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Morphological and glycan features of the camel oviduct epithelium

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SUMMARY

This study describes regional differences in the oviduct of the one-humped camel (*Camelus dromedarius*) during the growth phase (GP) and the mature phase (MP) of the follicular wave by means of morphometry, scanning electron microscopy (SEM) and glycohistochemistry investigations. Epithelium height significantly increased in the ampulla and decreased in the isthmus passing from the GP to the MP. Under SEM, non-ciliated cells displayed apical blebs (secretory) or short microvilli. Cilia glycocalyx expressed glycans terminating with sialic acid linked α2,6 to Gal/GalNAc (SNA affinity) throughout the oviducts of GP and MP and sialic acid linked α2,3 to Galβ1,3GalNAc (MAL II and KOH-sialidase (K-s)-PNA staining) throughout the MP oviducts. Non-ciliated cells displayed lectin-binding sites from the supra-nuclear cytoplasm to the luminal surface. Ampulla non-ciliated cells showed O-linked (mucin-type) sialoglycans (MAL II and K-s-PNA) during GP and MP and N-linked sialoglycans (SNA) during the MP. Isthmus non-ciliated cells expressed SNA reactivity in GP and MP, also K-s-PNA binders in MP, and MAL II and PNA affinity (Galβ1,3GalNAc) during GP. Galβ1,3GalNAc was sialilated in the non-ciliated cells of GP UTJ. Luminal surface lacked of Galβ1,3GalNAc in GP and MP, whereas it expressed α2,6- and α2,3-linked sialic acids. In GP intraluminal substance reacted with SNA, MAL II, K-s-PNA in ampulla and only with MAL II in the isthmus and UTJ. These results demonstrate that the morphology and the glycan pattern of the camel oviductal epithelium vary during the follicular wave and that could relate to the region-specific functions.

1. Introduction

The oviduct plays an essential role in mammalian reproduction because it is an active secretory organ providing a suitable microenvironment for the oocyte maturation, sperm capacitation, fertilization, early embryonic development and transportation (Hunter, 2003).

The mammalian oviduct can be divided into three anatomically and functionally different regions: infundibulum, ampulla and isthmus. The fimbriated infundibulum introduces the ovulated eggs to the oviductal fluid and pushes them into the ampulla, where fertilization and early cleavage-stage of embryo development takes place. The isthmus is considered to be a sperm reservoir as well as being involved in the regulation of sperm transport to the ampulla and in the passage of the embryo(s) into the uterus (Abe, 1996; Suarez, 2002).

The oviductal mucosa is lined by a simple columnar epithelium consisting of two types of cells: ciliated and non-ciliated cells. Ciliated cells play a role in the transport of germinal cells (Odor and Blandau, 1973; Kölle et al., 2009), whereas the non-ciliated cells are secretory cells mainly involved in the synthesis and release of glycoproteins (OGPs) that are dissolved in the oviductal fluid (Murray, 1992; Leese, 1988; Buhi et al., 2000; Hunter, 2003; Georgiou et al., 2007; Avilés et al., 2010; Desantis et al., 2011; Singh et al., 2012).

OGPs interacts physiologically in reproductive events. It has been demonstrated they are in association with the zona pellucida and perivitelline space of oviductal oocytes and embryos (Oikawa et al., 1988; Robitaille et al., 1988; Kan et al., 1990; Hunter, 1994; Gandolfi, 1995; Malette et al., 1995; Kolbe and Holtz, 2005; Lyng and Shur, 2009). Moreover, OGPs bind to sperm surface and may

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enhance sperm capacitation (Killian, 2004). Therefore OGPs play important roles in fertilization and early embryonic development (Coy et al., 2012).

Although reports exist on the gross anatomy and histology of the camel oviduct (Osman, 1967; Tibari and Anouassi, 1997), scarce and incomplete studies have been carried out on its ultrastructural features and glycan composition. In particular, one study was performed on the infundibulum region of the camel oviduct by electron microscopy (Nayak, 1977). In addition, the oviductal glycan composition has recently been studied (Salari et al., 2011) by conventional glycohistochemical investigation (PAS and Alcian Blue stainings). The lectins permit the characterization of the glycoconjugates (Spicer and Schulte, 1992). Thus, lectin histochemistry has been successfully used to demonstrate the *in situ* distribution of glycoconjugates in several mammalian oviducts such as mice (Lee et al., 1983), humans (Schulte et al., 1985; Kiss et al., 1998; Gheri et al., 2007), hares (Menghi et al., 1988), rats (Menghi et al., 1989), pigs (Raychoudhury et al., 1993; Walter and Bavdek, 1997), rabbits (Menghi et al., 1995), hamsters (El-Mestrah and Kan, 1999), monkeys (Jones et al., 2001) and horses (Ball et al., 1997; Desantis et al., 2004, 2005). Among the carbohydrates that constitute the oligosaccharide chains in glycoproteins, sialic acids are known to be a large family of nine-carbon carboxylated sugars that usually occupy the terminal position of the oligosaccharides and, due to their net negative charge, act as ligands in recognition phenomena (Schauer, 1985; Varki, 1997) as well as *in vitro* sperm capacitation (Banerjee and Chowdhury, 1994; Focarelli et al., 1995) and sperm-egg interaction (Geng et al., 1997; Cortés et al., 2004).

Camels are induced ovulators characterized by successive follicular waves, each consisting of growth phase, maturation phase and regression phase. During the growth phase usually one follicle becomes dominant from a cohort of small follicles and continues the growth. During the maturation phase, the dominant follicle ovulates if mating occurs, otherwise it could continue to grow (large anovulatory follicle) or regress (regression phase) (Skidmore, 2011). The follicular development depends on the concentration of oestrogen which reaches its maximum when one dominant follicle is present in the ovary (Skidmore, 2011). The aim of this study has been to investigate the morphology and the sialoglycan pattern of the camel oviduct during the growth and the maturation phase by means of scanning electron microscopy and lectin histochemistry, respectively.

2. Materials and methods

2.1. Tissue preparation

Oviducts and initial uterine horns from growth ($n = 3$) and mature ($n = 3$) phases of the follicular wave (Skidmore, 2011) were obtained from local abbatoirs in Cairo (El-Basateen, Cairo gov.; Kerdassa and El-Moneeb, Giza gov.). Ovarian phases were identified by visual observation of the two ovaries soon after the retrieval of female genitalia after slaughtering. The growth phase was identified when emergent small follicles (3–10 mm in diameter) were visible on the ovarian surface, while the mature phase was assigned when the ovary contained at least one dominant follicle (1.3–1.9 cm in diameter) (Skidmore, 2011). Immediately after collection, the ampulla, isthmus and papilla of oviduct-utero junction were cut into small pieces and fixed by immersion either in Bouin's fluid for 24 h at room temperature (RT) or in 3% glutaraldehyde buffered with 0.1 M sodium cacodylate (pH 7.2) for 4 h at 4 °C.

2.2. Light microscopy

The Bouin fixed tissues were then dehydrated in an ethanol series, cleared in xylene, and embedded in paraffin wax. Serial sections (4 μm thick) were cut and, after de-waxing with xylene and hydration in an ethanol series of descending concentrations, were stained with haematoxylin–eosin for morphological and morphometric studies and, by means of conventional histochemical procedures or the lectin histochemistry according to Desantis et al. (2004) for glycoconjugate characterization.

2.3. Conventional histochemistry

Sections were treated with Alcian blue at pH 2.5 (AB 2.5) for testing acidic glycoconjugates (Pearse, 1968) and periodic acid-Schiff (PAS) reaction for neutral glycoconjugates (Mc Manus, 1948).

2.4. Lectin histochemistry

De-waxed and re-hydrated tissue sections were immersed in 3% H_2O_2 for 10 min to suppress the endogenous peroxidase activity, rinsed in 0.05 M Tris–HCl buffered saline (TBS) pH 7.4 and incubated in lectin solution (20–25 μg/ml) for 1 h at room temperature (RT). Sialic acid residues were demonstrated directly by the binding with the biotinylated lectins MAL II and SNA (specific for Siaα2-3Galβ1-3(±Siaα2-6)GalNAc (Geiser and Jarvis 2011) and Neu5Acα2,6Gal/GalNAc (Shibuya et al., 1987), respectively, and indirectly with identification of its subterminal GalNAc residue by the binding with HGP-conjugated PNA lectin (specific for Galβ1,3GalNAc (Lotan et al., 1975), without and with prior sialidase digestion.

Sections incubated in biotinylated MAL II and SNA lectins were treated with streptavidin/peroxidase complex for 30 min and subsequently with 0.05% (w/v) 3,3′-diaminobenzidine (DAB) plus 0.003% (v/v) H_2O_2 in 0.05 M TBS (pH 7.6) for 10′, whereas those incubated in HGP-conjugated PNA were directly developed in DAB-H_2O_2 solution. Controls for lectin staining included (i) incubation with lectin-free substrate medium and (ii) incubation with each lectin in the presence of its hapten inhibitory sugar (0.2–0.5 M in Tris buffer).

2.5. Sialidase treatment

Before staining with PNA some sections were incubated at 37 °C for 16 h in 0.86 U/mg protein of sialidase (neuraminidase) (Type V, from *Clostridium perfringens*) dissolved in 0.1 M sodium acetate buffer, pH 5.5, containing 10 mM $CaCl_2$. As controls of the enzyme digestion procedure, sections were incubated in the enzyme-free buffer solution under conditions of the same duration and temperature. In control sections, cleavage of sialic acid was not evident.

2.6. Scanning electron microscopy

The glutaraldehyde fixed tissues, after rinsing, were post-fixed in 1% OsO_4 buffered with sodium cacodylate for 2 h at 4 °C, rinsed, dehydrated in an ethanol series, and then critical point dried using CO_2. Specimens were mounted on stubs, coated with gold–palladium in a sputter coater, and examined using a LEO S420 SEM.

2.7. Mophometry and statistical analysis

The height of epithelium lining the oviduct was measured on 15 microphotograph fields randomly detected and taken with a digital camera (DC 300, Leica, Cambridge, U.K.) connected to a light microscope (DMRBE, Leica, Cambridge, U.K), using a 100× lens and an image analysis software (QWIN, Leica, Cambridge, U.K.). For the measurement, we selected cells in which the plane of the section clearly passed through the cell nucleus, parallel to the longitudinal axis of the cells in each region.

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