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Morphology, histochemistry and glycosylation of the placenta and associated tissues in the European hedgehog (*Erinaceus europaeus*)

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ABSTRACT

Introduction: There are few descriptions of the placenta and associated tissues of the European hedgehog (*Erinaceus europaeus*) and here we present findings on a near-term pregnant specimen.
Methods: Tissues were examined grossly and then formalin fixed and wax-embedded for histology and immunocytochemistry (cytokeratin) and resin embedded for lectin histochemistry.
Results: Each of four well-developed and near term hoglets displayed a discoid, haemochorial placenta with typical labyrinth and spongy zones. In addition there was a paraplacenta incorporating Reichert's membrane and a largely detached yolk sac. The trophoblast of the placenta contained diverse populations of granule which expressed most classes of glycan. Intercellular membranes were also glycosylated and this tended to be heavier in the labyrinth zone. Fetal capillary endothelium had glycosylated apical surfaces expressing sialic acid and various other glycans. Glycogen was present in large cells situated between the spongy zone and the endometrium. Trophoblast cells in the placental disc and under Reichert's membrane, as well as yolk sac endoderm and mesothelium, were cytokeratin positive. Reichert's membrane was heavily glycosylated. Yolk sac inner and outer endoderm expressed similar glycans except for *N*-acetylgalactosamine residues in endodermal acini.
Discussion: New features of near-term hedgehog placenta and associated tissues are presented, including their glycosylation, and novel yolk sac acinar structures are described. The trophoblast of the placental disc showed significant differences from that underlying Reichert's membrane while the glycan composition of the membrane itself showed some similarity to that of rat thereby implying a degree of biochemical conservation of this structure.

1. Introduction

The West European hedgehog (*Erinaceus europaeus*) has iconic status among embryologists as it was one of the first species subjected to a detailed study of placentation [1]. Only two subsequent descriptions of the fetal membranes have been published [2,3]. Some information is available on the African hedgehogs, *Atelerix frontalis* [4,5] and *A. albiventris* [6] and the gymnure (*Echinosorex gymnura*) [7], which are also members of the Erinaceidae family.

In the present study, the placentae of a late-pregnant hedgehog were examined grossly, histologically, immunohistochemically and by lectin histochemistry to determine their structure and cellular architecture and to define the types of glycans expressed by the component maternal and fetal tissues. Such investigations are important as lectin histochemistry, applied to semi-thin sections, gives high resolution information on the cellular glycome, providing a useful tool in detecting subtle changes in the biochemistry and function of cells that are not evident using routine histological stains. Here it has been used to identify alterations in cell glycosylation that relate to the positions of cells within the placental disc and yolk sac, reflecting local differences in function and secretory ability.

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2. Material and methods

2.1. Animal

Examination of a recent road-kill hedgehog revealed a gravid uterus with 4 large pregnancy bulges, each containing a well-developed, near-term hoglet with crown-rump lengths that ranged from about 5.5 to 6.0 cm (Fig. 2A). The short umbilical cords were severed to expose the flattened discoid placentae. Wedges (1–2 cm wide) were cut from the outer edge towards the centre of each placental disc and fixed in 10% neutral buffered formalin for 4 days.

2.2. Histology and immunocytochemistry

Pieces of fixed tissue were trimmed and embedded in paraffin wax for sectioning at 5 μm prior to staining for conventional histology with haematoxylin and eosin (H & E) or Giemsa (for the yolk sac). Other sections for immunocytochemistry were dewaxed at 56 °C overnight, immersed in a high pH antigen masking solution (Dako PT link; Dako UK Limited, Ely, Cambs, UK) and heated to 97 °C for 20 min. After cooling, the slides were rinsed in PBS and transferred to a Dako Plus Autostainer (Dako UK) where a mouse monoclonal antibody generated against pancytokeratin (MNF-116, Dako UK at 1:200 dilution) and appropriate secondary antibody (Dako EnVision HRP labelled polymer anti rabbit and mouse antibody) were each applied for 30 min. The secondary antibody, blocking reagents, buffers, substrate, chromagen and nuclear stain were all EnVision FLEX reagents (Dako UK) optimised for use in the Autostainer Plus. After staining, slides were dehydrated, cleared and mounted in DPX. A negative control was run by replacing the primary antibody with an unrelated monoclonal antibody.

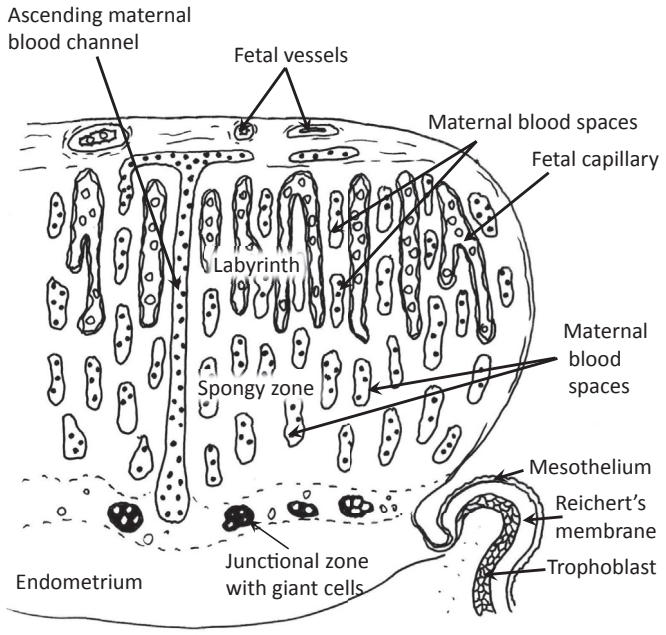

Fig. 1. Diagram of the placenta and paraplacenta of *Erinaceus* (not to scale). Note that fetal erythrocytes in the fetal capillaries are larger than the maternal red blood cells being carried in the maternal channels and passing through the placenta in the maternal blood spaces which are lined by trophoblast cells.

2.3. Lectin histochemistry

Strips of formalin-fixed placenta were embedded in epoxy resin (TAAB Laboratories Equipment Ltd., Aldermaston, UK) prior to cutting 0.75 μm sections with a 3 mm diamond knife and mounting them on multispot slides (C.A. Hendley, Essex, UK) Ltd, composed of four wells each 12 mm in diameter. These were dried for 2 days at 50 °C and stained with a panel of 25 lectins and an avidin-biotin revealing system as previously described [8] except that SNA-1 was used at a concentration of 50 μg/ml. Major binding specificities of the lectins are shown in Table 1. Sections stained with AHA, ECA, SBA, SNA-1, MAA, PAA and WGA were treated with 0.1 units/ml neuraminidase (0.1 units/ml, type VI from *Clostridium perfringens*, Sigma) for 2 h at 37 °C to cleave off terminal sialic acid before incubation in the lectin. Controls were carried out as previously described [8] and as a control for glycogen, sections were pre-digested with 1% amylase (Sigma) in distilled water at 37 °C for 30 min, then washed under running water before incubation in BSA-II. Sections were examined under an Olympus BX41 microscope (Tokyo, Japan) where staining intensity was assessed over 3 sections each of two full-depth blocks of tissue (placenta and adjoining paraplacenta) and 3 sections from one block of yolk sac and allocated a grade from 0 (negative) to 4 (intense staining) and granule density from +/− (sparse) to ++++ (closely packed). Where there was variation in staining intensity, the range is denoted in the tables.

3. Results

3.1. Histology of placental disc and placental bed

The placental disc comprised 3 principal zones (Fig. 1): a labyrinth and spongy zone, both cytokeratin positive (Figs. 2B and 3A) and a mixed population of cells at the fetal-maternal interface (Fig. 2F, G). The fetal-facing surface (chorionic plate) contained connective tissue and small to medium size branches of umbilical arteries and veins and overlay medium size maternal blood channels lined by trophoblast (Fig. 2C). In all vessels, the fetal and maternal erythrocytes could be distinguished by the larger size of the former (Fig 2D). Although connections between fetal capillaries could be seen entering into veins, we did not encounter arteries descending through the labyrinth to supply the capillaries. Occasional nucleated erythrocytes were present.

In the labyrinth, fetal capillaries ran parallel to trophoblast-lined maternal blood channels (Fig. 2D). The spongy zone, comprising trophoblast with maternal blood spaces (Fig. 2E), was less extensive in this near term specimen than that described for earlier stages by Hubrecht [1] and Morris [2,3]. Trophoblast cells here were often binucleate. At the fetal-maternal interface existed a mixed population of cells, most of which were small with a high nuclear-to-cytoplasm ratio and darkly staining nuclei, containing little or no cytokeratin. Interspersed with these were larger cells that stained strongly for cytokeratin and often contained granules or vacuoles (Fig. 2F,G). They occasionally formed clusters and may correspond to the trophoblast giant cells (TGCs) mentioned by Carter and Enders [5], or the deciduofracts described by Hubrecht [1].

A thick layer of decidualized endometrium existed beneath the placental disc followed by the myometrium. Many sections of maternal vessels were found in the placental bed. Sometimes they were surrounded by cytokeratin-positive cells, presumed to be trophoblast, but these cells were never found in the lumen, as they are in the gymnure [7], and only rarely in the tunica media.

Large maternal blood channels ran from the maternal side of the placental disc to the fetal surface where they branched at right angles (Figs. 1 and 3A); they supplied the smaller, trophoblast-lined