Biopsy techniques to study the human placental bed

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Title

Biopsy techniques to study the human placental bed.

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Disclosure Statement

The authors report no conflict of interest.

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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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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 EVT invasion facilitates 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
early and late normal and complicated pregnancies, taken by reproducible
techniques and coupled to detailed clinical characterisation of the study subjects.
A ‘true’ placental bed biopsy consists of both decidual and myometrial tissue and
contains EVTs. However, in order to study spiral artery remodelling a ‘successful’
placental bed biopsy must contain at least one spiral artery. To obtain a successful
placental bed biopsy is challenging, because the placental bed only contains around
30-60 spiral arteries (5-7). The success rate varies with the biopsy technique used
and interpretation of data is heavily dependent on subsequent tissue handling and
conservation. Choosing the right technique and sample processing is therefore of
pivotal importance.
In this review we provide a comprehensive overview of the techniques employed in
current studies of the human placental bed and the limited number of biopsy
collections worldwide. Techniques are discussed with their corresponding success
rates. Finally, we propose a uniform protocol for sampling the human placental bed
and subsequent tissue processing in order to increase the availability of comparable
and good quality specimens with which to unravel the mechanisms of trophoblast
invasion and spiral artery remodelling, and their failure in adverse pregnancy
outcomes.

Methods
Studies included were identified by a systematic search of the databases PubMed,
Embase and Cochrane. The following search terms were combined using the
Boolean operator OR: placental bed, spiral arteries, uteroplacental arteries and
preeclampsia. The complete search query is presented in Table 1. The search string
was restricted to title and abstract. All papers, written in English, that described
studies of the placental bed in humans, using placental bed biopsies or hysterectomy specimens taken after caesarean or vaginal delivery, were included. Case reports, expert opinions, studies concerning placental bed research in animals and reviews that did not describe actual placental bed studies were excluded. To identify relevant studies not covered by the search, cross-references were also picked up in the review process and screened for relevance. A total of 1027 papers were identified with the search query and after duplicates were removed. After screening title and abstract for the inclusion and exclusion criteria mentioned above, 130 papers remained for full text analysis. Finally, full text screening identified 91 studies that have used placental bed biopsies. Together with 3 additional studies found by cross-references a total of 94 papers were included. All papers were examined to give an overview of the biopsy techniques used. However, not all papers have reported success rates of the biopsy technique used and some studies have used archived collections of placental bed biopsies, in which case only the original study was included in the analysis of success rates. Twenty-five papers reported success rates of their biopsy technique. (8-32) Successful biopsies were defined as either sampling a myometrial spiral artery with trophoblast or any spiral artery and trophoblast. In Supplemental Figure 1 a flowchart of the selection progress is shown. If success rates were reported for each studied patient group separately an average rate for that paper was calculated. Average success rates for each technique were calculated including only papers that reported success rate per case (and not per biopsy). A weighted mean was calculated to include sample size in the equation. Statistical analyses were performed using SPSS (release 20.0; Chicago, IL).

Results
Historical overview placental bed research

From meticulous examinations of the human placenta in situ in the 16th century, it was concluded for the first time that there is no direct vascular connection between mother and foetus. (33) This finding presented indirect evidence for the existence of a vascular bed in the uterine wall adjacent to the placenta: the ‘placental bed’. The Hunter brothers published the first illustrations of the placental bed. Using coloured wax injected into specimens of human uteri with placentas in situ they revealed the first direct macroscopic evidence for spiral arteries in the placental bed. (34)

With the discovery of the microscope and its rapid advances, trophoblasts were identified in the second half of the 19th century. Not long thereafter the invasive nature of the trophoblast was first appreciated. (1) The advances in early placenta bed research were attributed to the availability of hysterectomy and post-mortem specimens of uteri with in situ placentas. Several of these specimens are preserved in histological collections and often accessibility is limited. The biggest collections of early pregnancy material are stored in the Boyd collection at the Centre for Trophoblast Research, University of Cambridge (35) and the Carnegie Collection at the Human Developmental Anatomy Center, Washington DC. (36) The Carnegie collection consists mainly of the specimens preserved by Hertig and Rock who carefully collected very early embryos and implantation sites from hysterectomies. The placenta-in-situ samples of the Boyd collection are overrepresented by low-lying placentas that may have caused the need for hysterectomy. Although no tissue blocks remain the collection holds in situ placenta hysterectomies from various gestational ages that are partly digitalized and accessible on the website of the
Centre for Trophoblast Research. (http://www.trophoblast.cam.ac.uk). Both collections were fundamental for the study of the placenta.

Ground breaking work on spiral artery remodelling in the placental bed has undoubtedly been performed by Brosens (9,10,37-39) and Pijnenborg et al. (4,40,41). The early histological studies Pijnenborg conducted on the placental bed revealed a higher density of extravillous trophoblasts (EVTs) in the proximity of spiral arteries with morphological signs of remodelling. The specimens that were used for these studies came from an impressive collection of 48 intact uteri with pregnancies ranging from 8 to 18 weeks gestational age. Advances in obstetric care and surgical techniques have made pregnant hysterectomy a rare procedure. Therefore, obtaining new hysterectomy samples is very uncommon, let alone in complicated pregnancies. However, the growing body of evidence implicating abnormalities of the placental bed in the genesis of pregnancy complications, such as preeclampsia, increases the need for representative samples to study the placental bed. (42,43)

“Modern” placental bed biopsy techniques

In the early 1950’s, Hertig(44) and, Zeek and Assali (45) among others described changes in decidual vessels that were recognized as having acute atherosis. However, the authors acknowledged the fact that the material was not adequate and that further study of maternal vessels deeper in the placental bed was required. The research on the role of spiral arteries was stimulated by Browne and Veall when they reported in a paper published in 1953 that defective placental perfusion might underlie hypertensive pregnancies. (42) In 1958 Dixon and Robertson introduced a technique to obtain representative placental bed material suitable for histological examination of such deeper myometrial maternal vessels. (46) Using a punch biopsy...
technique during caesarean section they were the first to collect myometrial parts of
the spiral arteries apart from the existing hysterectomy and post-mortem uterus
specimens. At the same time a group in Leuven independently collected placental
biopsies, using a sharpened ovum forceps they obtained biopsies by the transvaginal
route. Since that time several techniques for the sampling of the placental bed have
been used which we will discuss in the following section.

**Punch biopsy at caesarean section**

Since its introduction in 1958, the *punch biopsy* technique has been widely used.
Gerretsen et al studied the physiological changes of spiral arteries in normal
pregnancies and a variety of pregnancy complications. The authors used the
technique to obtain 1 sample of the placental bed of 175 patients. In 42% of the
cases they found at least one spiral artery and trophoblasts. Robson et al sampled
139 patients during caesarean section and collected successful biopsies in 45% of
the normal pregnancies and 52% of the pregnancies complicated by preeclampsia.
Eight biopsies were taken under direct vision of the placental bed after placental
removal and intravenous administration of 10IU oxytocin to facilitate uterine
contraction. Taking multiple biopsies from the placental bed seemed not to enhance
the chances of successfully sampling the bed.

Our group recently started a new biobank study in which the punch biopsy technique
during caesarean section has shown comparable success rates. Collecting 280
biopsies from 70 patients we managed to sample the placental bed containing at
least one spiral artery and EVT in 57% of the cases (unpublished data). Our
proposed standardized protocol will be discussed in detail later.
The studies that reported the likelihood of sampling a spiral artery and trophoblasts from the placental bed with punch biopsy at caesarean section are shown in Table 1.

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

**Transcervical punch biopsy**

The punch biopsy technique was not only used in the setting of caesarean sections. Gerretsen *et al.* also employed a comparable technique in 1981 after vaginal delivery and reported a success rate of 33%.(47) Success was defined as sampling tissue containing interstitial trophoblasts and spiral arteries at the endometrial-myometrial junction. In 1990 Michel *et al.* were the first to describe the ultrasound-guided placental bed biopsy.(48) Focusing on leucocytes in the first trimester they did not report the number of spiral arteries in their biopsies. By excluding the cases with insufficient myometrial vessels it is impossible to assess the success rate. The experiences from Robson and colleagues in sampling under ultrasound guidance were discussed in their publication in 2002. (28) They report success rates with both transcervical techniques as well as biopsies under direct vision during caesarean section as discussed earlier. Currently this is the largest series of early placental bed biopsies reported in the literature. Biopsies were attempted in 313 women who underwent termination of pregnancy (TOP) before 20 weeks of gestation and in 104 women in need of evacuation of retained products of conception (ERPC) after foetal death between 7 and 21 weeks of gestation. In short, after induction of general anaesthesia an ultrasound scan was performed for placenta localization and estimation of gestational age. After evacuation of the uterine contents a biopsy forceps was introduced under ultrasound guidance to the site of the presumed placental bed. Four biopsies were then taken from the central placental bed. With this
procedure 17% to 77% of the biopsies were successful in sampling a spiral artery. There was a significant positive correlation between gestational age and the likelihood of obtaining at least one spiral artery. The latter was probably related to the difficulty to clearly visualize the placental bed with ultrasound in very early pregnancy. There were no complications recorded that could be related to the biopsy procedure.

This biobank of placental bed biopsies has been extensively used to study spiral artery remodelling and related processes. They contributed, for example, to our knowledge on the role of adhesion molecules (49), nitric oxide (50), transforming growth factor beta (49), interleukin-6 and 8 (51), and vascular smooth muscle cell apoptosis (52) in trophoblast invasion and spiral artery remodelling.

Studies that reported the likelihood of sampling a spiral artery and trophoblasts from the placental bed with punch biopsy after vaginal delivery, TOP or ERPC are shown in Table 2. With this technique an average success rate of 54% is feasible.

**Scissors or scalpel biopsy technique**

Robertson and colleagues published their 30-year experience from three centres in sampling the placental bed in 1986. (53) Centres in Leuven Belgium, London United Kingdom and Dublin Ireland have collected placental bed samples with curved scissors or scalpel, a technique that is reported to sample true placental bed in more than 70% of the cases. However, it is not clear if success was defined as the presence of spiral arteries or presence of interstitial trophoblasts alone. Biopsies were taken at caesarean section under direct visualisation of the placental bed. After digitally marking the centre of the placenta, the placenta itself was peeled away and the supposed placental site and surroundings were carefully inspected. With curved
scissors a disk of 1.5 cm in diameter was removed from the central part of the placental bed. A depth of 0.5 cm proved to be more than sufficient to obtain the myometrial sections of spiral arteries. The same dimensions can be obtained when a surgical scalpel is used to collect a wedge-shaped biopsy. The resulting defect was closed with absorbable sutures. There were no reports of biopsy-related complications and this technique has been widely used ever since. A variant of the scalpel biopsy technique was published by Pijnenborg et al. (26). A 22-gauge marking needle was thrust through the serosal side of the uterus through the centre of the placental bed that was manually localized previously. A second needle was inserted from the decidual side into the uterus next to the marking needle that could then be removed. With a sharp scalpel a cone of 1-2 cm wide and 1 cm deep was obtained. In 68% of hypertensive women the biopsies contained at least one spiral artery, whereas normotensive women were accurately sampled in 35% of the cases. The difference between success rates was not addressed in the paper.

The studies that reported the likelihood of sampling a spiral artery and trophoblasts from the placental bed with scalpel or scissors are shown in Table 3. With this technique an average success rate of 58% is feasible.

Vacuum suction technique

More recently Harsem evaluated a new technique collecting decidual tissue by vacuum suction for functional and morphological studies described earlier by Staff et al. (17,54) Quality criteria included the presence of spiral arteries and extravillous trophoblasts. However, the collected decidual samples do not fill the criteria of true placental bed biopsies, as discussed earlier. Morphological features of the obtained biopsies were compared with archived placental bed material and basal plate
sections. During caesarean section 5IU oxytocin was given intravenously after
delivery of the baby to ensure uterine contraction. The placenta was then manually
located and gently removed from the uterine wall. Placental bed tissue was collected
with suction force (vacuum systems such as used for uterine evacuation) into a
tissue-sampling box, which in turn was flushed with 500 mL of sterile saline to
remove blood. A random portion was selected for morphological studies. In the
evaluation study 51 women undergoing caesarean sections and vacuum suctions
were included and compared to 33 placental bed punch biopsies and 33 placental
plate specimens sampled from the maternal side of the delivered placenta. In 86% of
the decidual suction samples one or more spiral arteries were found. Placental bed
biopsies and placental plate biopsies contained spiral arteries in 61% and 48%
respectively. In the long term follow up of 38-60 months after vacuum suction there
were no biopsy related complications reported. (17)
The studies that reported the likelihood of sampling a spiral artery and trophoblasts
from the decidua with vacuum suction are shown in Table 4. With this technique an
average success rate of 78% is feasible although the chances of obtaining
myometrial spiral arteries is only 13%. (4)

Other techniques
Several other techniques have been used to sample the placental bed. Most of the
studies did not report any success rates merely because the aim was to investigate
the metabolism or the immune system in relation to localisation within the foetal
placental unit. For instance, Schafer et al. collected decidual material from the
placental bed during caesarean section by wiping the uterine cavity with a tampon.
The number of spiral arteries or interstitial trophoblasts is not discussed. (55)
Transcervically obtaining spiral arteries from the placental bed by curettage after vaginal delivery has proven not to be successful. Only one out of 46 (2%) biopsies was successful in sampling an appropriate portion of spiral artery. The technique was therefore abandoned by Gerretsen et al. (47) Stallmach et al. used curettage of the placental bed under direct vision during caesarean section to sample the placental bed. (56) Their success rates seem to be significantly higher with an average of 6,2 spiral arteries per sample. However, no reports were made on samples without spiral arteries. Interestingly, although no incisions were made in the uterine wall, biopsies ‘usually’ contained myometrium.

It is beyond the scope of this review to discuss all studies that have sampled decidual tissue and basal plate on the delivered placenta. Although Khong and Chambers showed that *en face* specimens from the basal plate of the delivered placenta could sample spiral arteries with a 56.4% success rate we focussed on techniques of direct sampling of the placental bed. (57)

**Localization of the placental bed**

For accurate sampling of the ‘true placental bed’ it is the crucial to localize the placental bed either by ultrasound or under direct visualization. The transcervical biopsy procedure is accurately described in the publications by Robson et al. (28) It has proven difficult to visualize the early pregnancy placental bed with ultrasound before 8 weeks of gestation. Only 17% of those samples contained spiral arteries. By thrusting the biopsy forceps through the *in situ* placenta to the placental bed it was usually possible to obtain two or three biopsies in the study of Dixon and Robertson. (46) This technique very accurately localized the placental bed, but did not necessarily result in a high success rate.
The method of Pijnenborg et al (26) involved insertion of a 22-gauge needle from the serosal side of the uterus after localizing the centre of the placenta as previously described.

Finally, biopsies can be taken under direct visualization of the placental bed during caesarean section. Before the procedure the placental site should be identified using ultrasound, as routine practice. Immediately after delivery of the baby the centre of the placenta is manually localized and gently separated from the underlying myometrium by controlled cord traction. From our own experience it is possible to sometimes feel the slightly depressed and rough placental site, different from the smooth surrounding uterine wall. This could be helpful in making the distinction between the two when the placental bed is located in fundo or anterior.

Accurate localisation with ultrasound and palpation is especially important when the uterus contracts following the placental removal. The position of the placental bed might shift drastically, resulting in low successful biopsies. When the placenta is located on the anterofundal site of the uterus the uterine wall can be exteriorized by slightly inverting the incision side. When taking the biopsy it is important to record the location if possible: central, paracentral or periphery.

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

Although based on expert opinion, when sampling the placental bed from (complicated) pregnancies caution should be taken when the patient has any form of coagulopathy. For instance, when the pregnancy is complicated by the haemolysis, low platelets and elevated liver enzyme (HELLP) syndrome, one should assess platelet count before surgery. Platelet count <100,000/mm$^3$ is included in the classification of HELLP syndrome. (58) In our protocol discussed below we set the
limit of platelet count to 50,000/mm$^3$ with normal ranges being 150,000/ul-

400,000/mm$^3$. The limit was set in accordance with local protocols indicating that administration of platelets in patients requiring caesarean section was necessary if platelet count is <50,000/mm$^3$.

During caesarean section all procedures are as normal. It is at the obstetrician’s discretion to abandon the biopsy procedure in case of any complications like excessive blood loss, hemodynamic problems, etc. Robson et al. (28) as well as Harsem et al (17) have described the use of oxytocin after delivery of the foetus; 5 and 10IU were administered intravenously. In our own study we have used 5IU oxytocin in order to increase the chances of sampling a spiral artery and reducing blood loss. After delivery of the placenta and confirmation that all uterine contents were removed, uterine angles were first secured to ensure patient safety and a reduction of blood loss before the sampling procedure commenced.

From our experience is has never been necessary to use sutures to close any defects in the uterine wall after punch biopsy. It must be emphasized that it is not of vital importance to sample the myometrium in great depth. Serial sections of hysterectomy specimens demonstrated that the radial arteries divided approximately 0.5 cm beneath the endometrium (i.e., myometrial junctional zone) into 2 or 3 arteries with physiologic changes or transformation. (59) Hence, shallow biopsies no deeper than 5 mm are sufficient and reduce the need for sutures and possible complications. Using the scissors and scalpel biopsy technique it is reported that absorbable sutures are often needed. However, no complications have been reported in 30 years of experience. The few published adverse events could not be accredited to the biopsy procedure.(53)
Sample handling

In an attempt to optimize sample collection of placental (bed) studies, very recently an extensive review has been published on sample handling and tissue processing.\(^{(60)}\) In short, biopsies should be processed as soon as possible.

Depending on the substrate or organelle of interest there may or may not be any delay in processing the samples. For instance there is no change of levels of heat shock proteins within a time period of 45 minutes. However hypoxemia occurs already after 7-10 min in delivered placentas, resulting in dilation of several organelles already within 5 minutes.\(^{(61)}\)

Fixation of the obtained biopsies in an appropriate fixative is a crucial step for long-term conservation. Placental bed samples are small in size (0.5 cm\(^3\)-1.5 cm\(^3\)), so caution should be taken with the duration of fixation. Burton et al. recommend that placental samples of the same dimensions should be fixed in paraformaldehyde no longer than 12 hrs. when used for immunohistochemistry.\(^{(60)}\) It is advisable to always snap-freeze some of the biopsies for protein, DNA, RNA and metabolomics studies. Ideally one would have the fixative available in the operating theatre in order to reduce the interval between sampling and conservation to a minimum.

After fixation, accurate orientation of the biopsies for proper cutting of paraffin or frozen tissue sections is essential. Biopsies should ideally be positioned in such way that perpendicular sections from decidual surface to the myometrial base can be obtained. However, macroscopically decidua and myometrium are difficult to distinguish. Figure 1 shows an overview picture of a well-orientated placental bed biopsy obtained with the punch biopsy technique. Taking biopsies with the scalpel and scissors technique will result in samples that are easy to orientate as well. Some
authors have recommended placing the scalpel or scissor biopsy with the myometrial aspect down on a piece of filter paper for orientation. (53)

Organizing effective logistics is very important in creating a representative and consistent biobank. This includes accurate registration of patient characteristics and clinical data. Due to many possible-confounding factors as medication use, labour and severity of clinical disease, data can be easily misinterpreted. Nelson and Burton (62) have listed possible confounders in a paper encouraging authors to register basic clinical characteristics in placental (bed) research. A recent paper by Myatt et al. (63) made suggestions for minimal and optimal clinical datasets that should be included in a study of preeclampsia to further facilitate comparisons between studies.

**Evaluation of placental bed biopsy**

When evaluating the success of the placental bed biopsy immunohistochemistry staining with cytokeratin 7 (CK7) for trophoblast and preferable Periodic Acid Shiff (PAS) after diastase for fibrinoid can be used. Diastase refers to any enzyme that cleaves glycogen into the water-soluble sugars maltose and dextrose, which in turn can be washed away. The typical PAS staining of fibrinoid in the vascular wall facilitates detection of (partially) transformed spiral arteries, without any background staining of glycogen. Caution must be taken when interpreting the CK7 stain, because decidual gland epithelium is also detected. Success can be defined in different ways. We propose an uniform protocol by dividing specimens in 3 categories as described previously (28): (1) specimens without trophoblast and spiral artery; (2) specimens with only trophoblast but no spiral arteries; and (3) specimens with both trophoblast and spiral artery (=successful biopsy). In Tables 1, 2, 3 and 4
the success rates per technique are shown as deduced from papers that have reported their success rates in sampling the placental bed.

Collection protocol

The following protocol is derived from our experience and based on publications on placental bed biopsies discussed in this review. The bullet action points are arranged in chronological order and can be incorporated into a local Standard Operation Procedure. It is recommended that the presence of all materials used are checked and replenished regularly in order not to delay the processing of the biopsies. The protocol is mainly applicable for biopsy techniques during caesarean section. However, many of the actions are also applicable to transcervical punch biopsies after delivery.

Preparations

1. Obtain informed consent according to the declaration of Helsinki before start of the procedure, in acute situations this must have been done beforehand.
2. Localize placenta (bed) by (trans-abdominal) ultrasound
3. Make sure (sterile) biopsy materials are present in the operating theatre
4. Check platelet count (> 50,000/ul)
5. Collect clinical data in structured manner i.e. standardized list of pregnancy and patient characteristics.

During caesarean section

6. After delivery of the foetus give 5-10IU oxytocin intravenously.
7. Identify the outer angles of the incision in the uterus and clamp with haemostatic forceps to reduce bleeding

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

9. Confirm removal of all foetal tissues from the uterus

10. Manually and/or visually (indirectly by having seen the placental location) identify the location of the placental bed. It is possible to sometimes feel a slightly depressed and rough placental site, different from the smooth surrounding tissue.

11. Depending on preferred technique:

   - **Punch biopsy:** Introduce the biopsy forceps into the uterine cavity directing it to the uterine wall. Whenever possible angle the forceps to 45 degrees with the uterine wall. Place the other hand on the serosal side of the uterus just underneath the placental site in order to prevent perforation. Take the biopsies, possibly from the center as well as the periphery and place them on a small bandage gauze.

   - **Scalpel/Scissors biopsy:** Identical as punch biopsy, but use curved scissors or scalpel. Remove a disk of 1.5 cm in diameter from the central part of the placental bed or a wedge shaped sample of 1.5 cm in length. Both no deeper than 0.5 cm. Further details are described elsewhere. (53)

   - **Vacuum suction biopsy:** Identical procedures as punch biopsy, but applying suction force directly on a nylon net placed on the placental bed. Further details are described elsewhere. (17)

12. Record the location if possible: central, paracentral or periphery
13. Biopsy site is inspected for bleeding, if necessary an absorbable suture can be used to close the defect. The suture technique is at the obstetricians’ discretion. The remainder of the procedure can be completed as normal.

Processing biopsy

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

15. Place specimens in desired fixative preferably within 5-10 minutes, e.g., on the operating table itself to prevent any delay.

16. Snap-freeze immediately in liquid nitrogen or transfer to fixative such as fresh 4% buffered paraformaldehyde overnight. If regulations do not allow liquid nitrogen in the operating theatre, the sluice room will suffice to process the samples.

17. For embedding in Optimal Cutting Temperature (OCT) compound transfer to 20-30% sucrose overnight at 4°C

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

19. Embed in paraffin or OCT

Future perspectives

In this review we have presented a comprehensive overview of studies with placental bed biopsies. Unfortunately many studies did not report the likelihood of sampling a spiral artery, but only presented data of preselected biopsies containing a spiral artery and trophoblasts. Currently it is possible to obtain successful biopsies in 17% up to 70% of the cases, with an average of approximately 50% depending on the technique used. Success rates seem to be dependent on experience of the obstetrician in sampling the placental bed. (37) Careful localization, visualisation and
inspection of the placental bed is very important and experience will enhance the chances of success. Interestingly, taking multiple biopsies appears not to increase the chance of obtaining spiral arteries and trophoblasts from the placental bed. Gerretsen et al obtained a portion of a spiral artery from the deciduomyometrial junction in 42% of the cases with a single biopsy. (47) Whereas Bulmer et al had a comparable success rate of 47% per case after taking 8 biopsies using only a slightly different biopsy forceps. (28) Moreover, in our own study we show a success rate of 57% per case when 4 biopsies were obtained. The number of biopsies taken should be adjusted to the quantity appropriate for the research question and use of multiple processing techniques.

Researchers interested to collect placental bed biopsies must choose their technique carefully. Depending on the research question and tissues needed the less invasive decidual suction methods may well suffice for their research purpose. For instance, acute atherosis lesions are more likely to be found in the decidual parts of the spiral artery. (64) Therefore the suction technique or even basal plate sampling should be used instead of punch biopsy or scalpel biopsy. If the research question encompasses remodelling of the deeper myometrial spiral arteries one should consider techniques that sample the placental bed up to 0.5 cm of depth (scalpel and scissors or punch biopsy). Although no complications were reported, the scissors and scalpel technique often needs closure of the created defect in the uterine wall as opposed to the punch biopsy technique. Therefore the punch biopsy with its excellent success rates and reproducibility is the recommended choice for (myometrial) placental bed sampling.

Several factors influence interpretation of the placental bed biopsy. Orientation is very important and sections from decidual surface to the myometrial base should be
obtained. Secondly, making serial sections could add to correctly interpreting the samples in terms of orientation and success rate. Further, the placental bed that has been exposed to labour must be used with care as many biological processes may be affected by the acute hypoxia and reperfusion of the placental bed during contractions. For instance, recent studies have shown that endoplasmic reticulum stress and oxidative stress are higher in laboured placentas. (65,66)

The greatest challenge in advancing this field further is the inability to obtain high-quality tissue samples from the first and second trimester in ongoing pregnancies. Failure of spiral artery remodelling occurs between 8-14 weeks of gestation and possibly even later. (4) The only materials currently available are remainders of chorionic villous samples collected largely for serum screening related risk for chromosomal abnormalities. (67) However, newer techniques in detecting foetal cells in the maternal circulation (i.e. Non Invasive Prenatal Testing (NIPT)) may make invasive villous sampling obsolete in the nearby future. (68) Other techniques should be explored to overcome this problem. Although prediction models for preeclampsia still suffer from low sensitivity and specificity, recent studies indicate that early preeclampsia can be accurately predicted in the first trimester of pregnancy using maternal characteristics, biomarkers and Doppler flows of the uterine artery. (69,70)

Obtaining these parameters before termination of pregnancy might enable us to stratify for women at high risk of developing (early) preeclampsia if their pregnancy would have continued. Although the majority of terminations are performed medically, we encourage researchers to continue collecting placental bed samples in surgical terminations of (early) pregnancy if indicated and when possible to use prediction models for preeclampsia to stratify for women that are at higher risk of developing preeclampsia combined with judging the level of spiral artery remodeling. (71)
However, the predictive nature of such studies will still result in a demand for placental bed biopsies sampled in term (complicated) pregnancies. Standardisation of placental bed biopsy procedures will improve the quality and comparability of studies using placental bed biopsies and allow large collections of samples to be generated. We have proposed a standard in this paper. Placental bed samples, when combined with functional experiments on primary and/or immortalized trophoblasts, should give us further insight in the mechanisms of non-trophoblast dependent as well as trophoblast dependent spiral artery remodelling and subsequently in the pathology of pregnancy complications such as preeclampsia, IUGR, preterm birth and placental abruption.

References


(20) Madazli R, Budak E, Calay Z, Aksu MF. Correlation between placental bed biopsy findings, vascular cell adhesion molecule and fibronectin levels in pre-eclampsia. BJOG 2000 04;107:514-518.


(56) Stallmach T, Hebisch G, Orban P, Lu X. Aberrant positioning of trophoblast and lymphocytes in the feto-maternal interface with pre-eclampsia. Virchows Arch 1999 03;434:207-211.


Table 1: Search syntax

| pregnant*[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.
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
Table 1: PUNCH BIOPSY AT CAESAREAN SECTION

<table>
<thead>
<tr>
<th>Author</th>
<th>Patient population</th>
<th>Complications</th>
<th>Number of patients</th>
<th>Number of biopsies taken</th>
<th>Technique used</th>
<th>Successful biopsy: myometrial spiral artery and trophoblast (%)</th>
<th>Successful biopsy: any spiral artery and trophoblast (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Wolf et al (1)</td>
<td>1. Pregnancies complicated by IUGR but without hypertensive disease.</td>
<td>None</td>
<td>5</td>
<td>1</td>
<td>Punch biopsy</td>
<td>N/A</td>
<td>80</td>
</tr>
<tr>
<td>Gerretsen et al (2)</td>
<td>1. Normal pregnancies 2. Preeclampsia 3. Variety of complications (i.e. fetal infection, diabetes, drug abuse, etc)</td>
<td>None</td>
<td>175</td>
<td>1</td>
<td>Punch biopsy</td>
<td>N/A</td>
<td>42</td>
</tr>
<tr>
<td>Harsem et al (4)</td>
<td>Not described</td>
<td>None</td>
<td>33</td>
<td>1</td>
<td>Punch biopsy</td>
<td>61</td>
<td>61</td>
</tr>
<tr>
<td>McFayden et al (5)</td>
<td>1. Elective or emergency operation 2. Severe maternal hypertension or IUGR</td>
<td>None</td>
<td>109</td>
<td>1</td>
<td>Punch and Scalpel (mixed)</td>
<td>N/A</td>
<td>47</td>
</tr>
<tr>
<td>Robson et al (6)</td>
<td>1. Normal pregnancy 2. Preeclampsia</td>
<td>None</td>
<td>139</td>
<td>8</td>
<td>Punch biopsy</td>
<td>39</td>
<td>47</td>
</tr>
<tr>
<td>Veerbeek et al (submitted)</td>
<td>1. Normal pregnancy 2. Preeclampsia</td>
<td>None</td>
<td>68</td>
<td>4</td>
<td>Punch biopsy</td>
<td>N/A</td>
<td>57</td>
</tr>
<tr>
<td>Weir (7)</td>
<td>1. Normal pregnancy</td>
<td>None</td>
<td>32</td>
<td>1</td>
<td>Punch biopsy</td>
<td>N/A</td>
<td>84</td>
</tr>
</tbody>
</table>

* 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)

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

* 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

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

* 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

<table>
<thead>
<tr>
<th>Author</th>
<th>Patient population</th>
<th>Complications</th>
<th>Number of patients</th>
<th>Number of biopsies taken</th>
<th>Technique used</th>
<th>Successful biopsy: myometrial spiral artery and trophoblast (%)</th>
<th>Successful biopsy: any spiral artery and trophoblast (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harsem et al (23)</td>
<td>1. Normal pregnancies 2. Preeclampsia 3. Diabetes and superimposed preeclampsia</td>
<td>None</td>
<td>102</td>
<td>1</td>
<td>Vacuum suction</td>
<td>74</td>
<td>-</td>
</tr>
<tr>
<td>Harsem et al (4)</td>
<td>Not described</td>
<td>None</td>
<td>51</td>
<td>1</td>
<td>Vacuum suction</td>
<td>86</td>
<td>-</td>
</tr>
</tbody>
</table>

* Weighted means were calculated

References

(8) Ball E, Bulmer JN, Ayis S, Lyall F, Robson SC. Late sporadic miscarriage is associated with abnormalities in spiral artery transformation and trophoblast invasion. J Pathol 2006 03;208(0022-3417; 0022-3417; 4):535-542.
(15) Madazli R, Budak E, Calay Z, Aksu MF. Correlation between placental bed biopsy findings, vascular cell adhesion molecule and fibronectin levels in pre-eclampsia. BJOG 2000 04;107(1470-0328; 1470-0328; 4):514-518.
Supplemental figure 1. Flowchart of search.