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Biopsy techniques to study the human placental bed

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ABSTRACT

Background: 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.

Methods: 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.

Results: Depending on the technique used the likelihood of sampling a spiral artery and trophoblast from the placental bed is 51%–78% per case, without significant complications.

Conclusions: 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.

1. 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

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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.

2. 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 Fig. 1 a flowchart of the selection progress is shown.

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])

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).

3. Results

3.1. 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].

3.2. "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