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Lectin-binding sites in isolated equine cumulus-oocyte complexes: Differential expression of glycosidic residues in complexes recovered with compact or expanded cumulus

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Abstract

Equine cumulus-oocyte complexes (COCs) were analyzed by means of 13 lectins to evaluate their glycoconjugate patterns and to verify differences between COCs recovered with compact (Cp) and expanded (Exp) cumulus. Cumulus cells showed a similar staining pattern in both Cp and Exp COCs with all lectins used, except for a higher reactivity with SNA and GSA II in Cp COCs and SBA in Exp COCs. The zona pellucida (ZP) showed (1) uniform staining with MAL II, RCA_{120}, and SBA in both Cp and Exp COCs, (2) trilaminar binding pattern with WGA as well as higher Con A reactivity in the outer region of both types of COCs, (3) uniform staining with PNA only in Exp COCs, (4) uniform and trilaminar binding pattern with SNA in Cp and Exp COCs, respectively, and (5) major reactivity with GSA II in Exp COCs. Ooplasm showed similar staining intensity with Con A, HPA, GSA I-B_4, and WGA in both Cp and Exp COCs, with stronger reactivity to GSA II in Exp COCs, whereas SNA, UEA I, and LTA binding sites were present only in Cp COCs. Oocyte cortical granules of both Cp and Exp COCs reacted with Con A and WGA. These results suggest that, in the mare, viable (Cp) and atretic (Exp) COCs display different glycoconjugate staining pattern, which may account for the different maturation and developmental competence of COCs.

Keywords: Mare; Ovarian follicles; Glycoconjugates; Lectin histochemistry

1. Introduction

In the mare, the cumulus-oocyte complex (COC) is morphologically classified as having compact (Cp) or expanded (Exp) cumulus investment [1]. Oocytes with Cp cumuli, having a tight, complete compact cumulus with a distinct and smooth hillock, are recovered largely from viable follicles, whereas oocytes with Exp cumuli, characterized by a granular or expanded cumulus, are recovered widely from atretic follicles [2]. Oocytes with Cp cumuli show a lower meiotic competence [2,3], a slower rate of maturation [3–5], reduced ability to respond to an activation stimulus [6], and reduced male pronucleus formation rate after intracytoplasmic sperm injection (ICSI) [7] when compared with oocytes with

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Exp cumuli. Expanded oocytes fertilized by ICSI and transferred into the oviducts of recipient mares showed 85% cleavage and development to an average of 16 cells at 96 h after transfer, equivalent to normal development "in vivo" [8]. The physiologic differences between these two types of COCs have been attributed to their different initial chromatin configuration within the germinal vesicle (GV) [2,9,10].

Glycobiological investigations of reproductive biology in mammals suggest that oligosaccharides act as essential functional components of glycoproteins involved in fundamental steps, such as oocyte maturation [11–13], sperm-egg binding, and fertilization [13–18]. In particular, it has been reported that ooplasmic cortical granules of hamster preovulatory and ovulated oocytes show different expression of some glycoconjugates [11] and that the oligosaccharide pattern of the zona pellucida (ZP) changes between the stages of GV and metaphase II (MII) of porcine oocytes [12,13].

Lectins have specific binding affinity for sugar residues of glycoconjugates, therefore they represent useful tools for investigating glycoconjugate distribution as well as cell differentiation and functional maturation [19,20]. Lectin histochemistry has been successfully used to characterize in situ the oligosaccharide sequences of ovarian follicles in a number of mammalian species. The glycoconjugate pattern of cumulus cells has been investigated in the cat [21], dog [22,23], pig [24], and rat [25]. Oligosaccharide characterization of the ZP has been studied in the buffalo [26], cow [27,28], dog [22,23], hamster [11,29,30], mouse [30–32], pig [14,24], rat [25,30], typical Australian species (*Sminthopsis crassicaudata*, *Isoodon obesulus*, *Trichosurus vulpecula*, *Pseudocheirus peregrinus*, *Phascolarctos cinereus*, *Macropus giganteus*) [33], and human [34,35]. In the horse, the presence of *N*-acetylglucosamine (GlcNAc) in the ZP has been investigated by means of the lectin WGA [36]. Characterization of oligosaccharides in the ooplasm has been carried out in the cat [21], cow [37,38], dog [22,23], hamster [11,39], mouse [30,40], pig [14,24], rat [25], and human [34,35].

In the current study, the identification and localization of the oligosaccharide sequences of glycoconjugates in equine COCs were carried out by means of the 13 lectins most frequently used in glycohistochemistry in combination with enzymatic treatments. Because glycoconjugates play a key role in oocyte maturation and fertilization, the knowledge of the lectin staining pattern of oligosaccharides of equine COC could provide useful information about the different developmental potential between Cp and Exp COCs.

2. Materials and methods

2.1. Tissue preparation

Ovaries from mares of unknown reproductive history were obtained at a local abattoir, located at a distance of 20 km (30 min) from the laboratory. The ovaries were placed in physiologic saline (containing 0.9% NaCl and 40 mg/L gentamicin sulfate) within 30 min of slaughter and were transported to the laboratory in a thermal container set at 30 °C (1 to 2 h transport). Antral follicles were opened with a scalpel blade, and the granulosa cell layer was scraped with a curette following the method of Ref. 5. Granulosa cells were flushed from the curette into individual Petri dishes using *N*-2-Hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)-buffered tissue culture medium (Medium 199; Gibco BRL, Life Technologies Ltd., Paisley, Scotland) supplemented with 10% fetal calf serum (Sigma, Milan, Italy). Cumulus-oocyte complexes were identified in the collected mural granulosa cells by using a dissection microscope, and those classified as having compact (Cp) or expanded (Exp) cumulus investment [9] were selected, whereas oocytes surrounded by a partial cumulus and degenerating oocytes (having shrunken, dense, or fragmented cytoplasm) were recorded and discarded. Twenty-three COCs (n = 10 Cp COCs and n = 13 Exp COCs) were used for the study. The COCs were fixed in Bouin's fluid for 45 min at room temperature, dehydrated in an ethanol series, cleared in xylene, and embedded in paraffin wax. Five-micrometer-thick sections were cut and, after dewaxing with xylene and hydration in an ethanol series of descending concentrations, the sections were labeled with various lectins to investigate the binding sites of the corresponding 13 lectins [41].

2.2. Lectin histochemistry

The lectins used are listed in Table 1. The horseradish peroxidase (HRP)-conjugated lectins (PNA, RCA_{120}, DBA, SBA, HPA, Con A, WGA, GSA II, UEA I, and LTA) were obtained from Sigma Chemicals Co. (St. Louis, MO, USA). Biotinylated lectins (MAL II, SNA, and GSA I-B_4) were purchased from Vector Laboratories Inc. (Burlingame, CA, USA).

De-waxed and rehydrated tissue sections were immersed in 3% v/v solution of H_2O_2 in methanol 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 at appropriate dilutions (Table 1) for 1 h at room temperature. After three rinsings in TBS, the peroxidase activity of HRP-conjugated