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Glycan profile of oviductal isthmus epithelium in normal and superovulated ewes

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ABSTRACT

Glycans of oviductal isthmus are implicated in sperm-isthmus interaction, sperm storage, survival, and capacitation. Isthmus morphology and glycoprotein production are controlled by sex steroids, which could be responsible for alterations of some reproductive events in the superovulated ewes (SE). In this study, the oviductal isthmus epithelium was evaluated in normal and in SE using morphologic and lectin histochemical analysis. The epithelium of normal isthmi was significantly taller in folds than in crypts, whereas it significantly decreased in the folds of SE. Nonciliated cells (NCs) from normal, showed apical blebs revealing apocrine secretory activity, which was missing in SE. The quantitative analysis of lectin staining revealed higher Con A, DBA, and PNA reactivity but lower affinity to KOH-sialidase- (Ks)WGA, GSA II, LTA, UEA I, SBA, GSA I-B_4, RCA_{120}, KsPNA, MAL II, SNA in control isthmi compared with superovulated ones. The NCs apical blebs showed terminal fucose (Fuc), N-acetylgalactosamine (GalNAc), galactose (Gal), lactosamine, and O- and N-sialoglycans. In normal isthmi, the luminal surface of NCs and ciliated cells expressed Fuc, highly mannosilated N-glycans terminating with lactosamine as well as O-glycans ending with N-acetylglucosamine (GlcNAc) and GalNAc. Moreover, NCs microvilli contained Gal and α2-3-linked sialic acids. In SE, the luminal surface lacked Gal and GalNAcα1,3(LFucα1,2)Galβ1,3/4GlcNAcβ1, whereas it was enriched with Fuc in the folds and with α2-3sialo-mucins both in crypts and in folds. The apical surface showed additional O- and N-linked sialoglycans in NCs and αGal in the cilia, which expressed α2-6-linked sialic acid only in the folds. The cytoplasm of control NCs showed highly mannosilated N-glycans throughout the epithelium and GlcNAc in the folds. After superovulation treatment, NCs expressed cytoplasmic terminal Fuc, βGalNAc, lactosamine, α2-3-, and α2-6-linked sialic acids in the folds. The cytoplasm of normal ciliated cells cells displayed a binding pattern similar to normal NCs except for the absence of higly mannosilated N-glycans in the folds, which appeared in superovulated samples. This study demonstrates glycan zone-specific distribution along the isthmus epithelium that is influenced by the superovulation treatment. Whether an alteration in the glycan distribution is implicated in the low-rate fertilization after natural mating of the superovulated sheep remains to be addressed.

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1. Introduction

The mammalian oviduct plays a pivotal function in early reproductive events because its epithelial cells create a unique environment for gamete transport, sperm

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capacitation, fertilization, early embryonic development [1–4], and gender selection of the offspring [5].

The oviduct can be divided into three anatomically and functionally different regions: infundibulum, ampulla, and isthmus. Isthmus, the caudal portion of the oviduct, is recognized as the sperm reservoir in several mammals, including sheep [6], and it is the place where the last steps of sperm capacitation occur [7]. There is evidence that sperm-isthmus cells binding is mediated by species-specific glycans exposed on the apical surface of oviduct epithelial cells [6,8] and that isthmus glycoproteins affect sperm survival and capacitation state [7,9,10]. Moreover, this oviductal region plays a key role in sperm transport to the ampulla and in the transit of the embryo to the uterus [11,12].

The superovulation is an assisted reproductive technology widely used in small ruminant management for out of season estrus induction, enhancement of reproductive performance, and genetic improvement [13,14]. In sheep the most common ovarian hyperstimulation program includes the estrous synchronization on the basis of progestagen/progesterone and prostaglandin treatments [15,16] and superovulatory treatment with the administration of exogenous gonadotrophins. Among these hormones, progesterone perturbs the levels of estradiol-17β in the blood plasma, the oviductal wall [17], and the secretory activity of the oviduct [18–20] and is responsible for the lower fertility than naturally occurring estrus ewes [21]. Superovulated ewes show a lower fertilization rate after natural mating caused by a deficiency in sperm migration [22–24]. In addition, superovulated sheep exhibit embryo alterations that occur during the transition from the oviduct to the uterus causing a decreased viability. These alterations may be ascribed to the altered oviductal environment which influences the expression of genes involved in the regulation of metabolic activity in the embryo [25,26].

As in other oviductal regions, the epithelium lining the isthmus is made up of columnar ciliated cells (CCs) and nonciliated cells (NCs). CCs play a role in the ovum transport and in the mucociliary propagation toward the uterus [27]. NCs are secretory cells and their activity determines, together with a selective transudate of serum, the composition of the fluid in the oviduct. This fluid consists of amino acids, proteins, lipids, ions, energy substrates, hormones, and growth factors, which all have important roles in the physiology of the oviduct and embryo development [see 3,28 for review]. Among the components secreted by NCs in the oviductal fluid, there are the oviduct-specific glycoproteins (OGPs). These macromolecules have been found to play an important role in the fertilization process as they can associate with ovulated ova, spermatozoa, and early embryos [18,29,30].

The production and secretion of OGPs vary with hormonal changes during the ovulatory cycle [1,3,4,18]. In particular, estrogens stimulate the secretion of the oviductal epithelium which is very high in the proliferative phase [4,18]. Oviduct-specific glycoproteins accumulate as a mucus-like substance in the lumen of the isthmus [31]. The mucus viscosity depends on the sex steroid fluctuations during sexual cycle [31]. Thus, the aim of this study was to investigate for the first time the glycan pattern of the lining epithelium of sheep oviductal isthmus and the changes induced by an effective superovulatory treatment [32,33]. The glycan pattern was investigated by lectin histochemistry, the most suitable *in situ* technique to detect cellular glycosylation and has been successfully used to characterize oligosaccharides in the oviduct of several mammals, including sheep [34].

2. Materials and methods

2.1. Animals

Institutional Review Board approval of the study was obtained by the University of Bari Aldo Moro, Ethic Committee DETO, Italy, N° 002/2009. Procedures with animals were performed following good veterinary practice for animal welfare according to national laws in force (D.Lgs 116/92). Six adult ewes (42–59 kg body weight [bw]; n = 3 control, n = 3 treated animals) of the Comisana Breed were fed with high-quality hay and concentrate mixture (0.5 kg/head/day) twice a day with free access to fresh water in May (out of reproduction period). In treated ewes, the time of estrus was synchronized by a single intramuscular (IM) injection of 37.5 mg of D-cloprostenol at Day 0 (0.5 mL, Dalmazin, Fatro, Italy), and contemporaneous intravaginal insertion of FluorogestoneAcetate-impregnated sponge (CronoGest Spunge 40 mg, Intervet International), which was kept in place for the subsequent 12 days. Pluset (FSH + LH; Serono, Rome, Italy), was administered IM in decreasing doses, twice daily for 3 days (125, 75, and 50 IU/day) from Day 10 to day 13. On Day 12, at device removal, 1000 IU pregnant mare's serum gonadotropin (Folligon, Intervet International BV, Boxmeer, Holanda) was administered IM [35]. Treated animals underwent ovariohysterectomy 54 hours after device removal and when we ultrasonographycally observed multiple corpora lutea formation and ovulating follicles. This time lapse was chosen because it is considered the periovulation time (median time) of all ovulations when pregnant mare's serum gonadotropin is used [36]. In control ewes (non-superovulated animals), ovariohisterectomy was performed in the day when ovulation occurred (detected by ultrasonography). Anesthesia was obtained by intravenous administration of 2% xylazine hydrochloride (Rompun; Bayer, Italy) at 1 mg/10 kg/bw combined with IM injection of 0.4 mg/10 kg/bw atropine sulfate (ATI, Italy) followed by IM injection of 40 mg/10 kg/bw, Zolazepam and Tiletamine (Zoletil; Virbac, Italy).

2.2. Tissue preparation

After ovariohysterectomy, isthmus was separated from the other oviductal regions and fixed overnight in 4% (wt:vol) phosphate-buffered paraformaldehyde at room temperature. After fixation, tissues were washed and dehydrated in ethanol series, cleared in xylene, and embedded in paraffin wax. 4-μm thick sections were cut and, after dewaxing with xylene and hydration in an ethanol series of descending concentration, they were stained with Mayer's hematoxylin and eosin (to study the general morphology) or processed for lectin histochemistry [34].

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