Accepted Manuscript

Biopsy techniques to study the human placental bed

Jan H.W. Veerbeek, MD, Emiel D. Post Uiterweer, MD, Peter G.J. Nikkels, MD PhD, Steven V. Koenen, MD PhD, Marieke Van Der Zalm, Maria P.H. Koster, MD PhD, Graham J. Burton, MD PhD, Bas B. Van Rijn, MD PhD, Arie Franx, MD PhD

Title

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Authors

Dr. Jan H.W. VEERBEEK, MD [a,b]

Dr. Emiel D. POST UITERWEER, MD [a]

Dr. Peter G.J. NIKKELS, MD PhD [c]

Dr. Steven V. KOENEN, MD PhD [a]

Dr. Maria P.H. KOSTER, MD PhD [a]

Prof Graham J BURTON, MD PhD [b]

Prof. Fiona LYALL, PhD [d]

Dr. Bas B. VAN RIJN, MD PhD [a,e]

Prof. Arie FRANX, MD PhD [a]

Affiliations

[a] Birth Centre, University Medical Centre Utrecht, Utrecht, the Netherlands

[b] Centre for Trophoblast research, Department of Physiology, Development & Neuroscience, University of Cambridge, Cambridge, United Kingdom

[c] Department of Pathology, University Medical Centre Utrecht, Utrecht, the Netherlands

[d] Institute of Medical Genetics, College of Medicine, Veterinary and Life Sciences, University of Glasgow School of Medicine, Glasgow, United Kingdom

[e] Academic Unit of Human Development and Health, University of Southampton, Southampton, United Kingdom

Disclosure Statement

The authors report no conflict of interest.

Corresponding Author

J.H.W. Veerbeek, MD

Department of Obstetrics

UMC Utrecht, loc. WKZ

PO Box: 85090 (room: KE04.123.1)

3508 AB Utrecht

The Netherlands

Phone: +31(0) 88-7553940

Fax: +31(0) 88-7555320

Email: J.H.W.Veerbeek@umcutrecht.nl

Word count

Abstract: 241

(Main text: 5490 (excluding tables))

Abstract

The physiologic transformation of uterine spiral arteries in the human placental bed is essential for a healthy pregnancy. Failure of this transformation due to deficient trophoblast invasion is widely believed to underlie pregnancy complications such as preeclampsia, foetal growth restriction, miscarriage and preterm labour. Understanding of invasive behaviour and remodelling properties of trophoblasts in the uterine wall is essential in elucidating the aetiology of these pregnancy complications. However, there is a lack of satisfactory specimens of the placental bed to enhance our knowledge on the mechanisms that control trophoblast invasion. Several techniques can be used to obtain biopsies from the placental bed and sample handling can be executed differently depending on the research question. This systematic review provides an overview of all studies investigating the placental bed and sampling techniques used. Papers that described surgical techniques, specimen handling, complications and/or success rate of the placental bed biopsy procedures were included. Placental bed biopsies are an essential and feasible technique to study abnormalities in the placental bed associated with pregnancy complications. Depending on the technique used the likelihood of sampling a spiral artery and trophoblast from the placental bed is 51% to 78% per case, without significant complications. Caution is needed when interpreting data if the placental bed is subjected to labour. We propose a uniform sampling technique and conservation protocol for the study of the placental bed and provide tools for selection of the appropriate technique for future placental bed collections.

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5 Jan H.W. VEERBEEK, MD [a,b]

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7 Peter G.J. NIKKELS, MD PhD [c]

8 Steven V. KOENEN, MD PhD [a]

9 Marieke VAN DER ZALM [a]

10 Maria P.H. KOSTER, MD PhD [a]

11 Graham J BURTON, MD PhD [b]

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13 Arie FRANX, MD PhD [a]

14

15 **Affiliations**

16 [a] Birth Centre, University Medical Centre Utrecht, Utrecht, the Netherlands

17 [b] Centre for Trophoblast research, Department of Physiology, Development &

18 Neuroscience, University of Cambridge, Cambridge, United Kingdom

19 [c] Department of Pathology, University Medical Centre Utrecht, Utrecht, the

20 Netherlands

21 [d] Academic Unit of Human Development and Health, University of Southampton,

22 Southampton, United Kingdom

23

24

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28   **Corresponding Author**

29   J.H.W. Veerbeek, MD

30   Department of Obstetrics

31   UMC Utrecht, loc. WKZ

32   PO Box: 85090 (room: KE04.123.1)

33   3508 AB Utrecht

34   The Netherlands

35   Phone: +31(0) 88-7553940

36   Fax: +31(0) 88-7555320

37   Email: j.h.w.veerbeek@umcutrecht.nl

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Introduction

Over the last five decades extravillous trophoblast (EVT) dependent spiral artery remodelling has emerged as a vital process in establishing a functional human haemochorial placenta (1). It is thought that this process is preceded by decidua associated and trophoblast independent vascular remodelling. (1) Despite extensive research it remains largely unknown how EVT invasion is regulated in vivo and how EVTs facilitate the extensive so called "physiological change" of the spiral arteries of the placental bed (2). In recent years maternal immune cells such as the abundant uterine natural killer (NK) cells and macrophages have been attributed as key regulators of the placental bed, regulating both EVT invasion and spiral artery remodelling (3). How failure of deep EVT invasion and spiral artery remodelling contributes to the aetiology of placental disorders such as recurrent miscarriage, preeclampsia, intra uterine growth restriction (IUGR), and preterm birth remains to be elucidated.

Major modifications of the placental bed take place during early pregnancy (4). The study of early developmental processes in the placental bed *during* pregnancy is not feasible, and many studies have relied on samples of the placenta bed *after* (preterm) delivery of the placenta. However, functional studies on these specimens are very limited and most investigators have resorted to *in vitro* models of trophoblast invasion with the use of modified human trophoblast cell lines. Although these studies have given us a better understanding of the molecular mechanisms regulating invasion and remodelling of the placental bed spiral arteries, the interpretation of data must be executed with caution as they do not reflect the *in vivo* situation. Therefore, representative placental bed biopsies are still needed from both

75 early and late normal and complicated pregnancies, taken by reproducible

76 techniques and coupled to detailed clinical characterisation of the study subjects.

77 A 'true' placental bed biopsy consists of both decidual and myometrial tissue and

78 contains EVTs. However, in order to study spiral artery remodelling a 'successful'

79 placental bed biopsy must contain at least one spiral artery. To obtain a successful

80 placental bed biopsy is challenging, because the placental bed only contains around

81 30-60 spiral arteries (5-7). The success rate varies with the biopsy technique used

82 and interpretation of data is heavily dependent on subsequent tissue handling and

83 conservation. Choosing the right technique and sample processing is therefore of

84 pivotal importance.

85 In this review we provide a comprehensive overview of the techniques employed in

86 current studies of the human placental bed and the limited number of biopsy

87 collections worldwide. Techniques are discussed with their corresponding success

88 rates. Finally, we propose a uniform protocol for sampling the human placental bed

89 and subsequent tissue processing in order to increase the availability of comparable

90 and good quality specimens with which to unravel the mechanisms of trophoblast

91 invasion and spiral artery remodelling, and their failure in adverse pregnancy

92 outcomes.

93

94 **Methods**

95 Studies included were identified by a systematic search of the databases PubMed,

96 Embase and Cochrane. The following search terms were combined using the

97 Boolean operator OR: placental bed, spiral arteries, uteroplacental arteries and

98 preeclampsia. The complete search query is presented in Table 1. The search string

99 was restricted to title and abstract. All papers, written in English, that described

100 studies of the placental bed in humans, using placental bed biopsies or hysterectomy

101 specimens taken after caesarean or vaginal delivery, were included. Case reports,

102 expert opinions, studies concerning placental bed research in animals and reviews

103 that did not describe actual placental bed studies were excluded. To identify relevant

104 studies not covered by the search, cross-references were also picked up in the

105 review process and screened for relevance. A total of 1027 papers were identified

106 with the search query and after duplicates were removed. After screening title and

107 abstract for the inclusion and exclusion criteria mentioned above, 130 papers

108 remained for full text analysis. Finally, full text screening identified 91 studies that

109 have used placental bed biopsies. Together with 3 additional studies found by cross-

110 references a total of 94 papers were included. All papers were examined to give an

111 overview of the biopsy techniques used. However, not all papers have reported

112 success rates of the biopsy technique used and some studies have used archived

113 collections of placental bed biopsies, in which case only the original study was

114 included in the analysis of success rates. Twenty-five papers reported success rates

115 of their biopsy technique. (8-32) Successful biopsies were defined as either sampling

116 a myometrial spiral artery with trophoblast or any spiral artery and trophoblast. In

117 Supplemental Figure 1 a flowchart of the selection progress is shown.

118 If success rates were reported for each studied patient group separately an average

119 rate for that paper was calculated. Average success rates for each technique were

120 calculated including only papers that reported success rate per case (and not per

121 biopsy). A weighted mean was calculated to include sample size in the equation.

122 Statistical analyses were performed using SPSS (release 20.0; Chicago, IL).

123

124 **Results**

125

Historical overview placental bed research

127 From meticulous examinations of the human placenta *in situ* in the 16th century, it

128 was concluded for the first time that there is no direct vascular connection between

129 mother and foetus. (33) This finding presented indirect evidence for the existence of

130 a vascular bed in the uterine wall adjacent to the placenta: the 'placental bed'. The

131 Hunter brothers published the first illustrations of the placental bed. Using coloured

132 wax injected into specimens of human uteri with placentas *in situ* they revealed the

133 first direct macroscopic evidence for spiral arteries in the placental bed. (34)

134 With the discovery of the microscope and its rapid advances, trophoblasts were

135 identified in the second half of the 19th century. Not long thereafter the invasive

136 nature of the trophoblast was first appreciated. (1) The advances in early placenta

137 bed research were attributed to the availability of hysterectomy and post-mortem

138 specimens of uteri with *in situ* placentas. Several of these specimens are preserved

139 in histological collections and often accessibility is limited. The biggest collections of

140 early pregnancy material are stored in the Boyd collection at the Centre for

141 Trophoblast Research, University of Cambridge (35)   and the Carnegie Collection at

142 the Human Developmental Anatomy Center, Washington DC.(36)) The Carnegie

143 collection consists mainly of the specimens preserved by Hertig and Rock who

144 carefully collected very early embryos and implantation sites from hysterectomies.

145 The placenta-in-situ samples of the Boyd collection are overrepresented by low-lying

146 placentas that may have caused the need for hysterectomy. Although no tissue

147 blocks remain the collection holds *in situ* placenta hysterectomies from various

148 gestational ages that are partly digitalized and accessible on the website of the

6

149 Centre for Trophoblast Research. (http://www.trophoblast.cam.ac.uk). Both

150 collections were fundamental for the study of the placenta.

151 Ground breaking work on spiral artery remodelling in the placental bed has

152 undoubtedly been performed by Brosens (9,10,37-39) and Pijnenborg et al. (4,40,41)

153 The early histological studies Pijnenborg conducted on the placental bed revealed a

154 higher density of extravillous trophoblasts (EVTs) in the proximity of spiral arteries

155 with morphological signs of remodelling. The specimens that were used for these

156 studies came from an impressive collection of 48 intact uteri with pregnancies

157 ranging from 8 to 18 weeks gestational age. Advances in obstetric care and surgical

158 techniques have made pregnant hysterectomy a rare procedure. Therefore, obtaining

159 new hysterectomy samples is very uncommon, let alone in complicated pregnancies.

160 However, the growing body of evidence implicating abnormalities of the placental bed

161 in the genesis of pregnancy complications, such as preeclampsia, increases the

162 need for representative samples to study the placental bed. (42,43)

163

164 **"Modern" placental bed biopsy techniques**

165 In the early 1950's, Hertig(44) and, Zeek and Assali (45) among others described

166 changes in decidual vessels that were recognized as having acute atherosis.

167 However, the authors acknowledged the fact that the material was not adequate and

168 that further study of maternal vessels deeper in the placental bed was required. The

169 research on the role of spiral arteries was stimulated by Browne and Veall when they

170 reported in a paper published in 1953 that defective placental perfusion might

171 underlie hypertensive pregnancies. (42) In 1958 Dixon and Robertson introduced a

172 technique to obtain representative placental bed material suitable for histological

173 examination of such deeper myometrial maternal vessels.(46) Using a punch biopsy

174 technique during caesarean section they were the first to collect myometrial parts of

175 the spiral arteries apart from the existing hysterectomy and post-mortem uterus

176 specimens. At the same time a group in Leuven independently collected placental

177 biopsies, using a sharpened ovum forceps they obtained biopsies by the transvaginal

178 route. Since that time several techniques for the sampling of the placental bed have

179 been used which we will discuss in the following section.

180

181 *Punch biopsy at caesarean section*

182 Since its introduction in 1958, the *punch biopsy* technique has been widely used.

183 Gerretsen et al studied the physiological changes of spiral arteries in normal

184 pregnancies and a variety of pregnancy complications.(14) The authors used the

185 technique to obtain 1 sample of the placental bed of 175 patients. In 42% of the

186 cases they found at least one spiral artery and trophoblasts. Robson et al sampled

187 139 patients during caesarean section and collected successful biopsies in 45% of

188 the normal pregnancies and 52% of the pregnancies complicated by preeclampsia.

189 (28) Eight biopsies were taken under direct vision of the placental bed after placental

190 removal and intravenous administration of 10IU oxytocin to facilitate uterine

191 contraction. Taking multiple biopsies from the placental bed seemed not to enhance

192 the chances of successfully sampling the bed.

193 Our group recently started a new biobank study in which the punch biopsy technique

194 during caesarean section has shown comparable success rates. Collecting 280

195 biopsies from 70 patients we managed to sample the placental bed containing at

196 least one spiral artery and EVT in 57% of the cases (unpublished data). Our

197 proposed standardized protocol will be discussed in detail later.

198 The studies that reported the likelihood of sampling a spiral artery and trophoblasts

199 from the placental bed with punch biopsy at caesarean section are shown in Table 1.

200 With this technique an average success rate of 51% is feasible.

201

202 *Transcervical punch biopsy*

203 The punch biopsy technique was not only used in the setting of caesarean sections.

204 Gerretsen *et al.* also employed a comparable technique in 1981 after vaginal delivery

205 and reported a success rate of 33%.(47) Success was defined as sampling tissue

206 containing interstitial trophoblasts and spiral arteries at the endometrial-myometrial

207 junction. In 1990 Michel *et al.* were the first to describe the ultrasound-guided

208 placental bed biopsy.(48)) Focusing on leucocytes in the first trimester they did not

209 report the number of spiral arteries in their biopsies. By excluding the cases with

210 insufficient myometrial vessels it is impossible to assess the success rate. The

211 experiences from Robson and colleagues in sampling under ultrasound guidance

212 were discussed in their publication in 2002. (28) They report success rates with both

213 transcervical techniques as well as biopsies under direct vision during caesarean

214 section as discussed earlier. Currently this is the largest series of early placental bed

215 biopsies reported in the literature. Biopsies were attempted in 313 women who

216 underwent termination of pregnancy (TOP) before 20 weeks of gestation and in 104

217 women in need of evacuation of retained products of conception (ERPC) after foetal

218 death between 7 and 21 weeks of gestation. In short, after induction of general

219 anaesthesia an ultrasound scan was performed for placenta localization and

220 estimation of gestational age. After evacuation of the uterine contents a biopsy

221 forceps was introduced under ultrasound guidance to the site of the presumed

222 placental bed. Four biopsies were then taken from the central placental bed. With this

223 procedure 17% to 77% of the biopsies were successful in sampling a spiral artery.

224 There was a significant positive correlation between gestational age and the

225 likelihood of obtaining at least one spiral artery. The latter was probably related to the

226 difficulty to clearly visualize the placental bed with ultrasound in very early

227 pregnancy. There were no complications recorded that could be related to the biopsy

228 procedure.

229 This biobank of placental bed biopsies has been extensively used to study spiral

230 artery remodelling and related processes. They contributed, for example, to our

231 knowledge on the role of adhesion molecules (49), nitric oxide (50), transforming

232 growth factor beta (49), interleukin-6 and 8 (51), and vascular smooth muscle cell

233 apoptosis (52) in trophoblast invasion and spiral artery remodelling.

234 Studies that reported the likelihood of sampling a spiral artery and trophoblasts from

235 the placental bed with punch biopsy after vaginal delivery, TOP or ERPC are shown

236 in Table 2. With this technique an average success rate of 54% is feasible.

237

238 *Scissors or scalpel biopsy technique*

239 Robertson and colleagues published their 30-year experience from three centres in

240 sampling the placental bed in 1986. (53) Centres in Leuven Belgium, London United

241 Kingdom and Dublin Ireland have collected placental bed samples with curved

242 scissors or scalpel, a technique that is reported to sample true placental bed in more

243 than 70% of the cases. However, it is not clear if success was defined as the

244 presence of spiral arteries or presence of interstitial trophoblasts alone. Biopsies

245 were taken at caesarean section under direct visualisation of the placental bed. After

246 digitally marking the centre of the placenta, the placenta itself was peeled away and

247 the supposed placental site and surroundings were carefully inspected. With curved

10

248   scissors a disk of 1.5 cm in diameter was removed from the central part of the

249   placental bed. A depth of 0.5 cm proved to be more than sufficient to obtain the

250   myometrial sections of spiral arteries. The same dimensions can be obtained when a

251   surgical scalpel is used to collect a wedge-shaped biopsy. The resulting defect was

252   closed with absorbable sutures. There were no reports of biopsy-related

253   complications and this technique has been widely used ever since. A variant of the

254   scalpel biopsy technique was published by Pijnenborg et al. (26). A 22-gauge

255   marking needle was thrust through the serosal side of the uterus through the centre

256   of the placental bed that was manually localized previously. A second needle was

257   inserted from the decidual side into the uterus next to the marking needle that could

258   then be removed. With a sharp scalpel a cone of 1-2 cm wide and 1cm deep was

259   obtained. In 68% of hypertensive women the biopsies contained at least one spiral

260   artery, whereas normotensive women were accurately sampled in 35% of the cases.

261   The difference between success rates was not addressed in the paper.

262   The studies that reported the likelihood of sampling a spiral artery and trophoblasts

263   from the placental bed with scalpel or scissors are shown in Table 3. With this

264   technique an average success rate of 58% is feasible.

265

266   *Vacuum suction technique*

267   More recently Harsem evaluated a new technique collecting decidual tissue by

268   vacuum suction for functional and morphological studies described earlier by Staff et

269   al. (17,54) Quality criteria included the presence of spiral arteries and extravillous

270   trophoblasts. However, the collected decidual samples do not fill the criteria of true

271   placental bed biopsies, as discussed earlier. Morphological features of the obtained

272   biopsies were compared with archived placental bed material and basal plate

273 sections. During caesarean section 5IU oxytocin was given intravenously after

274 delivery of the baby to ensure uterine contraction. The placenta was then manually

275 located and gently removed from the uterine wall. Placental bed tissue was collected

276 with suction force (vacuum systems such as used for uterine evacuation) into a

277 tissue-sampling box, which in turn was flushed with 500 mL of sterile saline to

278 remove blood. A random portion was selected for morphological studies. In the

279 evaluation study 51 women undergoing caesarean sections and vacuum suctions

280 were included and compared to 33 placental bed punch biopsies and 33 placental

281 plate specimens sampled from the maternal side of the delivered placenta. In 86% of

282 the decidual suction samples one or more spiral arteries were found. Placental bed

283 biopsies and placental plate biopsies contained spiral arteries in 61% and 48%

284 respectively. In the long term follow up of 38-60 months after vacuum suction there

285 were no biopsy related complications reported. (17)

286 The studies that reported the likelihood of sampling a spiral artery and trophoblasts

287 from the decidua with vacuum suction are shown in Table 4. With this technique an

288 average success rate of 78% is feasible although the chances of obtaining

289 myometrial spiral arteries is only 13%. (4)

290

291 *Other techniques*

292 Several other techniques have been used to sample the placental bed. Most of the

293 studies did not report any success rates merely because the aim was to investigate

294 the metabolism or the immune system in relation to localisation within the foetal

295 placental unit. For instance, Schafer et al. collected decidual material from the

296 placental bed during caesarean section by wiping the uterine cavity with a tampon.

297 The number of spiral arteries or interstitial trophoblasts is not discussed. (55)

298 Transcervically obtaining spiral arteries from the placental bed by curettage after

299 vaginal delivery has proven not to be successful. Only one out of 46 (2%) biopsies

300 was successful in sampling an appropriate portion of spiral artery. The technique was

301 therefore abandoned by Gerretsen et al. (47) Stallmach et al. used curettage of the

302 placental bed under direct vision during caesarean section to sample the placental

303 bed. (56) Their success rates seem to be significantly higher with an average of 6,2

304 spiral arteries per sample. However, no reports were made on samples without spiral

305 arteries. Interestingly, although no incisions were made in the uterine wall, biopsies

306 'usually' contained myometrium.

307 It is beyond the scope of this review to discuss all studies that have sampled decidual

308 tissue and basal plate on the delivered placenta. Although Khong and Chambers

309 showed that *en face* specimens from the basal plate of the delivered placenta could

310 sample spiral arteries with a 56.4% success rate we focussed on techniques of direct

311 sampling of the placental bed. (57)

312

313 **Localization of the placental bed**

314 For accurate sampling of the 'true placental bed' it is the crucial to localize the

315 placental bed either by ultrasound or under direct visualization. The transcervical

316 biopsy procedure is accurately described in the publications by Robson et al. (28) It

317 has proven difficult to visualize the early pregnancy placental bed with ultrasound

318 before 8 weeks of gestation. Only 17% of those samples contained spiral arteries.

319 By thrusting the biopsy forceps through the *in situ* placenta to the placental bed it

320 was usually possible to obtain two or three biopsies in the study of Dixon and

321 Robertson. (46) This technique very accurately localized the placental bed, but did

322 not necessarily result in a high success rate.

323   The method of Pijnenborg et al (26) involved insertion of a 22-gauge needle from the

324   serosal side of the uterus after localizing the centre of the placenta as previously

325   described.

326   Finally, biopsies can be taken under direct visualization of the placental bed during

327   caesarean section. Before the procedure the placental site should be identified using

328   ultrasound, as routine practice. Immediately after delivery of the baby the centre of

329   the placenta is manually localized and gently separated from the underlying

330   myometrium by controlled cord traction. From our own experience it is possible to

331   sometimes feel the slightly depressed and rough placental site, different from the

332   smooth surrounding uterine wall. This could be helpful in making the distinction

333   between the two when the placental bed is located in fundo or anterior.

334   Accurate localisation with ultrasound and palpation is especially important when the

335   uterus contracts following the placental removal. The position of the placental bed

336   might shift drastically, resulting in low successful biopsies. When the placenta is

337   located on the anterofundal site of the uterus the uterine wall can be exteriorized by

338   slightly inverting the incision side. When taking the biopsy it is important to record the

339   location if possible: central, paracentral or periphery.

340

341   **Safety, complication rates and post-biopsy procedures**

342   Although based on expert opinion, when sampling the placental bed from

343   (complicated) pregnancies caution should be taken when the patient has any form of

344   coagulopathy. For instance, when the pregnancy is complicated by the haemolysis,

345   low platelets and elevated liver enzyme (HELLP) syndrome, one should assess

346   platelet count before surgery. Platelet count <100.000/mm^3 is included in the

347   classification of HELLP syndrome. (58) In our protocol discussed below we set the

348 limit of platelet count to 50.000/mm^3 with normal ranges being 150.000/ul-

349 400.000/mm^3. The limit was set in accordance with local protocols indicating that

350 administration of platelets in patients requiring caesarean section was necessary if

351 platelet count is <50.000/mm^3.

352 During caesarean section all procedures are as normal. It is at the obstetrician's

353 discretion to abandon the biopsy procedure in case of any complications like

354 excessive blood loss, hemodynamic problems, etc. Robson et al. (28) as well as

355 Harsem et al (17) have described the use of oxytocin after delivery of the foetus; 5

356 and 10IU were administered intravenously. In our own study we have used 5IU

357 oxytocin in order to increase the chances of sampling a spiral artery and reducing

358 blood loss. After delivery of the placenta and confirmation that all uterine contents

359 were removed, uterine angles were first secured to ensure patient safety and a

360 reduction of blood loss before the sampling procedure commenced.

361 From our experience is has never been necessary to use sutures to close any

362 defects in the uterine wall after punch biopsy. It must be emphasized that it is not of

363 vital importance to sample the myometrium in great depth. Serial sections of

364 hysterectomy specimens demonstrated that the radial arteries divided approximately

365 0.5 cm beneath the endometrium (i.e., myometrial junctional zone) into 2 or 3 arteries

366 with physiologic changes or transformation. (59) Hence, shallow biopsies no deeper

367 than 5 mm are sufficient and reduce the need for sutures and possible complications.

368 Using the scissors and scalpel biopsy technique it is reported that absorbable sutures

369 are often needed. However, no complications have been reported in 30 years of

370 experience. The few published adverse events could not be accredited to the biopsy

371 procedure.(53)

372

373 **Sample handling**

374 In an attempt to optimize sample collection of placental (bed) studies, very recently

375 an extensive review has been published on sample handling and tissue

376 processing.(60) In short, biopsies should be processed as soon as possible.

377 Depending on the substrate or organelle of interest there may or may not be any

378 delay in processing the samples. For instance there is no change of levels of heat

379 shock proteins within a time period of 45 minutes. However hypoxemia occurs

380 already after 7-10 min in delivered placentas, resulting in dilation of several

381 organelles already within 5 minutes.(61)

382 Fixation of the obtained biopsies in an appropriate fixative is a crucial step for long-

383 term conservation. Placental bed samples are small in size (0.5 cm^3-1.5 cm^3), so

384 caution should be taken with the duration of fixation. Burton et al. recommend that

385 placental samples of the same dimensions should be fixed in paraformaldehyde no

386 longer than 12 hrs. when used for immunohistochemistry. (60) It is advisable to

387 always snap-freeze some of the biopsies for protein, DNA, RNA and metabolomics

388 studies. Ideally one would have the fixative available in the operating theatre in order

389 to reduce the interval between sampling and conservation to a minimum.

390 After fixation, accurate orientation of the biopsies for proper cutting of paraffin or

391 frozen tissue sections is essential. Biopsies should ideally be positioned in such way

392 that perpendicular sections from decidual surface to the myometrial base can be

393 obtained. However, macroscopically decidua and myometrium are difficult to

394 distinguish. Figure 1 shows an overview picture of a well-orientated placental bed

395 biopsy obtained with the punch biopsy technique. Taking biopsies with the scalpel

396 and scissors technique will result in samples that are easy to orientate as well. Some

16

397 authors have recommended placing the scalpel or scissor biopsy with the myometrial

398 aspect down on a piece of filter paper for orientation. (53)

399 Organizing effective logistics is very important in creating a representative and

400 consistent biobank. This includes accurate registration of patient characteristics and

401 clinical data. Due to many possible-confounding factors as medication use, labour

402 and severity of clinical disease, data can be easily misinterpreted. Nelson and Burton

403 (62) have listed possible confounders in a paper encouraging authors to register

404 basic clinical characteristics in placental (bed) research. A recent paper by Myatt et

405 al. (63) made suggestions for minimal and optimal clinical datasets that should be

406 included in a study of preeclampsia to further facilitate comparisons between studies.

407

408 **Evaluation of placental bed biopsy**

409 When evaluating the success of the placental bed biopsy immunohistochemistry

410 staining with cytokeratin 7 (CK7) for trophoblast and preferable Periodic Acid Shiff

411 (PAS) after diastase for fibrinoid can be used. Diastase refers to any enzyme that

412 cleaves glycogen into the water-soluble sugars maltose and dextrose, which in turn

413 can be washed away. The typical PAS staining of fibrinoid in the vascular wall

414 facilitates detection of (partially) transformed spiral arteries, without any background

415 staining of glycogen. Caution must be taken when interpreting the CK7 stain,

416 because decidual gland epithelium is also detected. Success can be defined in

417 different ways. We propose an uniform protocol by dividing specimens in 3

418 categories as described previously (28): (1) specimens without trophoblast and spiral

419 artery; (2) specimens with only trophoblast but no spiral arteries; and (3) specimens

420 with both trophoblast and spiral artery (=successful biopsy). In Tables 1, 2, 3 and 4

421 the success rates per technique are shown as deduced from papers that have

422 reported their success rates in sampling the placental bed.

423

424 **Collection protocol**

425 The following protocol is derived from our experience and based on publications on

426 placental bed biopsies discussed in this review. The bullet action points are arranged

427 in chronological order and can be incorporated into a local Standard Operation

428 Procedure. It is recommended that the presence of all materials used are checked

429 and replenished regularly in order not to delay the processing of the biopsies. The

430 protocol is mainly applicable for biopsy techniques during caesarean section.

431 However, many of the actions are also applicable to transcervical punch biopsies

432 after delivery.

433

434 *Preparations*

435 1. Obtain informed consent according to the declaration of Helsinki before start of

436   the procedure, in acute situations this must have been done beforehand.

437 2. Localize placenta (bed) by (trans-abdominal) ultrasound

438 3. Make sure (sterile) biopsy materials are present in the operating theatre

439 4. Check platelet count (> 50.000/ul)

440 5. Collect clinical data in structured manner i.e. standardized list of pregnancy and

441   patient characteristics.

442

443 *During caesarean section*

444 6. After delivery of the foetus give 5-10IU oxytocin intravenously.

18

445 7. Identify the outer angles of the incision in the uterus and clamp with haemostatic

446   forceps to reduce bleeding

447 8. Ascertain location of placenta manually and remove by controlled cord traction

448 9. Confirm removal of all foetal tissues from the uterus

449 10. Manually and/or visually (indirectly by having seen the placental location) identify

450   the location of the placental bed. It is possible to sometimes feel a slightly

451   depressed and rough placental site, different from the smooth surrounding tissue.

452 11. Depending on preferred technique:

453   • Punch biopsy: Introduce the biopsy forceps in to the uterine cavity

454   directing it to the uterine wall. Whenever possible angle the forceps to

455   45 degrees with the uterine wall. Place the other hand on the serosal

456   side of the uterus just underneath the placental site in order to prevent

457   perforation. Take the biopsies, possibly from the center as well as the

458   periphery and place them on a small bandage gauze.

459   • Scalpel/Scissors biopsy: Identical as punch biopsy, but use curved

460   scissors or scalpel. Remove a disk of 1,5 cm in diameter from the

461   central part of the placental bed or a wedge shaped sample of 1,5 cm

462   in length. Both no deeper than 0,5cm. Further details are described

463   elsewhere. (53)

464   • Vacuum suction biopsy: Identical procedures as punch biopsy, but

465   applying suction force directly on a nylon net placed on the placental

466   bed. Further details are described elsewhere. (17)

467 12. Record the location if possible: central, paracentral or periphery

468   13. Biopsy site is inspected for bleeding, if necessary an absorbable suture can be

469   used to close the defect. The suture technique is at the obstetricians' discretion.

470   The remainder of the procedure can be completed as normal.

471

472   *Processing biopsy*

473   14. Directly rinse collected specimens in sterile saline to remove excessive blood

474   and blood clots.

475   15. Place specimens in desired fixative preferably within 5-10 minutes, e.g., on the

476   operating table itself to prevent any delay.

477   16. Snap-freeze immediately in liquid nitrogen or transfer to fixative such as fresh 4%

478   buffered paraformaldehyde overnight. If regulations do not allow liquid nitrogen in

479   the operating theatre, the sluice room will suffice to process the samples.

480   17. For embedding in Optimal Cutting Temperature (OCT) compound transfer to 20-

481   30% sucrose overnight at 4℃

482   18. Register the biopsy-fixation time and store in biobank

483   19. Embed in paraffin or OCT

484

485   **Future perspectives**

486 In this review we have presented a comprehensive overview of studies with placental

487 bed biopsies. Unfortunately many studies did not report the likelihood of sampling a

488 spiral artery, but only presented data of preselected biopsies containing a spiral

489 artery and trophoblasts. Currently it is possible to obtain successful biopsies in 17%

490 up to 70% of the cases, with an average of approximately 50% depending on the

491 technique used. Success rates seem to be dependent on experience of the

492 obstetrician in sampling the placental bed. (37) Careful localization, visualisation and

493 inspection of the placental bed is very important and experience will enhance the

494 chances of success. Interestingly, taking multiple biopsies appears not to increase

495 the chance of obtaining spiral arteries and trophoblasts from the placental bed.

496 Gerretsen et al obtained a portion of a spiral artery from the deciduomyometrial

497 junction in 42% of the cases with a single biopsy.(47) Whereas Bulmer et al had a

498 comparable success rate of 47% per case after taking 8 biopsies using only a slightly

499 different biopsy forceps. (28) Moreover, in our own study we show a success rate of

500 57% per case when 4 biopsies were obtained. The number of biopsies taken should

501 be adjusted to the quantity appropriate for the research question and use of multiple

502 processing techniques.

503 Researchers interested to collect placental bed biopsies must choose their technique

504 carefully. Depending on the research question and tissues needed the less invasive

505 decidual suction methods may well suffice for their research purpose. For instance,

506 acute atherosis lesions are more likely to be found in the decidual parts of the spiral

507 artery. (64) Therefore the suction technique or even basal plate sampling should be

508 used instead of punch biopsy or scalpel biopsy. If the research question

509 encompasses remodelling of the deeper myometrial spiral arteries one should

510 consider techniques that sample the placental bed up to 0.5 cm of depth (scalpel and

511 scissors or punch biopsy). Although no complications were reported, the scissors and

512 scalpel technique often needs closure of the created defect in the uterine wall as

513 opposed to the punch biopsy technique. Therefore the punch biopsy with its excellent

514 success rates and reproducibility is the recommended choice for (myometrial)

515 placental bed sampling.

516 Several factors influence interpretation of the placental bed biopsy. Orientation is

517 very important and sections from decidual surface to the myometrial base should be

518 obtained. Secondly, making serial sections could add to correctly interpreting the

519 samples in terms of orientation and success rate. Further, the placental bed that has

520 been exposed to labour must be used with care as many biological processes may

521 be affected by the acute hypoxia and reperfusion of the placental bed during

522 contractions. For instance, recent studies have shown that endoplasmic reticulum

523 stress and oxidative stress are higher in laboured placentas. (65,66)

524 The greatest challenge in advancing this field further is the inability to obtain high-

525 quality tissue samples from the first and second trimester in on-going pregnancies.

526 Failure of spiral artery remodelling occurs between 8-14 weeks of gestation and

527 possibly even later. (4) The only materials currently available are remainders of

528 chorionic villous samples collected largely for serum screening related risk for

529 chromosomal abnormalities. (67) However, newer techniques in detecting foetal cells

530 in the maternal circulation (i.e. Non Invasive Prenatal Testing (NIPT)) may make

531 invasive villous sampling obsolete in the nearby future. (68) Other techniques should

532 be explored to overcome this problem. Although prediction models for preeclampsia

533 still suffer from low sensitivity and specificity, recent studies indicate that early

534 preeclampsia can be accurately predicted in the first trimester of pregnancy using

535 maternal characteristics, biomarkers and Doppler flows of the uterine artery. (69,70)

536 Obtaining these parameters before termination of pregnancy might enable us to

537 stratify for women at high risk of developing (early) preeclampsia if their pregnancy

538 would have continued. Although the majority of terminations are performed medically,

539 we encourage researchers to continue collecting placental bed samples in surgical

540 terminations of (early) pregnancy if indicated and when possible to use prediction

541 models for preeclampsia to stratify for women that are at higher risk of developing

542 preeclampsia combined with judging the level of spiral artery remodeling.(71)

543 However, the predictive nature of such studies will still result in a demand for

544 placental bed biopsies sampled in term (complicated) pregnancies.

545 Standardisation of placental bed biopsy procedures will improve the quality and

546 comparability of studies using placental bed biopsies and allow large collections of

547 samples to be generated. We have proposed a standard in this paper. Placental bed

548 samples, when combined with functional experiments on primary and/or immortalized

549 trophoblasts, should give us further insight in the mechanisms of non-trophoblast

550 dependent as well as trophoblast dependent spiral artery remodelling and

551 subsequently in the pathology of pregnancy complications such as preeclampsia,

552 IUGR, preterm birth and placental abruption.

553

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31

Table 1: Search syntax

pregnan*[tiab] AND ("placental bed"[tiab] OR "placenta bed"[tiab] OR "spiral

artery"[tiab] OR "spiral arteries"[tiab] OR "uteroplacental arteries"[tiab] OR

"uteroplacental artery"[tiab])

Figure 1. Well-orientated biopsy perpendicular embedded and sectioned from decidual surface to the myometrial base (PAS after diastase stain). Arrowhead: non-remodeled spiral artery; M: myometrium; D: decidua.

D

D

M

1 mm

10X
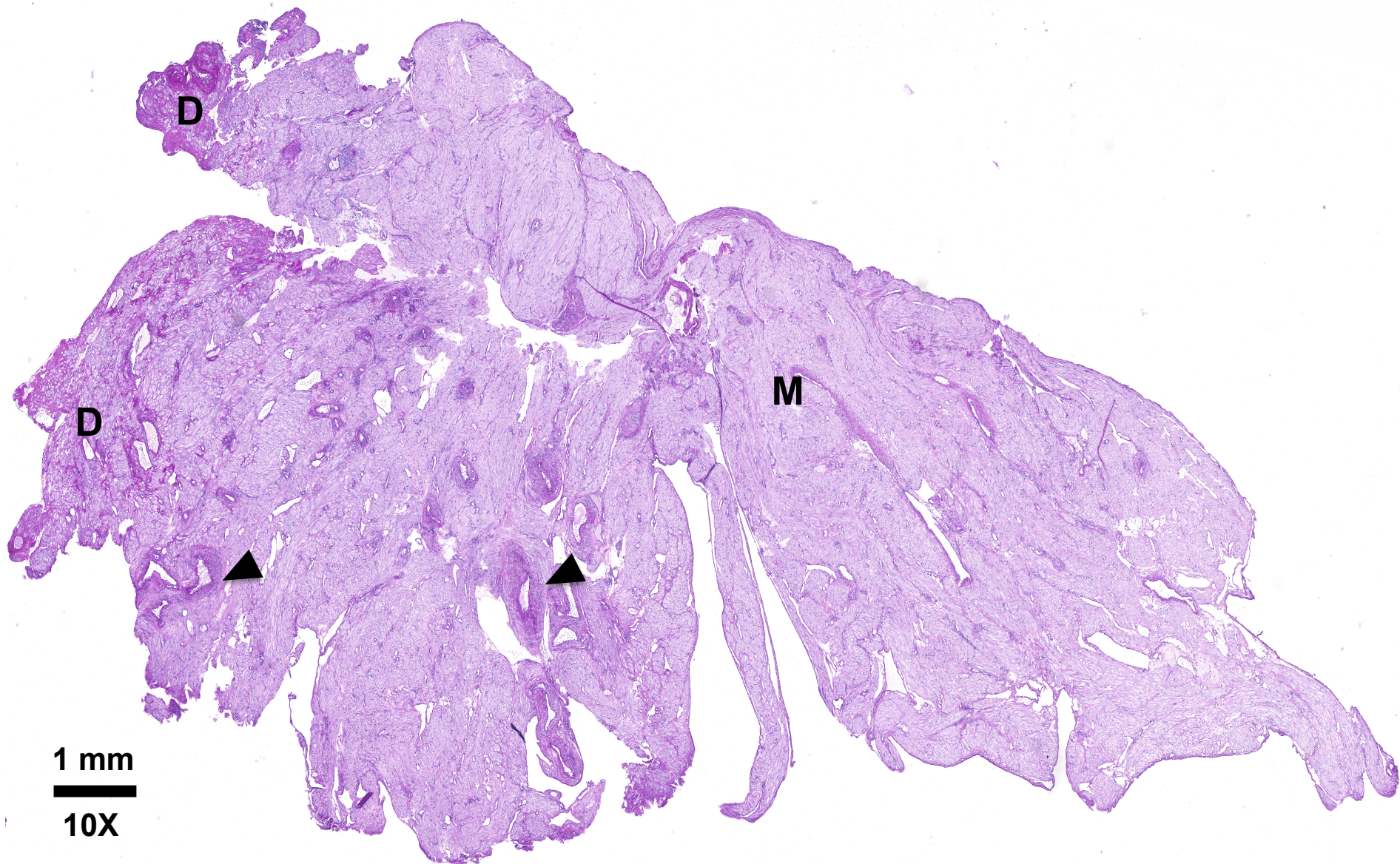
Highlights

- We summarized all studies using placental bed biopsy techniques
- Average success rates were calculated per technique
- We suggested a uniform protocol for sampling the placental bed

Supplemental tables 1, 2, 3 and 4

Table 1: PUNCH BIOPSY AT CAESAREAN SECTION							
Author	Patient population	Complications	Number of patients	Number of biopsies taken	Technique used	Successful biopsy: myometrial spiral artery and trophoblast (%)	Successful biopsy: any spiral artery and trophoblast (%)
De Wolf et al (1)	1. Pregnancies complicated by IUGR but without hypertensive disease.	None	5	1	Punch biopsy	N/A	80
Gerretsen et al (2)	1. Normal pregnancies 2. Preeclampsia 3. Variety of complications (i.e. fetal infection, diabetes, drug abuse, etc)	None	175	1	Punch biopsy	N/A	42
Gosseye et al (3)	1. Normal blood pressure 2. Moderate hypertension 3. Chronic essential hypertension 4. Preeclampsia	None	38	≥1	Punch biopsy	N/A	63
Harsem et al (4)	Not described	None	33	1	Punch biopsy	61	61
McFayden et al (5)	1. Elective or emergency operation 2. Severe maternal hypertension or IUGR	None	109	1	Punch and Scalpel (mixed)	N/A	47
Robson et al (6)	1. Normal pregnancy 2. Preeclamspia	None	139	8	Punch biopsy	39	47
Veerbeek et al (submitted)	1. Normal pregnancy 2. Preeclampsia	None	68	4	Punch biopsy	N/A	57
Weir (7)	1. Normal pregnancy	None	32	1	Punch biopsy	N/A	84
					Success rate (%)	43*	51*

Success rate calculated per biopsy

* Weighted means were calculated including only studies that reported success rate per case

PIH: Pregnancy Induced Hypertension; APS: Anti Phospholipid Syndrome; IUGR: Intra Uterine Growth Restriction

Table 2: PUNCH BIOPSY AT TOP/ERPC (VAGINAL)							
Author	Patient population	Complications	Number of patients	Number of biopsies taken	Technique used	Successful biopsy: myometrial spiral artery and trophoblast (%)	Successful biopsy: any spiral artery and trophoblast (%)
Ball et al (8)	1. TOP 2. Sporadic early miscarriage	None	252	3 to 4	Punch biopsy	41	56
Carbillon et al (9)	1. TOP and missed abortions	None	5	≥1	Punch biopsy	0	0
Gerretsen et al (2)	1. Normal pregnancies 2. Preeclampsia 3. Variety of complications (i.e. fetal infection, diabetes, drug abuse, etc)	None	55	1	Punch biopsy	N/A	33
Robson et al (6)	1. TOP 2. ERPC	None	417	4	Punch biopsy	35	55
Talaulikar et al (10)	1. TOP	None	10	≥1	Punch biopsy (Hysteroscopy)	40	70
					Success rate (%)	37*	54*

* Weighted means were calculated including only studies that reported success rate per case

TOP: Termination of Pregnancy; ERPC: Evacuation of Retained Products of Conception; IUGR: Intra Uterine Growth Restriction

Table 3: SCALPEL OR CURVED SCISSORS BIOPSY AT CAESAREAN SECTION								
Author	Patient population	Complications	Number of patients	Number of biopsies taken	Technique used	Successful biopsy: myometrial spiral artery and trophoblast (%)	Successful biopsy: any spiral artery and trophoblast (%)
Brosens et al (11)	1. Normal weight foetuses 2. IUGR foetuses	None	108	≥1	Scalpel or curved scissors (Robertson et al)	N/A	73
Dommisse et al (12)	1. Placental abruption	None	18	1	Scalpel (10mmx5mm)	N/A	67
Frusca et al (13)	1. Normal pregnancy 2. Preeclampsia	None	38	≥1	Scalpel	42[#]	42[#]
Hanssens et al (1997)	Abstract only	?	201	1 to 2	Scalpel	46	N/A
Katabuchi et al (14)	1. Normal pregnancy 2. Preeclampsia	None	26	1 to 2	Scalpel (15x5 mm; Robertson et al)	N/A	75[#]
Madazli et al (15)	1. Normal pregnancy 2. Preeclampsia	None	60	2 to 3	Scalpel, (10x10x10mm)	N/A	64
McFayden et al (5)	1. Elective or emergency operation 2. Severe maternal hypertension or IUGR	None	109	1	Punch and Scalpel (mixed)	N/A	47
Meekins et al (16)	1. Normal pregnancy 2. Preeclampsia	None	45	2 to 3	Scalpel or curved scissors (Robertson et al)	90[#]	N/A
Naicker et al (17)	1. Normal pregnancy 2. Hypertensive pregnancy	None	76	1	Curved scissors (Robertson et al)	N/A	72
Olofsson et al (18)	1. Normal pregnancy 2. IUGR 3. IUGR and PIH or preeclampsia 4. Preeclampsia	None	55	1	Scalpel	N/A	63
Pijnenborg et al (19)	1. Normal pregnancy 2. Hypertensive pregnancy	None	82	1	Scalpel conisation	N/A	52
Reister et al (20)	1. Normal pregnancy 2. Preeclamspia	None	59	1	Scalpel (10x5x5 mm)	N/A	27
Stone et al (21)	1. Normal pregnancies 2. APS	None	36	2	Curved scissors (Robertson et al)	N/A	60
Vuorela et al (22)	1. Normal pregnancy 2. Preeclampsia	None	35	1	Scalpel	50	50
					Success rate (%)	47*	58*

[#] Success rate calculated per biopsy

* Weighted means were calculated including only studies that reported success rate per case

PIH: Pregnancy Induced Hypertension; APS: Anti Phospholipid Syndrome; IUGR: Intra Uterine Growth Restriction

Table 4. VACUUM SUCTION AT CAESAREAN SECTION							
Author	Patient population	Complications	Number of patients	Number of biopsies taken	Technique used	Successful biopsy: <u>myometrial spiral artery</u> and trophoblast (%)	Successful biopsy: <u>any spiral artery</u> and trophoblast (%)
Harsem et al (23)	1. Normal pregnancies 2. Preeclampsia 3. Diabetes and superimposed preeclamspia	None	102	1	Vacuum suction	N/A	74
Harsem et al (4)	Not described	None	51	1	Vacuum suction	13	86
					Success rate (%)	-	**78***

* Weighted means were calculated

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Supplemental figure 1. Flowchart of search.