Comparison of uteroplacental glycosylation in the camel (*Camelus dromedarius*) and alpaca (*Lama pacos*)

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The recent birth of a camel–llama hybrid, after numerous failed attempts, has prompted an investigation into the glycosylation of apposing fetal and maternal tissues of pregnant camels and alpacas. This study was undertaken to determine whether interspecies differences in glycans are factors that may account in part for the difficulty in producing a viable hybrid. Specimens of camel placentae from day 60 to day 375 of gestation and alpaca placentae from day 22 to term (approximately 345 days) were fixed and embedded in resin, and sections were stained with a panel of 19 biotinylated lectins and an avidin–peroxidase revealing system. Several qualitative interspecies differences in tissue glycosylation were found, mainly in the trophoblast, and

especially with respect to bi/tri-antennary bisected *N*-glycan, fucosylated structures, β -galactosyl residues and sialyl termini. In the maternal uterine epithelium, differences were found mainly in bi/tri-antennary bisected complex *N*-glycan and β -galactosyl residues, indicating that there is more conservation of glycosylation in maternal tissues compared with trophoblast. There were also many quantitative differences in the distribution of glycans. It is possible that a failure to effect the normal glycan–glycan complementation that occurs at the cell surface between maternal and fetal tissues during the implantation processes of apposition and adhesion may account in part for the difficulty in establishing a viable pregnancy between these two species.

Introduction

In recent years there has been much interest in the scientific and popular media following the birth of a camel–llama hybrid in Dubai (Gee, 1999; Roemer *et al.*, 1999; Skidmore *et al.*, 1999; Wright, 1999; Taylor, 2000). This was the first viable hybridization between the Old and New world camelids (Skidmore *et al.*, 1999), and the result of 50 attempts to produce a cross between species that diverged 11 million years ago (Stanley *et al.*, 1994). Jones *et al.* (2000) suggested that glycan–glycan interactions at the fetomaternal interface may play a role in successful implantation and subsequent maintenance of pregnancy, and that interspecies differences in glycosylation may be one mechanism for blocking inappropriate hybridization. In the present study, lectin histochemistry was used to compare the glycosylation of the adhering fetal and maternal tissues of the one-humped camel (*Camelus dromedarius*) and alpaca (*Lama pacos*), the latter species being able to interbreed with the llama (*Lama guanicoe*)

(Gray, 1972). Such a comparison may help to determine whether there are significant differences between glycans of the camel and alpaca fetomaternal interfaces that may account in part for the difficulty encountered in producing a viable interspecies hybrid.

Materials and Methods

Camels

Nine uteri from pregnant camels were obtained from the Cairo (Egypt) abattoir. Placentae from fetuses at days 60, 70, 90, 145, 170, 210, 265, 350 and 375 of gestation, calculated from curved crown–rump lengths of 4.5–115.0 cm (Elwishy *et al.*, 1981), were perfusion fixed in 3% (v/v) glutaraldehyde in 0.1 mol phosphate buffer l⁻¹ (pH 7.3). The placentae were then immersion fixed for 2 h and washed in buffer. Full depth slices of placental tissue were dehydrated in an ascending alcohol series, treated with propylene oxide and infiltrated with Taab epoxy resin (Taab Laboratories Equipment Ltd, Aldermaston), and were embedded in flat-bottomed capsules and polymerized at 60°C for 72 h.

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Alpacas

Breeding and pregnancy determination. During the breeding season, for a period of 2 h (from 06:00 h to 08:00 h), a total of 20 pairs was isolated from the group and left in pairs in a fenced area of 10 m × 5 m. The animals were observed and after coitus they were marked properly. The day of coitus was considered as day 1 of pregnancy. Fifteen days later, pregnancy was confirmed by non-receptive behaviour in the presence of males, uterine rectal palpation and ultrasonography. In total, seven animals were selected for this study at days 22, 45, 150, 264, 283, 296 and 347 of pregnancy. All procedures were performed in accordance with the ethical principles for animal research adopted by the Brazilian College of Animal Experimentation and the experimental protocol was approved by the Biomedical Sciences Institute, University of São Paulo Ethical Committee for Animal Research (no. 027/98).

Collection and processing of tissue from the embryonic-maternal interface. Laparotomies were performed on all animals under general anaesthesia using sodium pentobarbital (30 mg kg⁻¹ administered i.v.) and cervical denervation. The reproductive organs were exposed through a mid-ventral incision of about 10 cm in length and the uteri and ovaries were removed. The ovaries were analysed for counting of the corpora lutea and the uteri were cut into small fragments of approximately 10 mm × 3 mm × 2 mm for light microscopic studies and 2 mm × 3 mm × 2 mm for ultrastructural analysis. These fragments were fixed rapidly by immersion in 2.5% (v/v) glutaraldehyde in 0.1 mol phosphate buffer I⁻¹, pH 7.4. For lectin histochemistry, tissues were washed in PBS, dehydrated in a graded ethanol series and embedded in Spurr resin (medium grade; Electron Microscopic Science Co., St Louis, MO). The camel (day 60) and alpaca (day 150) tissues that had been post-fixed in 1% (w/v) osmium tetroxide and processed for electron microscopy were also sectioned and stained with uranyl acetate and lead citrate before examination in a Zeiss EM109 or Philips EM301 to determine the morphology of secretory granules at the fetomaternal interface.

Lectin histochemistry

Suitable areas from non-osmicated blocks of both species were identified on 0.5 µm thick sections stained with 1% (w/v) toluidine blue in 1% (w/v) aqueous sodium tetraborate. Sections (0.75 µm thickness) were cut for lectin histochemistry, mounted on 3-aminopropyltriethoxysilane (APES)-coated slides (Maddox and Jenkins, 1987) and stained with a panel of 19 biotinylated lectins and an avidin-peroxidase revealing system as described by Jones *et al.* (2000). Resin was removed with saturated sodium ethoxide diluted 1:1 with absolute ethanol for 15 min and, after washing in ethanol followed by distilled water, endogenous peroxidase was blocked with 10% (v/v) hydrogen peroxide (100 volumes; BDH, Poole) before

exposure to 0.03% (w/v) trypsin (Type II-S; Sigma, Poole) in 0.05 mol Tris-buffered saline (TBS) I⁻¹, pH 7.6, for 4 min at 37°C. After washing, the sections were incubated with 10 µg biotinylated lectin ml⁻¹ (Sigma; SNA from Boehringer Mannheim, Lewes; MAA, DSA and STA from Vector Laboratories, Peterborough; LFA from EY Laboratories, San Mateo, CA; see Table 1 for major sugar specificities) in 0.05 mol TBS I⁻¹ containing 1 mmol calcium chloride I⁻¹ for 1 h at 37°C, washed in the same buffer, then treated with 5 µg avidin peroxidase ml⁻¹ (Sigma) in 0.125 mol TBS I⁻¹, pH 7.6, with 0.347 mol sodium chloride I⁻¹ for 1 h at 37°C (Jones *et al.*, 1987). Sections were washed and sites of lectin binding were revealed using 0.05% (w/v) diaminobenzidine tetrahydrochloride dihydrate (Aldrich Chemical Co., Gillingham) in 0.05 mol TBS I⁻¹, pH 7.6, and 0.015% (v/v) hydrogen peroxide (100 volumes) for 5 min at 18.0 ± 0.5°C. The sections were rinsed, air-dried and mounted in neutral synthetic mounting medium (BDH).

Controls were performed as described by Jones *et al.* (1995). Sections were assessed using a semi-quantitative ranking system of analysis in which staining intensity was allocated a grade from 0 (negative) to 4 (intense staining), and granule density was scored from sparse (+/-) to numerous (++++).

Results

Histological structure of the fetomaternal interface

Both species of camelid had an epitheliochorial type of placentation, with close interdigitation of the microvillous surface of the fetal trophoblast with that of the maternal uterine epithelium. Initially, this interaction created a more or less flat or slightly undulating interface (Fig. 1a,b), but at progressive stages of gestation the profile changed so that the trophoblast produced blunt placental outgrowths and the maternal epithelium had equivalent crypts. In the camel, these remained relatively simple until term, but in the alpaca the villous outgrowths were more complex. Occasional trophoblast giant cells were seen in some of the camel specimens (days 60, 90, 170, 210, 350 and 375 of gestation), with many nuclear profiles, and these cells were more prevalent in alpacas, with several giant cells often visible in a single section (Fig. 1h). Blood vessels were found underlying the uterine epithelial cells and also indenting the trophoblast. The developmental changes that occur during gestation in camel (a,c,e,g) and alpaca (b,d,f,h) are shown (Fig. 1).

Ultrastructure of granules

At the ultrastructural level, camel maternal epithelium contained a mainly supranuclear population of round secretory droplets (Fig. 2a), whereas the trophoblast (Fig. 2b) contained a range of different-sized granules, some of which were very pleomorphic. Many mitochondria, well-developed Golgi bodies and large numbers of cisternae of rough endoplasmic reticulum were also present to a

Table 1. Lectins used and their major specificities

Acronym	Source	Major specificity	Reference
PSA	<i>Pisum sativum</i> Garden pea	α -D-mannose in non-bisected bi/tri-antennary, complex N-linked sequences	Trowbridge, 1974 Debray <i>et al.</i> , 1981
e-PHA	<i>Phaseolus vulgaris</i> (erythroagglutinin) Kidney bean	Bi/tri-antennary bisected complex N-linked sequences	Cummings and Kornfeld, 1982 Yamashita <i>et al.</i> , 1983
I-PHA	<i>Phaseolus vulgaris</i> (leukoagglutinin) Kidney bean	Tri/tetra-antennary, non-bisected complex N-linked sequences	Hämmarstrom <i>et al.</i> , 1982
DSA	<i>Datura stramonium</i> Jimson weed	β 1,4GlcNAc, N-acetyl lactosamine > chitotriose	Green and Baenziger, 1987 Crowley <i>et al.</i> , 1984 Yamashita <i>et al.</i> , 1987
STA	<i>Solanum tuberosum</i> Potato	β 1,4GlcNAc oligomers	Allen and Neuberger, 1973 Debray <i>et al.</i> , 1981
LEA	<i>Lycopersicon esculentum</i> Tomato	β 1,4GlcNAc oligomers	Nachbar <i>et al.</i> , 1980
ECA	<i>Erythrina cristagalli</i> Coral tree	Gal β 1,4GlcNAc β 1-	Iglesias <i>et al.</i> , 1982 De Boeck <i>et al.</i> , 1984
AHA	<i>Arachis hypogaea</i> Peanut	Gal β 1,3GalNAc β 1- > Gal β 1,4GlcNAc β 1-	Lotan and Sharon, 1978 Sueyoshi <i>et al.</i> , 1988
MPA	<i>Maclura pomifera</i> Osage orange	Gal β 1,3GalNAc α 1- > GalNAc α 1-	Sarkar <i>et al.</i> , 1981
HPA	<i>Helix pomatia</i> Roman snail	Terminal GalNAc α 1-	Hammarström <i>et al.</i> , 1977 Torres <i>et al.</i> , 1988
SBA	<i>Glycine max</i> Soybean	Terminal GalNAc α 1- > Gal α 1	Pereira <i>et al.</i> , 1974 Bhattacharyya <i>et al.</i> , 1988
WFA	<i>Wisteria floribunda</i> Wisteria	GalNAc α 1,6Gal β 1- > GalNAc α 1,3Gal β 1-	Sugii and Kabat, 1980 Baker <i>et al.</i> , 1983
DBA	<i>Dolichos biflorus</i> Horse gram	GalNAc α 1,3(Lfuc α 1,2)Gal- β 1,3/4GlcNAc β 1-	Etzler and Kabat, 1970 Baker <i>et al.</i> , 1983
UEA-1	<i>Ulex europaeus-1</i> Gorse	H type 2 antigen (α L-Fuc(1,2) Gal β 1,4GlcNAc β 1-) and Le ^x	Hindsgaul <i>et al.</i> , 1985 Baldus <i>et al.</i> , 1996
LTA	<i>Tetragonolobus purpureus</i> Lotus	L-fucosyl terminals (especially where clustered), Fuc α 1,6GlcNAc > Fuc α 1,2Gal β 1,4(Fuc α 1,3)-GlcNAc β	Pereira and Kabat, 1974 Debray <i>et al.</i> , 1981
SNA	<i>Sambucus nigra</i> Elderberry bark	NeuNAc α 2,6Gal/GalNAc-	Shibuya <i>et al.</i> , 1987
MAA	<i>Maackia amurensis</i>	NeuNAc α 2,3Gal β 1-	Wang and Cummings, 1988 Knibbs <i>et al.</i> , 1991
LFA	<i>Limax flavus</i> Yellow slug	Certain sialyl termini	Miller <i>et al.</i> , 1982 Knibbs <i>et al.</i> , 1993
WGA	<i>Triticum vulgaris</i> Wheatgerm	Di-N-acetyl chitobiose, N-acetyl lactosamine (especially if clustered) and some sialyl residues	Gallagher <i>et al.</i> , 1985

significant degree, reflecting cellular secretory activity. The alpaca placenta was broadly similar (Fig. 2c–e) to that of the camel except that, at the stage examined (day 150), the granules in the maternal epithelium were very sparse (Fig. 2c) and in the trophoblast the pleomorphic granules were situated mainly in the basal part of the cell (Fig. 2d), whereas small electron-lucent vesicles were found apically. Examination of the fetomaternal interface demonstrated the intimate association between the two tissues (Fig. 2e) and the presence of cytoplasmic filaments, which indicate a possible contractile function of the microvilli. In places, vesicles or dilatations of the intercellular space were found in association with the basal areas of the microvilli, especially in the trophoblast.

Lectin histochemistry

The binding properties of the placenta are summarized (Table 2). Not much variation in glycosylation was observed in either camel or alpaca during the course of pregnancy and, hence, two time points have been chosen to illustrate early and late gestation: days 70 and 350 in camel, and days 45 and 347 in alpaca. Both species had areolae associated with the trophoblast layer, but these have not been included in the analysis.

Maternal uterine epithelium

Camel. There was an increase with time in the binding of LTA to granules (Fig. 3a), which was significant only from

Fig. 1. (a,c,e,g) Development of the camel placenta. Low power views at days (a) 70, (c) 170, (e) 210 and (g) 375 of gestation stained with the lectin from *Lycopersicon esculentum* (LEA) to show blunt villous development. Trophoblast (t) and maternal epithelium (m) can be seen. (b,d,f,h) Development of the alpaca placenta. Low power views at days (b) 45, (d) 150, (f) 283 and (h) 347 of gestation stained with LEA. The villi show a more complex branching structure than those of the camel. Two giant cells (*) are visible in (h). Scale bars represent 50 μ m.

Fig. 2. (a,b) Ultrastructure of the camel fetomaternal interface at day 60 of gestation. (a) Maternal uterine epithelium showing round apical secretory droplets (arrows). (b) Trophoblast: inclusions (arrow) are more pleomorphic and irregular in size. (c–e) Ultrastructure of the alpaca fetomaternal interface at day 150 of gestation. (c) Maternal epithelium showing sparse, small, electron-dense secretory droplets (arrows). (d) Trophoblast: large, pleomorphic inclusions (arrows) are found towards the basal aspect of the cell. (e) Interdigitating microvillous membrane showing the intimate connection between the fetal (f) and maternal (m) surfaces. The maternal microvilli appear to be more sparse than those of the trophoblast and are longer than their fetal equivalents, with longitudinal cytoplasmic filaments running within them (arrows). The microvilli of the trophoblast tend to lie against the surface of the maternal epithelium and may have small vesicles or dilations of the intercellular space associated with the basal areas (arrowheads). Scale bars represent (a,b) 1 μ m, (c,d) 2 μ m and (e) 250 nm.

Table 2. Lectin binding properties of camel and alpaca trophoblast and uterine epithelium at early and late gestation

Lectin	Uterine epithelium				Trophoblast			
	Early gestation		Late gestation		Early gestation		Late gestation	
	Camel (day 70)	Alpaca (day 45)	Camel (day 350)	Alpaca (day 347)	Camel (day 70)	Alpaca (day 45)	Camel (day 350)	Alpaca (day 347)
PSA	3 ++++	3–4 +	3 ++	4 +/-	3 ++	3–4 ++	2 +++	3–4 ++
E-PHA	2–3 +++	2–3 ++	2 ^a	0 ^a	2*	4 +/-	2 ^a	0 ^a
L-PHA	0	1 +/-	0	0	0	0	0	0
DSA	3 +++	3–4 ++++	2–3 ++	3–4 +++	3 ++	3–4 +++	3–4 ++	3–4 +++
STA	3–4 +++	2–3 +++	3 ++	3–4 +++	2–4 ++	3–4 +++	3–4 ++	3–4 +++
LEA	3–4 +++	3–4 +++	3 +++	3–4 +++	2–4 +	2–4 ++	3–4 ++	3–4 +++
ECA	3 +++	3 +	3 ++	3 +	4 +/-	2–3 +/-	2–4 +	1 +
AHA	3 +	3 +/-	4 +++ ^a	0 ^a	0 ^a	3–4 +++ ^a	3 +/-	2–3 ++
MPA	3 +++	4 ++	2 ++	2 +/-	3 +++	2–4 ++	3 ++++	3 +++
HPA	2–3 +	3–4 ++	3 +	4 +	3–4 ++	2–4 ++	3–4 ++	3–4 +++
SBA	3 +++	3 ++	4 ++	3 ++	4 +/-	3 +/-	3 ++	2–3 ++
WFA	4 +++	3–4 +++	4 ++	4 ++	4 +	3–4 ++	4 +++	3–4 ++
DBA	3 +	1–2 +++	0	0	0 ^a	2–3 +++ ^a	0 ^a	1–2 +++ ^a
UEA-1	3 ++	3 +	3 ++	4 ++	0	3 +/-	4 +/-	4 +/-
LTA	1 +	0	3 ++	4 +	2–3 ++	2–3 ++	3 ++	2–3 +++
SNA	0	2 +	0	0	0	3 +/-	0	0
MAA	0	3 +	0	2 +/-	0 ^a	2–4 +++ ^a	0 ^a	2–3 +++ ^a
LFA	0	0	0	0	0	0	0	0
WGA	4 +++	4 ++	3 ++	3 +	4 +	2–4 ++	2–4 +++	3–4 ++

Staining: 0: negative; 1: weak; 2: moderate; 3: strong; 4: intense.

Granule density: +/- : occasional; +: sparse; ++: few; +++: many; ++++: numerous.

*The staining with e-PHA was uniform and not granular in these cases.

^aIndicates significant differences between the two species at that stage of gestation.

day 145 of gestation, whereas binding of SBA decreased from about day 265, and binding of WFA (Fig. 3c) and WGA decreased from day 350. There was no binding of L-PHA, SNA, MAA or LFA at any stage, whereas DBA stained occasional granules up to day 110 of gestation and was then negative until day 375, when weak staining was again observed. Binding of several lectins, namely PSA (Fig. 3e), ePHA (Fig. 4a), UEA-1, MPA, AHA (Fig. 4c,e), ECA (Fig. 5a), LEA (Fig. 5c), DSA, STA (Fig. 5e) and HPA was fairly consistent throughout gestation. The maternal epithelium became somewhat flattened during the course of pregnancy.

Alpaca. There was a loss in the binding of e-PHA (Fig. 4b), L-PHA and SNA after day 45 of gestation, at which point LTA staining was observed (Fig. 3b). AHA bound to a scarce subpopulation of granules up to day 150 only (Fig. 4d,f) and DBA bound weakly, or not at all, to a subpopulation of granules. LFA did not bind in this tissue and many lectins, namely PSA (Fig. 3f), LTA (Fig. 3b), WFA (Fig. 3d), UEA-1, MPA and ECA (Fig. 5b), HPA, SNA, MAA and WGA stained a subpopulation of granules only. More widespread staining was observed with SBA, DSA, LEA and STA (Fig. 5d,f).

Trophoblast

Camel. There was no binding of L-PHA, DBA (except for a very occasional granule at day 170), SNA, MAA or LFA.

Many granules stained with PSA, MPA, DSA and STA (Figs 3e and 5e), whereas subpopulations bound LEA (Fig. 5c) and HPA in early pregnancy. There was diffuse cytoplasmic staining with e-PHA (Fig. 4a), with some granular staining between day 90 and day 265 of gestation, and an increase in the binding of LTA, SBA, WFA and WGA to granules was observed over the course of gestation (Fig. 3a,c). Occasional granules were stained with UEA-1 at days 145, 170 and 350 of gestation, and the density of granules within cells also varied with AHA; few granules were present at day 60 and from day 145 to day 375 (Fig. 4e). ECA binding was absent at day 60, but occasional granules bound thereafter (Fig. 5a). The giant cells were sparse and were not observed in every specimen; in general they stained in a similar way to the rest of the trophoblast. MPA bound less strongly. There was a tendency for the staining of giant cells, as with the trophoblast in general, to be somewhat polarized, with the most intense stain being near the microvillous border (Fig. 5e); this often led to a variegated appearance to the trophoblast in sections.

Alpaca. There was no binding of L-PHA or LFA, and only very occasional granules stained with SNA at days 22 and 45 of gestation. Granules bound LTA (Fig. 3b), WFA (Fig. 3d), PSA (Fig. 3f), MPA, AHA (Fig. 4d,f), DSA, LEA (Fig. 5d), STA (Fig. 5f), HPA, MAA and WGA, and pleomorphism was

Fig. 3. (a,b) *Tetragonalobus purpureus* agglutinin (LTA). (a) At day 375 of gestation, sparse granules are observed in both the maternal epithelium (m) and the trophoblast (t) of camel, with a more strongly staining interdigitating microvillous membrane. (b) In alpaca at day 347 of gestation, the trophoblast shows moderate staining with only occasional granules in the maternal epithelium (arrow). The giant cell (*) shows a gradation in binding, which is concentrated near the microvillous surface. (c,d) *Wisteria floribunda* agglutinin (WFA). (c) At day 170 of gestation there is strong binding of this lectin to both maternal epithelium and trophoblast of camel at this stage of pregnancy. (d) At day 150 of gestation there is less binding of WFA to both maternal epithelium and trophoblast of alpaca. (e,f) *Pisum sativum* agglutinin (PSA). (e) At day 70 of gestation the maternal epithelium has many lectin-binding granules in camel, whereas in the trophoblast, granules are more pleomorphic and sparse. (f) At day 45 of gestation there are only very occasional PSA-positive granules in the maternal epithelium in alpaca, but a variety of different granules, some of which stain strongly, in the trophoblast. Scale bars represent 50 μ m.

often apparent, some granules attaining considerable size, especially in the early stages of gestation (Figs 3f, 4d and 5d). There was a loss of binding of e-PHA at day 264 of gestation (Fig. 4b) and of UEA-1 at day 283 of gestation, although binding had been very sparse in the latter case. DBA staining became weaker over the course of pregnancy and ECA bound to a subpopulation of sparse granules only (Fig. 5b), with hardly any staining at day 296 and term. There was a slight increase in the number of granules binding SBA from day 264. In general, the giant cells showed the same binding characteristics as the rest of the trophoblast (Fig. 5f), although they sometimes appeared slightly paler with more homogeneous granulation (Fig. 4f). There was often a clear zonation within the cell: most staining was observed in an area underneath the

microvillous membrane, whereas the rest of the cell was stained less intensely (Fig. 3b). However, at term, the staining was generally stronger than in the rest of the trophoblast; this was particularly marked with PSA, MPA, SBA, WFA, HPA and WGA.

Interdigitating microvillous membrane

In general, there was no lectin binding to the membrane when both tissues were unstained. An exception was DBA binding to the alpaca microvillous membrane, which sometimes stained intensely even though the two tissue layers had little reactivity, indicating the possibility of glycosyl transferase activity at the cell surface. However, usually the membrane was stained only by lectins where

Fig. 4. (a,b) *Phaseolus vulgaris* erythroagglutinin (e-PHA). (a) Staining with e-PHA is very diffuse in the camel trophoblast (t) and maternal epithelium (m) at day 375 of gestation. (b) At day 347 of gestation in alpaca, there is no binding of e-PHA, although blood capillaries stain strongly. (c,d) *Arachis hypogaea* agglutinin (AHA). (c) At day 90 of gestation in camel, AHA shows moderate binding to granules in the maternal epithelium only. (d) At day 45 of gestation in alpaca, there is some binding to the maternal epithelium and many different granules are stained in the trophoblast. (e,f) *Arachis hypogaea* agglutinin (AHA). (e) At day 375 of gestation in camel there is still binding of AHA to the maternal epithelium and occasional positive granules in the trophoblast. (f) At day 347 of gestation there is no binding to the maternal epithelium but moderate staining of the trophoblast in alpaca. A giant cell (*) appears slightly paler than the rest of the trophoblast. Scale bars represent 50 μ m.

one of the two cell layers (maternal or fetal) contained lectin-positive granules, which were probably secreted on the microvillous surface. The vesicles or dilatations associated with the basal areas of the trophoblastic microvilli may be involved in the secretion or uptake of material at this interface. The staining properties of the interdigitating microvillous membrane depended to some extent on the closeness of the attachment between the two tissues. It became clear from examination of free surfaces (where they had detached from each other during specimen

preparation) that some steric hindrance often occurred in the intact tissue, which reduced the binding of the lectin. This resulted in much weaker staining than when the same surfaces were free.

Controls

Substitution of buffer for the lectin resulted in the absence of staining, whereas incubation of the lectin in the presence of its inhibitory sugar reduced or eliminated the

Fig. 5. (a,b) *Erythrina cristagalli* agglutinin (ECA). (a) At day 90 of gestation in camel, there is moderate binding to the maternal epithelium (m) but only very occasional granules that stain in the trophoblast (t, arrow). (b) At day 45 of gestation in alpaca, neither the maternal epithelium nor the trophoblast shows much staining. (c,d) *Lycopersicon esculentum* (LEA). (c) At day 90 of gestation in camel, the maternal epithelium stains quite strongly and a few pleomorphic granules can be seen in the trophoblast. (d) At day 45 of gestation in alpaca, there is binding to the maternal epithelium and numerous weakly stained granules in the trophoblast, together with some intensely stained pleomorphic inclusions. (e,f) *Solarium tuberosum* agglutinin (STA). (e) A giant cell (*) in camel at day 170 of gestation shows more binding near the microvillous border and the maternal epithelium is stained more strongly. (f) A giant cell in alpaca at day 347 of gestation clearly shows the bizarre profiles of the nuclei. Scale bar represents 50 μm .

staining in the tissues. Neuraminidase treatment of sections before application of the sialic acid-binding lectins (MAA, SNA and LFA) also reduced their ability to bind.

Discussion

These findings show that, despite their common ancestry and identical diploid chromosome number ($2n = 74$), there are several differences in the glycosylation of the fetomaternal interfaces of camels and alpacas. The most striking qualitative differences within the trophoblast are in the occurrence of bi/tri-antennary bisected *N*-glycan

(e-PHA), fucosylated structures bound by DBA ($\text{GalNAc}\alpha 1,3(\text{Fuc}\alpha 1,2)\text{Gal}\beta 1,3/4\text{GlcNAc}\beta 1-$), β -galactose (AHA) and $\alpha 2,3$ -linked sialic acid (MAA). In the maternal uterine epithelium, differences are found in bisected bi/tri-antennary *N*-glycan (e-PHA) and β -galactosyl residues (AHA). Several other minor quantitative interspecies differences were also evident. Fucosyl residues are very variable, even between closely related species (Jones *et al.*, 2000). They are also important in cyclic changes of the endometrium in both humans and baboons (Aplin *et al.*, 1997; Jones *et al.*, 1998), where they may be under steroidal control based on altered binding by DBA. Fucosyl residues

are added by fucosyl transferases. The FUT-1 gene (H-type $\alpha(1-2)$ fucosyl transferase) is hormonally modulated in mouse endometrium (Sidhu and Kimber, 1999), whereas the fucosylated H-type-I antigen has been implicated in rodent blastocyst–endometrial interactions (Kimber *et al.*, 1995; Sidhu and Kimber, 1999). Similar mechanisms may also operate in other species. There is less sialylation in camels than in alpacas, and treatment with neuraminidase (data not shown) has little effect on the lectin binding to uterine epithelium or trophoblast in either species, confirming the scarcity of sialyl termini there. Tri/tetra-antennary bisected complex N-glycan, shown by I-PHA staining, is also sparse in both species, whereas N-acetyl lactosamine (DSA, WGA), N-acetyl galactosamine (SBA, WFA) and N-acetyl glucosamine oligomers (STA, LEA) are generally abundant.

The results of the present study indicate that there are more differences in the glycans found in the trophoblast than in the uterine epithelium, a feature that was also noted in our previous study comparing camels and horses with horses and donkeys (Jones *et al.*, 2000). These results indicate that uterine tissues are more conserved than trophoblast with respect to glycosylation. Murphy (2000) noted that similar basolateral and apical plasma membrane alterations occur in the uterine epithelium of many species during pregnancy, despite variation in the type of placenta that is formed ultimately. There are also differences in the general structure of the two placentae: alpacas have more complex branching of the placental villi than do camels, and also have many more giant cells, although these were not described in the ultrastructural study of alpaca placenta by Steven *et al.* (1980). The giant cells increase in number and size during gestation and show polyploidy (L. Oliveira, personal observation); polyploidy is also observed in the giant cells of camels (Gorokhovskii *et al.*, 1975). However, it has yet to be determined whether the nuclear profiles that are observed in camel giant cells are individual structures or part of one, or a few, large tortuous structures, as suggested by van Lennep (1961).

The similarity in lectin binding in the giant cells and uninuclear trophoblast of camels was reflected in their similar ultrastructure, as described by Skidmore *et al.* (1996); no obvious secretory granules were seen in their study, although lysosomes were described, which may account for some of the granular binding observed in the present study. Smooth endoplasmic reticulum was observed, which may relate to the steroid biosynthesis reported in camels (Ozturk *et al.*, 1999), and also in rat and ruminant giant cells (Hoffman and Wooding, 1993). Much of the lectin staining is localized to very fine granular areas in the cell, which may be a reflection of glycosylation associated with subcellular organelles such as cisternae of endoplasmic reticulum, mitochondria, small electron-lucent vesicles and Golgi-associated structures, which are beyond the resolution of the light microscope and were observed at the ultrastructural level in the present study.

There have been many attempts to produce hybrids between unrelated species; other than the camel–llama cross there have also been attempts to cross laboratory and wild

mice species (Rossant *et al.*, 1982, 1983), mice and bank voles (Mystkowska, 1975), and sheep and goats (Fehilly *et al.*, 1984; Meinecke-Tillmann and Meinecke, 1984). In most cases, the embryos do not survive to term. Rossant *et al.* (1983) contended that the trophoblast was the critical factor in embryo survival as, in the mouse system, the donor inner cell mass could survive indefinitely if injected into maternal host blastocysts. This was also true in the sheep–goat cross, as mixing donor with maternal host blastocysts, or surrounding them with a host zona pellucida, so that the chorionic epithelium of the placenta developed from cells of the maternal host, resulted in the birth of sheep–goat hybrids (Fehilly *et al.*, 1984; Meinecke-Tillmann and Meinecke, 1984). This finding implies that the surface characteristics of the trophoblast are of significance in establishing and maintaining a viable pregnancy, whether due to an immune response or other factors that contribute to the formation of a stable interface or intercellular adhesion, which in camels is a prolonged and complex process (Abd-Elnaeim *et al.*, 1999). These factors must be appropriately ‘matched’ to the maternal tissues and appear to operate not just at the time of implantation but throughout the period of gestation, as some embryonic hybrids between camels and llamas were resorbed between day 25 and day 40 of gestation, whereas others survived up to days 260, 291 and 302 (Skidmore *et al.*, 1999). Two other hybrids were stillborn at day 365 and one live hybrid was born prematurely at day 328 of gestation. Problems in maintenance of pregnancy were found in both directions (female camel $\times$ male alpaca and male camel $\times$ female alpaca) of cross.

This failure to maintain pregnancy is similar to observations of extraspecific donkey-in-horse pregnancy (the transfer of donkey embryos to horse mares), in which there is minimal or no microvillous attachment between fetal and maternal epithelial surfaces (Allen, 1982; Allen *et al.*, 1987), and the endometrium does not undergo the architectural modifications that would normally provide crypts to accommodate the placental villi. Instead of the normal microvillous interdigitation, the trophoblast simply rests on the surface of the uterine epithelium, as if, according to the author (Allen, 1982) ‘...each epithelial surface was trying to repel rather than unite with the other...’. Trophoblast cells also produced excess secretions that formed a barrier between the two cell layers. An immunological response also developed, apparently directed against the trophoblast, and about 70% of these pregnancies were aborted (Allen *et al.*, 1987; Allen and Short, 1997). These occurrences indicate that there may be an abnormality in the intercellular responses between maternal uterine epithelium and trophoblast, which may implicate glycan–glycan interactions, as these structures are so abundant at the two apposing cell surfaces. Oligosaccharides are capable of possessing an enormous coding capacity that can mediate cell adhesion (Solís *et al.*, 2001). Whyte and Allen (1985) and Jones *et al.* (2000) have suggested that any aberration of this recognition system may cause a failure in blastocyst attachment at implantation. There are many factors that influence such

intercellular adhesions and steroid-modulated events are of prime importance in implantation (Carson *et al.*, 1990; Aplin, 1997), especially in relation to glycoproteins and their glycosylation (Schlafke and Enders, 1975; Cross *et al.*, 1994; Wegner and Carson, 1994; Bowen *et al.*, 1996; Aplin, 1997; Aplin *et al.*, 1997; Jones *et al.*, 1998; Johnson *et al.*, 2000). The failure of such interactions may contribute to the difficulty in establishing interspecies hybrids, both between the camel and llama, and among equids.

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