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Functional immunocytochemistry of Tragulus placenta: Implications for ruminant evolution

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ABSTRACT

Introduction and methods: Tragulus, the mouse deer, is considered the most primitive ruminant, with a diffuse placenta grossly quite unlike the cotyledonary type of the other ruminants. This immunocytochemical investigation of placental transporters was designed to elucidate possible mechanisms of evolution to the cotyledonary form.

Results and discussion: Tragulus expresses several of the major transport systems characteristic of the ruminants: the trophoblast binucleate cell (BNC) dynamics, the requirement for two isoforms, GT1 and GT3, for glucose transport, the provision of Aquaporin 3 for water control, and uterine milk and histiotrophic secretion from uterine glands. However whereas the expression of the 9kD Calcium Binding Protein (9CBP) for calcium transport in ruminants is restricted to the intercotyledonary trophoblast with its areolae, Tragulus, having no intercotyledonary area, expresses 9CBP throughout the villus trophoblast. There is some localised development of areolar-like structures in the mid term Tragulus but it is insignificant at term. The strong expression of Glucose Transporter 1 (GT1) in the BNC granules is unique to Tragulus.

Conclusion: Tragulus relies on essentially similar transport and BNC dynamics as the other ruminants. Thus the evolutionary pressures driving the development of the cotyledonary placenta probably lie in the increase in body size and the consequent need for a larger placental area to ensure sufficient glucose for the fetus. The delivery in Tragulus of GT1 to the maternal facing side may be this species unique solution to maintain the glucose supply.

1. Introduction

All of the molecular and structural evidence indicates that the Tragulids are representative of the most primitive ruminants [1–4]. One of the significant differences is the diffusely organised nature of the placental membranes. In all of the other ruminants so far examined the proliferations of the membranes occur at discrete sites, forming the characteristic cotyledonary placenta [6]. A second characteristic feature of the ruminant placenta is the production throughout pregnancy of trophoblast binucleate cells (BNC) which subsequently migrate to, and fuse with, uterine epithelial cells or derivatives [6]. We have shown that Tragulus has a very similar system of trophoblast BNC dynamics [5], and now report on further immunocytochemical investigations of the transport of glucose, calcium and water to see how far these systems differ from those in the conventional ruminant placenta.

2. Materials and methods

Three mouse deer [see 5] were used in this study: one non pregnant (No.17, non pregnant) one mid pregnancy (No.11, fetal weight 15 g) and one close to term. (No.16, fetal weight 170 g).

Animals were killed by intraperitoneal injection of an overdose of ketamine hydrochloride. Numbers 11 and 17 were fixed by immersing the pregnant uterus in either10% formalin (No.17) or Bouin's solution (No.11) followed by dissection into small pieces and storage in buffer at 4 °C. Number 16 was fixed immediately after death by perfusion with 0.1 M phosphate buffered 4% formaldehyde, firstly through the uterine arteries and secondly through the umbilical arteries for 15 min. The placenta was then cut into 0.5–1 cm blocks and fixed for 17 h in either 4% formaldehyde or 1% glutaraldehyde + 4% formaldehyde and finally stored in buffer at 4 °C.

Wax embedding was carried out using Paraplast, 8–10 mμ sections were cut onto aminopropyltriethoxysilane (APES) coated slides.

Dehydration and embedding was at room temperature for araldite resin. Semithin sections were cut from the araldite blocks and picked up on coverglass squares coated with APES. Resin was removed from semithin araldite sections by incubation in sodium ethoxide solution (15 g of sodium hydroxide pellets dissolved in 15 mls of

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absolute alcohol) for 15 min followed by alcohol and water washes before staining with toluidine blue for cellular identification using light microscopy. For immunocytochemistry the coverglass squares were floated section side down on drops of antibody followed by immunogold colloid (Goat anti rabbit G4, Jackson Immunoresearch Labs, USA), then intensified with silver reagent (Aurion, Wageningen, Netherlands) followed if necessary by 0.05% fast green counterstain (FG) for light microscopy.

The dewaxed sections were treated in the same sequence with drops of reagents on the tops of the slides without counterstaining. Immunocytochemical controls, in which the primary antibody was omitted and replaced with buffer or a non specific antibody at the same concentration, were carried out routinely, alongside the experimental samples. Controls showed an insignificant level of staining, as can be seen, for example, on comparing Fig. 4(13) with Fig. 4(13) CONTROL.

Antibodies used: see Table 1

3. Results

In Tragulus we have previously shown that the maternofetal interface is synepitheliochorial. The trophoblast is made up of uninucleate (UNC) and binucleate (BNC) cells which have a microvillar interdigitation with a maternofetal hybrid syncytium formed by migration and fusion of the BNC with the original uterine epithelium [5].

In the present study, trophoblast UNC on wax sections show strong expression of GTI as does the base of the syncytium (Fig. 1(1) and (2)), whereas the GT3 is restricted to the interdigitated microvilli forming the maternofetal junction (Fig. 1(3) and (4)). A

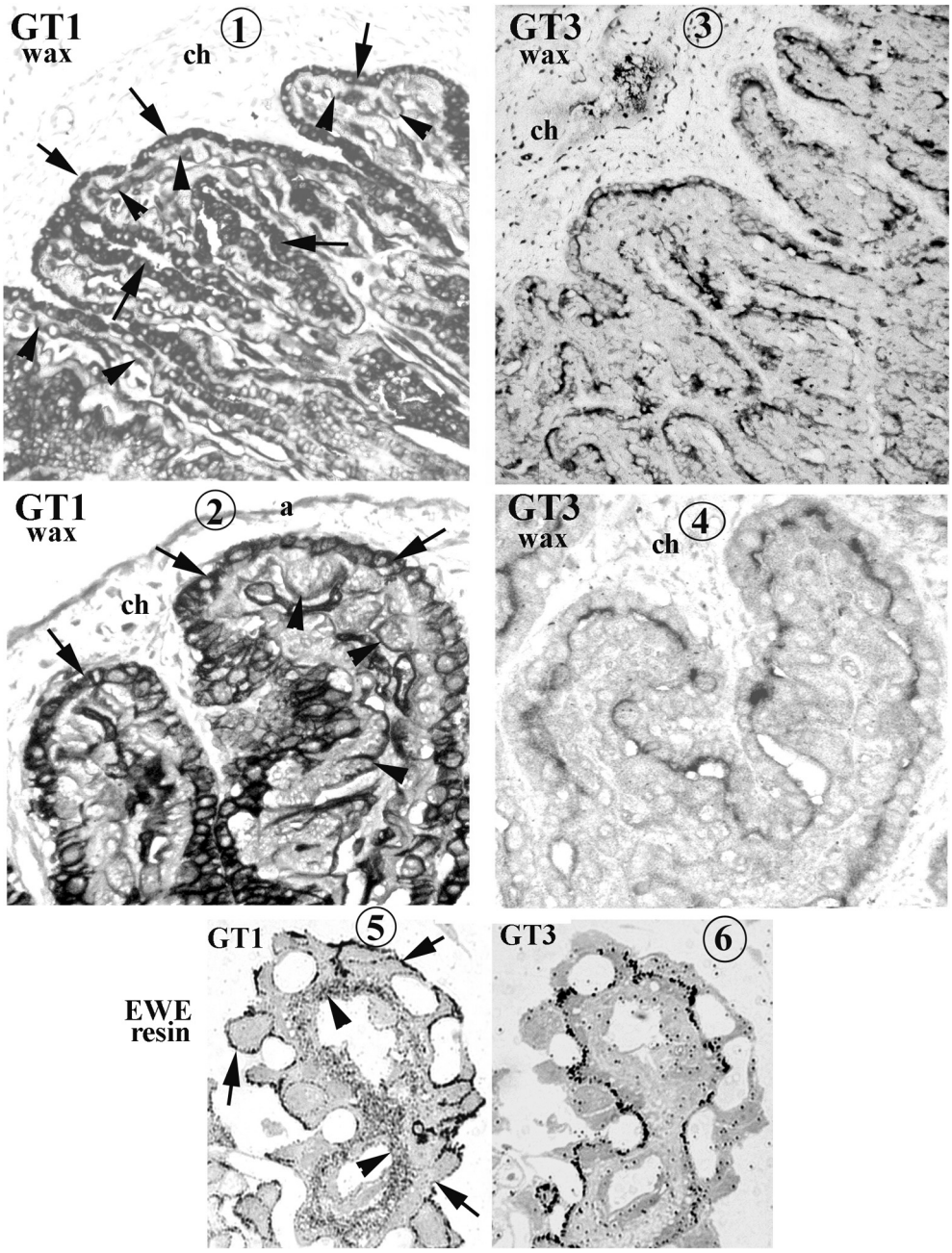

Fig. 1. (1), (2). Wax section. Mid pregnancy. Glucose transporter 1 (GT1) localisation, with no counterstain, identifies only the trophoblast [arrows] and basal membrane of the syncytium [arrowheads] at the base of the fetal villus. This pattern is found throughout the villi, see Fig. 2(7) for detail. a: allantois, ch: chorion. (3) and (4). Wax section. Mid pregnancy. Glucose transporter 3 (GT3) localised in a linear distribution along the apex of the trophoblast which identifies the microvillar junction between trophoblast and syncytium, which is not defined on these sections. (5) and (6). Resin sections of Sheep placentome. Compare the GT1 and GT3 localisations with those in Tragulus – (1)–(4) above. mv: maternal, fv: fetal blood vessel, Sections from Ref. [7],with permission.