Placenta 62 (2018) 34–42

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Trinucleate uterine epithelial cells as evidence for White-tail Deer trophoblast binucleate cell migration and as markers of placental binucleate cell dynamics in a variety of wild ruminants

F.B. Peter Wooding [a, *], David Osborn [b], Gary J. Killian [c]

[a] Physiology Development and Neuroscience Dept, Cambridge University, Cambridge, CB2 3EG, UK
[b] Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA, GA3060, USA
[c] Dept of Dairy and Animal Science, Pennsylvania State University, Pennsylvania 16802, USA

ARTICLE INFO

Article history:
Received 25 September 2017
Received in revised form
11 December 2017
Accepted 12 December 2017

Keywords:
Ruminant placenta
Fetomaternal tissue
Fetal binucleate cell
Fusion
Maternal trinucleate cells

ABSTRACT

Introduction: The unicellular trophoblast epithelium of all ruminants so far investigated contains 15–20% binucleate cells with numerous secretory granules. Electron microscope (EM) studies of the domesticated cow, ewe, goat and deer species have established that these BNC migrate out of the trophoblast epithelium to fuse with the apposed maternal uterine epithelial cells or derivative to form fetomaternal tissue throughout pregnancy. However there is one careful EM study of the trophoblast of a wild ruminant, the White-tail deer, which found the usual number of BNC but no evidence of any migration or fusion. Since there are up to 200 species of wild ruminants, it was important to establish whether there really are two possible scenarios for BNC function.
Materials and methods: This paper reports a light microscope (LM) immunocytochemical study of cell dynamics in ruminant placentas using 1-2 mμ deresinated sections.
Results: The results clearly demonstrate that the White-tail deer and all of the other 15 (see Table 1) randomly selected wild ruminants show the same BNC migration and fusion pattern.
Discussion: These results suggest that this remarkable cellular behaviour is fundamental to the ruminant evolutionary success.

1. Introduction

Trophoblast binucleate cells ("giant cells") are a unique and uniform feature of ruminant trophoblast throughout pregnancy. They are present in the earliest ruminants to evolve, the Tragulids [1], and in all of the species of ruminant so far investigated histologically [2,3], although this forms only a handful of the wide variety known.

These studies established that probably any uninucleate trophoblast cell can undergo two successive divisions producing a replacement uninucleate cell and a binucleate cell. There is no published histological or ultrastructural evidence for any stem cell population. The binucleate cell then differentiates within the epithelium, out of contact with either the basement layer or the tight junctions of the trophoblast. The nuclei become polyploid [4] and the cell produces a large number of granules from the Golgi body, containing pregnancy associated glycoproteins (PAGs) and placental lactogen (PL) hormone [2]. When fully granulated, the cells migrate up to and pass through the apical tight junction maintaining the seal as they do so. They then fuse with the apposed uterine epithelial cell, forming a fetomaternal hybrid cell. The granules stream down the now trinucleate cell and are released by exocytosis to the maternal tissues. The system provides a fetomaternal buffer between the two immunological systems as well as a means of direct fetomaternal communication throughout pregnancy [2].

However this remarkable and unique system has only been clearly demonstrated in domesticated and zoologically captive species, and Sinha et al. reported [5] a careful histological and electron microscopical investigation of the placenta of White-tail deer which found no evidence for any such migration in this wild species even though the BNC were present in the normal frequency. Since there are nearly 200 wild ruminant species it was important to discover if there were two separate systems for BNC function

* Corresponding author.
E-mail address: fbpw2@cam.ac.uk (F.B.P. Wooding).

https://doi.org/10.1016/j.placenta.2017.12.012
0143-4004/

with more wild species showing no evidence of such a migration and fusion system as in the White-tail.

This paper reports an immunocytochemical method of investigation of conventionally immersion fixed placentas using the identification of trinucleate uterine epithelial cells with associated granule content labelling as markers for the migration and fusion system in Whitetail and a range of other wild ruminants.

2. Methods and materials

2.1. Animals

Mid to late pregnant animals were shot as part of Wild life management or culling procedures and placentomes removed and immersion fixed within 20 min of the death of the animals. Fixatives used included Bouins, phosphate buffered Paraformaldehyde or Glutaraldehyde and Surgipath (methyl alcohol and formaldehyde). The quality of fixation varied, but crucially, all produced comparable immunocytochemical results with the PAG antibodies used on the placentome sections.

Details of the animals and origins. At least two placentomes from a single animal were used in the case of the Nilgai, Wildebeest, Axis Deer and Muntjac, at least two or more animals from each of the other species were used (see Table 1).

2.2. Immunocytochemistry

A central slice was cut from each fixed placentome. "Matchstick" samples from the central region of each slice from maternal to fetal edge were used. Whatever the original fixation, all samples were postfixed overnight in 4% (para)formaldehyde in PBS with or without 1% glutaraldehyde before standard epoxy embedding. Semithin sections were cut, picked up on cover glass squares treated with APES, and deresinated in sodium ethoxide. The cover glass squares were then floated section side down on drops of antibody followed by immunogold colloid (goat anti-rabbit G5, Jackson Immunoresearch Labs, USA) then intensified with silver reagent (Aurion, Wagenigen, Netherlands). The antibodies used were to Pregnancy Associated Glycoproteins Ovine PAG-2 and Bovine PAG-1 [6] used at a dilution of 1:1000. Both antibodies identified BNC granules in all species used, the one producing the least background was used to demonstrate the BNC to TNC process in each species Controls with buffer substituted for antibody showed no significant labelling. The two postfixations produced similar results.

3. Results

The trophobast BNC migration and fusion to form trinucleate cells (TNC) throughout pregnancy can be unequivocally recognised on LM sections using two criteria: cells with three nuclear cross sections in the otherwise uninuclear uterine epithelium together with the presence of immunocytochemical reaction product specific for BNC granules. This paper uses antibodies to the pregnancy associated glycoproteins (PAGs) to identify the BNC granules. In all species except the giraffe (see Ref [6]) the BNC show uniform distribution throughout the placentome (Fig. 2 a, b), with TNC also being found at all levels of the maternal septa. Since the plane of section through any TNC is random, the chance of including three recognisable nuclear profiles is low, but such a TNC provides the only conclusive evidence for BNC fusion with a single uterine epithelial (UE) cell. However cells in the UE showing PAG antibody reaction product with one two or no nuclear profiles do provide strong supporting evidence of such fusion since there is no evidence that the UE can synthesize PAGs.

Successful perfusion fixation maintains the fetomaternal apposition as it is in vivo (Fig. 1a). EM studies of such tissue have shown in the cow, ewe and deer the details of the BNC to TNC process [2,7]. Perfusion fixation is not practical with wild ruminants, tissues are normally only available after immersion fixation which usually disrupts the apposition. Fortunately, immersion fixation of cow placentomes, although resulting in the artifactual separation of trophoblast and uterine epithelium, can show TNC formation in the uterine epithelium at the Light Microscope level (arrowheads, Fig. 1b–e). In our immersion fixed White-tail deer material very similar images of TNC formation (arrowheads) can be found (Fig. 1 f–l) in both animals from different sources. The Whitetail samples (Fig. 1f–l) illustrate fusing of BNC to form the TNC (Fig. 1f and g) as well as TNC as part of the UE (Fig. 1h–l).

All species studied except the giraffe (see Ref [6]) show a fairly uniform distribution of BNC throughout the fetal placentomal villi, as can be seen in the White-tail and Red Deer examples, Fig. 2a and b. 2c is a higher magnification of 2b confirming that many of the black dots on 2b do show the characteristic two nuclei highlighted by the PAG reaction product in the BNC. At even higher magnification the red deer show the formation of TNC within the UE (Fig. 2 d, e arrowheads) with the BNC still attached to the trophoblast.

In the Wapiti (2f, g) the TNC are no longer attached to the trophoblast, and there are also patches of PAG reaction product indicating recent migration to the uterine epithelium with fewer nuclei on this plane of section (asterisks).

Table 1

Species	Origin
Cow (*Bos taurus*), Ewe (*Ovis aries*)	Dr FBP Wooding, Babraham Institute, Cambridge
White-Tail Deer(*Odocoileus virginia*)	D. Osborn, Warnell School of Forestry and Natural Resources, University of Georgia, USA and Dr G Killian, Pennsylvania State University, USA
Fallow deer (*Dama dama*) and Roe Deer (*Capreolus capreolus*)	R.Witta, Ranger, Thetford Forest, Norfolk, UK
Red deer (Cervus elaphus)	Regents Park, London UK and Dr CL Adam, Rowett Research Institute, Aberdeen,UK
Axis Deer (*Axis axis*); Chinese Water Deer (*Hydropotes inermis*); Congo Buffalo (*Syncerus caffer nanus*); Muntjac (*Muntiacus reevesi*); Nilgai (*Boselaphus tragocamelus*).	Veterinary Dept, ZSL Whipsnade Zoo, Dunstable, UK.
Wildebeest (*Connochaetes taurinus*) and Giraffe (*Giraffa camelopardalis*).	Dr WR Allen, The Paul Mellon Laboratory, Newmarket, UK
Impala (*Aepyceros melampus*) Springbok (*Antidorcas marsupialia*)	Dr RD Van Aarde, Dr JD Skinner, Mammal Research Institute, University of Pretoria, South Africa.
Tragulus (*Tragulus* spp.)	Dr J Kimura, Seoul National University, South Korea.
Pronghorn (*Antilocapra americana*) Wapiti (*Cervus canadensis*) American Plains Bison (*Bison bison*)	Dr WJ Silvia, CH Hamilton, Dept of Animal and Food Science, University of Kentucky, USA and Dr TW Geary, US Dept of Agricultural Research Service, Miles City, MT, USA