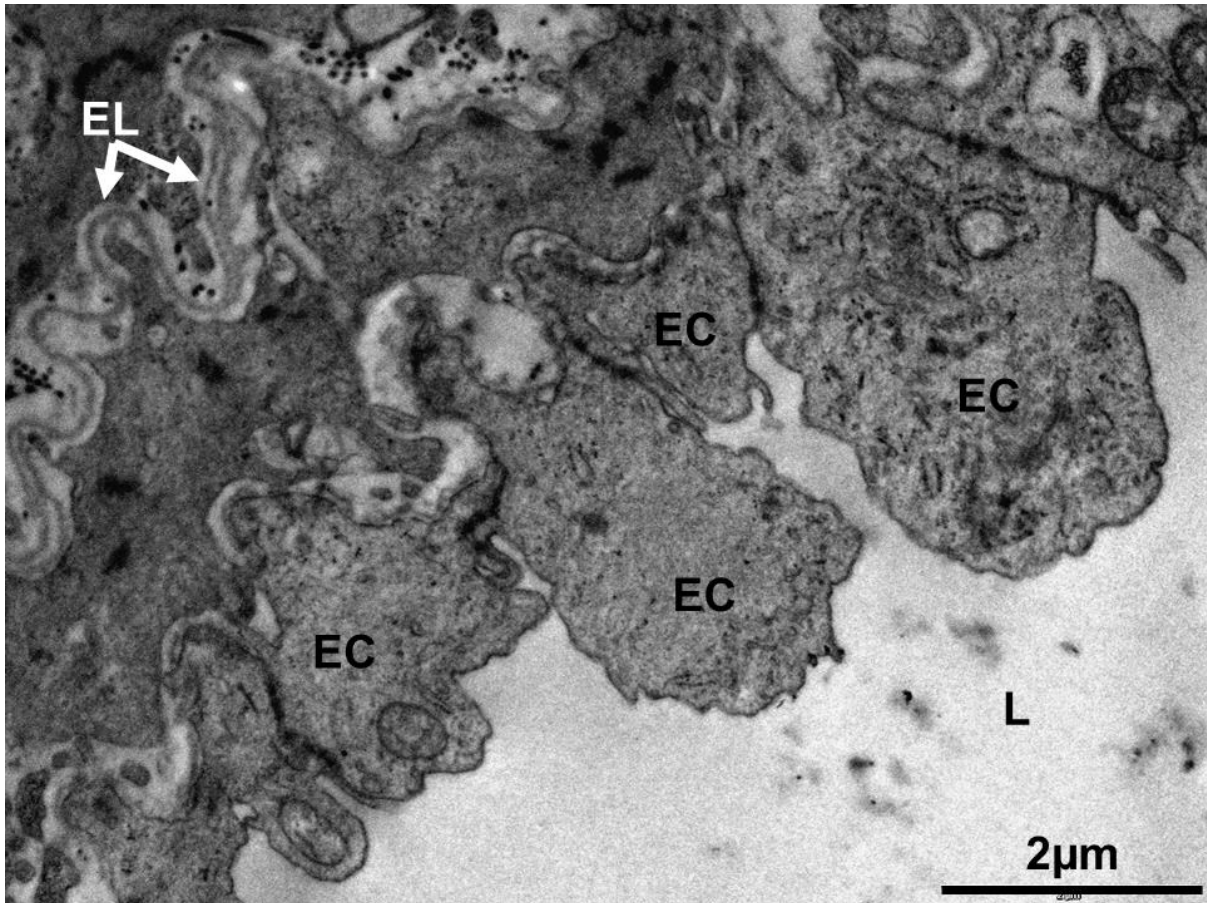
Figure 6: TEM showing a subsection of a stem villous artery with intact endothelium. EC: Endothelial cell; EL: Elastic lamina; L: Lumen.

521 **Tables**

522

523 Table 1: Patient demographics for collected placentae. Table shows mean (standard deviation) or total numbers. N=33.
524 yrs: years; wks: weeks. Customised weight centiles were calculated using Weight Centile Calculator from GROW
525 software version 8.0.4 (UK), 2019 [49, 50].

Age [yrs]	32.1 (6.2)
BMI (at booking)	30.1 (7.9)
Gravida	3.1 (1.5)
Parity	1.4 (1.1)
Gestational week at delivery [wks]	38.5 (1.2)
Birthweight [g]	3491.8 (548.1)
Customised weight centile	61.6 (28.2)
Sex baby	22 female, 11 male

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