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Differential location and structural specificities of sialic acid-ß-D-Gal sequences belonging to sialoderivatives of rabbit oviduct under hormonal treatment

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Summary. Sialoderivatives expressed in the rabbit oviduct under hormonal treatment have been investigated *in situ* by lectin histochemistry with a view to specifying further regional and temporal specializations which enable ampulla and isthmus to play distinct roles in the reproductive events. Application of MAL II and SNA lectins allowed sialoglycoconjugates containing Sia(α2,3)Gal and Sia(α2,6)Gal groups to be discriminated. Sialic acid residues linked to Gal(ß1,3)-D-GalNAc sequences were identified using PNA combined with sialidase digestion. Information on structural features of sialic acids were acquired by deacetylation and differential oxidation pretreatments. In both oviductal portions, Sia(α2,6) groups were restricted to the luminal surface of the lining epithelium while Sia(α2,3) groups were specifically located in the secretory, non-ciliated cells. In the ampullary epithelium, non-acetylated sialic acids α2,3-linked to Gal(ß1,3)-D-GalNAc sequences were largely present. Only at ovulation time were sialic acid residues containing acetyl substituents on C_4 also found. A great variety of sialic acids were found in the isthmic epithelium which showed the highest expression of acetylated forms at the first hours after the hormonal treatment. The heterogeneity of sialoderivatives differently expressed in the ampulla and isthmus as well as their distinct cycle-dependent modulation suggest that sialylated components may contribute to the molecular and functional specificities within the oviductal epithelium.

Key words: Sialoglycoderivatives, Oviduct, Rabbit, Lectin histochemistry.

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Introduction

It is well established that the oviduct plays an important role in mammalian reproduction by providing suitable conditions for gamete transport and maturation, fertilization, and early embryo development (Abe, 1996). For the success of these events glycoconjugates of oviductal origin, which are believed to mediate male and female gamete recognition and interaction, greatly contribute (Kan et al., 1990; Geng et al., 1997). Thus, the research interest has paid considerable attention to the glycosidic composition of either the oviductal epithelium or its secretory products (Menghi et al., 1984, 1988a,b, 1989; Kapur and Johnson, 1988; Kan et al., 1990; Gandolfi et al., 1991). Using lectin histochemistry, we previously investigated the occurrence and distribution of glycoconjugates in the rabbit oviductal epithelium (Menghi et al., 1985, 1986). After treatment with human chorionic gonadotropin (HCG), distinct lectin binding profiles were visualized. In particular, sialoglycoconjugates, characterized by the most common terminal sequences, sialic acid-galactose and sialic acid-N-acetylgalactosamine, exhibited differential expression in the oviductal ampulla and isthmus (Menghi et al., 1995). Sialic acids are a large family of 9-carbon carboxylated sugars. As terminal residues, they are not only responsible for the negative charge of the cell surface but also act as specific ligands in intercellular and/or intermolecular recognition phenomena (Schauer et al., 1995; Kelm and Schauer, 1997). In mammalian species, the most common modification of sialic acids is the addition of one or more O-acetyl esters to hydroxyl groups (Varki, 1997). The structural diversity of sialic acids also arises from their linkage to the penultimate sugar, which can occur in three main configurations: α2,3, α2,6, and α2,8. It has been suggested that the great variety of sialic acids is correlated to their different properties as well as to functional properties of sialoderivatives (Schulte and Spicer, 1985; Schulte et al., 1985). Thus, the knowledge of the structural features of

sialic acids and their linkage to the subterminal sugar may help to elucidate the functional meaning of specific sialoglycoconjugate expression. Lectins have been successfully employed for studying sialic acids. For this aim, two approaches have been developed for *in situ* investigations. First, an increasing number of lectins with high specificity for sialic acids present in a particular glycosidic linkage are becoming available (Toma et al., 2001; Sasaki et al, 2002; Zuber et al., 2003). As a second approach, appropriate lectins can be combined with sialidase predigestion in order to explore not only the occurrence of terminal sialic acid but also the type of its subterminal acceptor sugar. Additional chemical procedures allow the presence and position of acetylated substituents in sialic acid residues to be established (Menghi and Materazzi, 1994; Alonso et al., 2001). In the present study, both methodologies have been used for the identification and characterization of sialoderivatives which are expressed in the ampullary and isthmic regions of the rabbit oviduct after HCG administration. Application of *Maackia amurensis* II lectin (MAL II) and *Sambucus nigra* agglutinin (SNA) was aimed to discriminate between α2,3 and α2,6 sialoglycoconjugates (Shibuya et al., 1987; Wang and Cummings, 1988). Using *Arachis hypogaea* agglutinin (PNA), combined with sialidase predigestion and chemical treatments, expression of Sia-D-Gal(ß1,3)-D-GalNAc sequences as well as structural features of sialic acid residues were investigated.

Our aim was to contribute additional aspects to the structural and molecular heterogeneity which is known to exist between the oviductal ampulla and isthmus, in accordance with their distinct roles in reproductive events.

Materials and methods

Animals

Sexually mature, New Zealand strain female rabbits weighing 3 Kg were maintained in individual cages at 22 °C with a light/dark photoperiod of 14 and 10 hours, respectively. The oviducts, as well as other tissue samples for various experiments, were collected at 6, 9, 10, 12, 18, 24, 48, 72 h (3 animals for each group) after administration of 100 IU of human chorionic gonadotropin (USB, Cleveland, OH, USA). Two rabbits in estrus and anestrus served as non-pregnant controls. The estrous and anestrous conditions were established by preliminary examination of vulva and vaginal smears and, subsequently, by direct inspection of ovaries. The animals were sacrificed by cervical dislocation under sodium pentobarbital anesthesia and treated under the supervision of authorized investigators.

Tissue preparation

Oviducts were excised and the ampulla and isthmus were immediately separated, immersion-fixed in Carnoy's fluid for 12 h and post-fixed in 2% calcium acetate – 4% paraformaldehyde solution (1:1 v/v) for 3 h. After dehydration in graded ethanols, tissues were embedded in paraffin wax. Sections, serially cut at 5 µm of thickness, were mounted on Superfrost Plus slides (Bio-Optica, Milano,I).

Lectin histochemistry

Biotinylated lectins specific for sialic acid

The biotinylated lectins MAL II (100 µg/ml) and SNA (100 µg/ml), purchased from Vector Laboratories (Burlingame, CA, USA), were applied to rehydrated sections for 30 min at room temperature, following treatment with 0.3 % H_2O_2 in methanol to inactivate endogenous peroxidase. Endogenous avidin-binding activity was blocked using the avidin-biotin blocking kit (Vector Lab.). The sections were then incubated with avidin-biotin-peroxidase complex (ABC, Vector Lab.) at 1:100 dilution with 0.05 M Tris-buffered saline (TBS), pH 7.6, for 30 min. After washing in TBS, peroxidase activity sites were visualized with 3-3'-diaminobenzidine DAB kit (Vector Lab.) for 5 min. The sections were rinsed in tap water, dehydrated and finally mounted in Eukitt.

Lectin labelling combined with enzymatic and chemical pretreatments

After endogenous peroxidase inactivation, adjacent sections were incubated with horseradish peroxidase (HRP)-conjugated PNA lectin (Sigma Chemical Co., St.Louis, MO, USA), specific for the terminal D-Gal(ß1,3)-D-GalNAc sequence, with and without prior sialidase digestion, deacetylation and differential oxidation. Incubation with HRP-PNA (50 µg/ml) was performed for 1 h at room temperature. After washes in TBS, the sections were treated for 5 min with DAB kit, rinsed in tap water, dehydrated and mounted.

For enzymatic predigestion, sections were treated with 0.67 U/ml sialidase from *Clostridium perfringens* (Type V, Sigma), in 0.1 M acetate buffer, pH 5.5, containing 0.1% $CaCl_2$, overnight at 37 °C.

Deacetylation was performed by incubating sections with 0.1% KOH in 70% ethanol for 20 min at room temperature. This treatment, by detaching acetyl substituents, renders sialic acid residues, which contain acetylated groups on C_4 of the pyranose ring, susceptible to sialidase digestion (Moschera and Pigman, 1975).

For differential oxidation, both 1 mM aqueous periodic acid (1 mM PO) and 44 mM aqueous periodic acid (44 mM PO) were used for 15 min at room temperature. Mild oxidation abolishes the staining with sialidase/PNA or KOH/sialidase/PNA when sialic acids do not contain C_7- and/or C_8- and/or C_9-O-acetyl groups in the side chain. Strong oxidation blocks the subsequent staining with sialidase/PNA or KOH/sialidase/PNA except for C_9 acetylated sialic acids linked via an α2,3

bond to the penultimate ß-galactose (Roberts, 1977).

Controls

Controls for lectin specificity were performed either by substitution of lectin-conjugates with the respective unconjugated lectins or by preincubation of lectins with the corresponding hapten sugars (0.1 M α2,3 sialyllactose for MAL II, 0.1 M α2,6 sialyllactose for SNA, 0.2 M D-galactose for PNA). Control of sialidase digestion consisted of incubation of sections in enzyme-free buffer (Plendl et al., 1989). The efficacy of digestion was tested by treating adjacent sections, with and without prior deacetylation, with the enzyme solution and then by submitting them to MAL II and SNA labelling. Some control sections were subjected to a desulphation procedure which was performed by placing the slides in 0.15 N HCl in methanol for 5 h at 60 °C and then in 1% potassium hydroxide in 70% ethanol for 15 min at room temperature (Martinez-Menarguez et al., 1992).

Results

Direct visualization of sialic acid residues

MAL II binding sites, indicative of the occurrence of sialic acids α2,3 linked to galactose, were detected in many secretory, non-ciliated cells of the oviductal ampulla at the different stages examined (Fig. 1). Only from 10 to 18 hours after HCG treatment, a reduction up to disappearance of the lectin binding was observed (Fig. 1b). At any stage, the isthmic epithelium showed no MAL II reactivity unless in desulphated sections which exhibited little deposits of the reaction product (not shown).

SNA, which recognizes sialic acid α2,6 linked to galactose or N-acetylgalactosamine, produced a

Table 1. PNA reactivity, with and without sialidase predigestion and chemical treatments, in the rabbit ampulla at different stages after HCG administration and in control animals.

	PNA	Sial/PNA	KOH/Sial/PNA	1mMPO/Sial/PNA	44mMPO/Sial/PNA	1mMPO/KOH/ Sial/PNA	44mMPO/KOH/ Sial/PNA
6 h	0-1	1-2	1-2	0-1	0	0-1	0
9 h	0-1	2	3	0-1	0	0-1	0
10 h	0-1	2	3	0-1	0	0-1	0
12 h	0-1	2	3	0-1	0	0-1	0
18 h	0	2	2	0	0	0	0
24 h	0-1	1-2	1-2	0-1	0	0-1	0
48 h	0-1	1	1	0-1	0	0-1	0
72 h	0-1	1	1	0-1	0	0-1	0
Anestrus	1	2	2	0-1	0	0-1	0
Estrus	0-1	2	2	0-1	0	0-1	0

Results are expressed in arbitrary units ranging from (0) to (3) for negative to continuous, strong staining.

Table 2. PNA reactivity, with and without chemical and enzymatic treatments in the rabbit isthmus at different stages after HCG administration and in control animals.

	PNA	Sial/PNA	KOH/Sial/PNA	1mMPO/Sial/PNA	44mMPO/Sial/PNA	1mMPO/KOH/ Sial/PNA	44mMPO/KOH/ Sial/PNA
6 h	0	0-1	2	0-1	0	1-2	0
9 h	0	1	3	0-1	0	2	0
10 h	0	1	1-2	0-1	0	1	0
12 h	0	1	1-2	0-1	0	1-2	0
18 h	0	1	2	0-1	0	1-2	0-1
24 h	0	1	2	0-1	0	1-2	0-1
48 h	0	1	2	0-1	0	1-2	0-1
72 h	0	1	2	0	0	1	0-1
Anestrus	0	2-3	3	1	0	2	0-1
Estrus	0	1	2	1	0	1	0

Results are expressed in arbitrary units ranging from (0) to (3) for negative to continuous, strong staining.

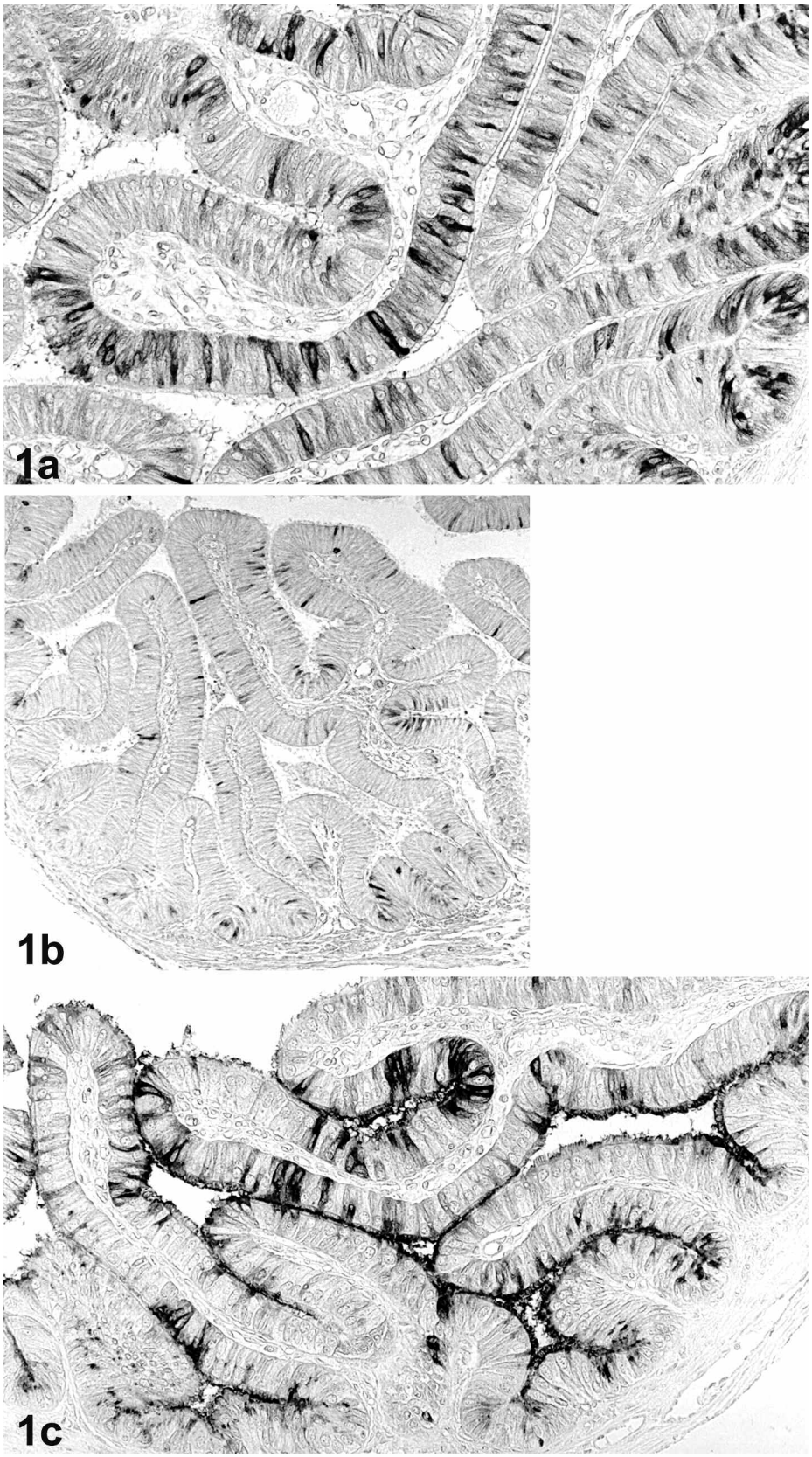

Fig. 1. MAL II binding pattern in the rabbit ampulla. MAL II reactivity is largely present in secretory, non-ciliated cells of the ampullary epithelium at 9h (**a**, x 380) and 48 h (**c**, x 250) after HCG treatment. Only a few stained cells can be found in the ampulla at 12 h after HCG treatment (**b**, x 140).

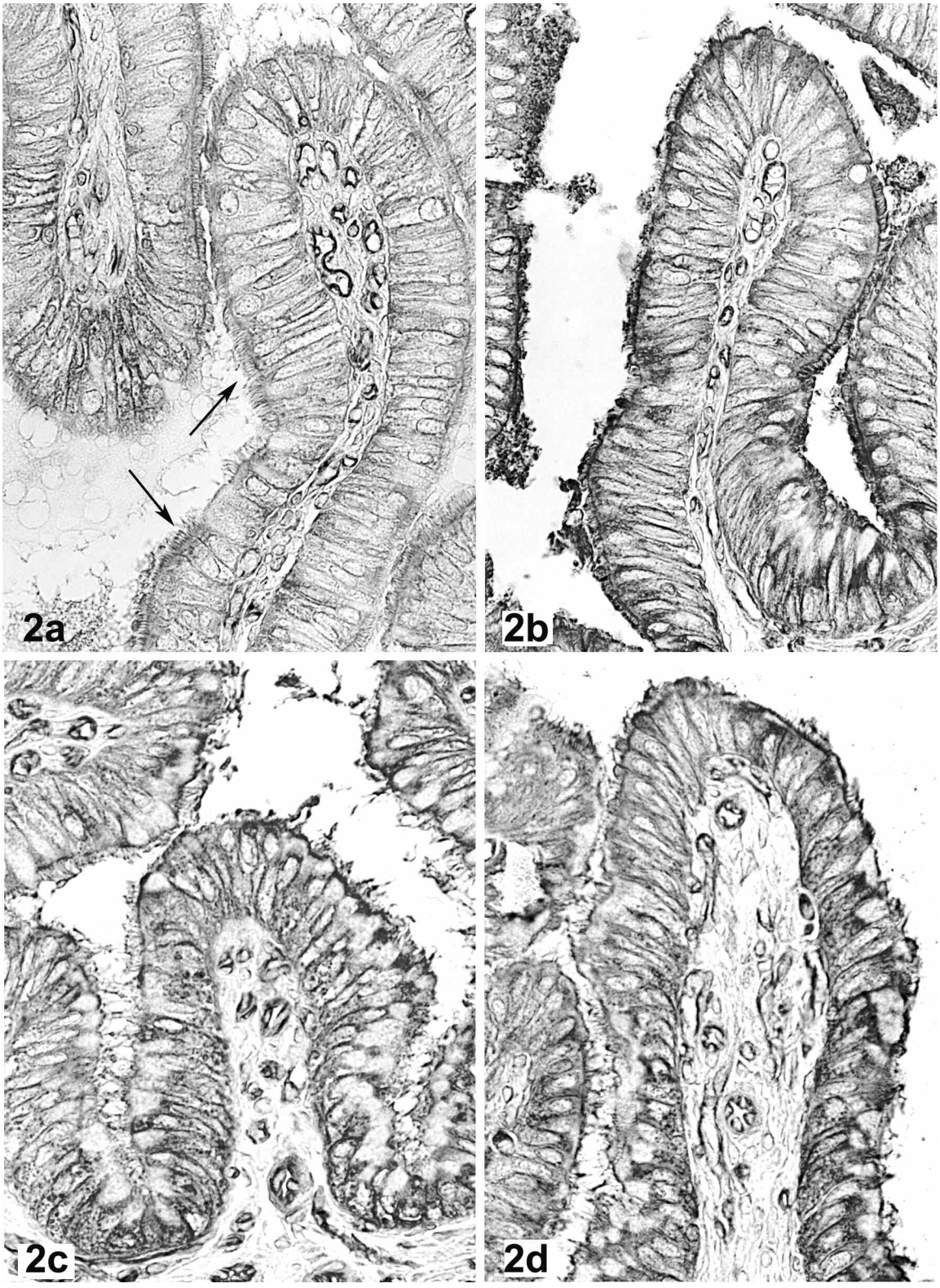

Fig. 2. SNA reactivity in the oviductal ampulla **(a, b)** and isthmus **(c, d)**. **a.** In the ampulla, a weak staining, mainly located at the cilia of ciliated cells (arrows), is present at estrous condition. **b.** The intensity of SNA staining at the luminal surface of the epithelium and cilia does increase at 10 h after hormonal treatment. x 450. In the isthmus, the binding pattern of SNA shows a similar distribution of affinity sites, mainly at the apical surface of the epithelium, at 10 h **(c)** and 48 h **(d)** after HCG administration. x 450.

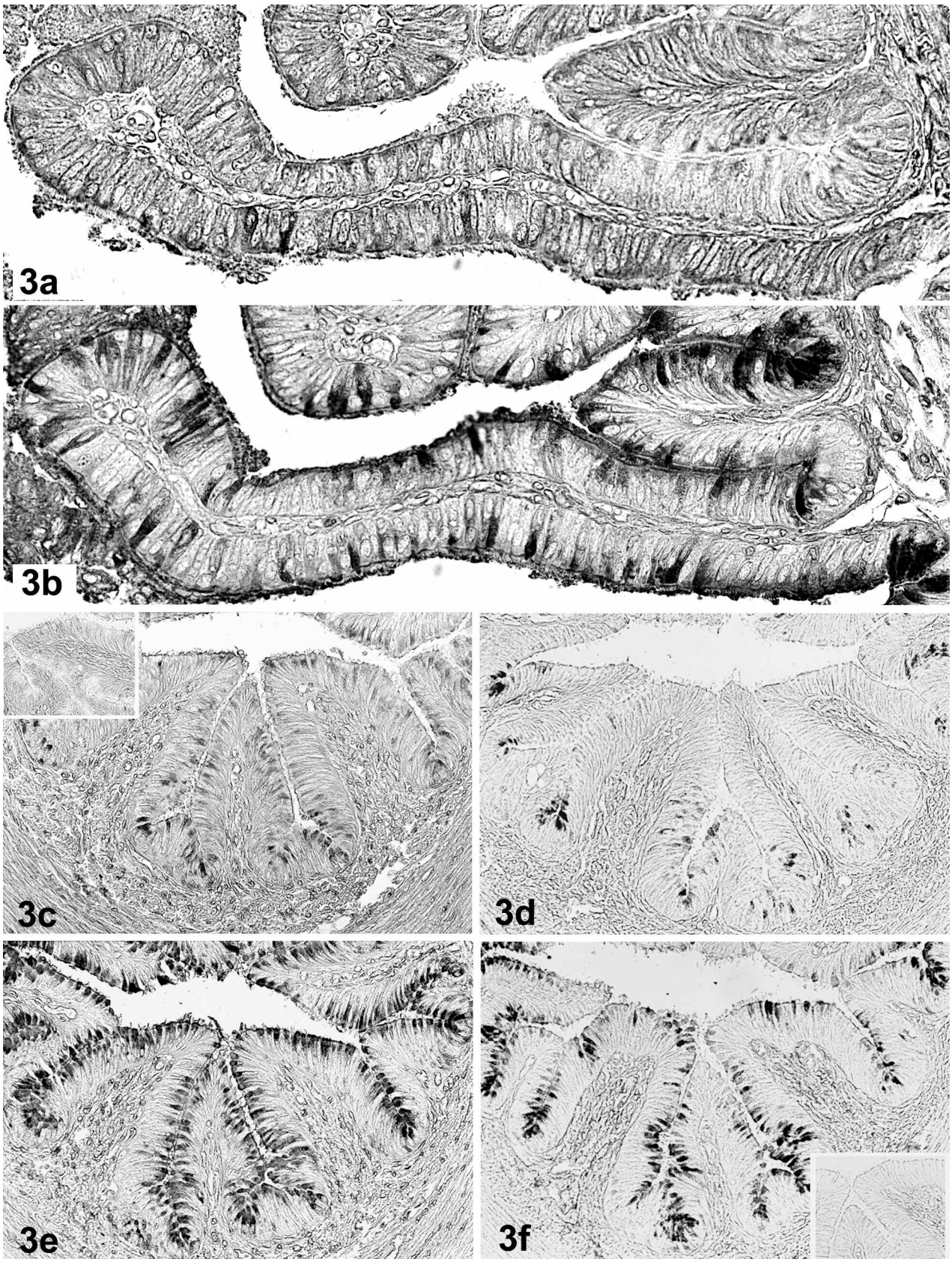

Fig. 3. PNA reactivity in the ampulla **(a, b)** and isthmus **(c - f)** of the rabbit oviduct at 9 h after HCG administration. **a.** PNA: a very few secretory cells of the ampulla are labelled. x 400. **b.** Sialidase/PNA: the number of PNA affinity sites in the ampulla greatly increases after sialidase predigestion. x 400. **c.** Sialidase/PNA: in the isthmus, sialidase digestion discovers a few PNA affinity sites compared with the negative binding pattern of PNA in untreated tissue (inset, x 100). **d.** 1mMPO/sialidase/PNA: the reactivity in the isthmic epithelium resists to mild oxidation. **e.** KOH/sialidase/PNA: a large amount of new PNA binding sites becomes evident. They are partially affected by mild oxidation **(f)** and removed by strong oxidation (inset in f, x 100). x 150

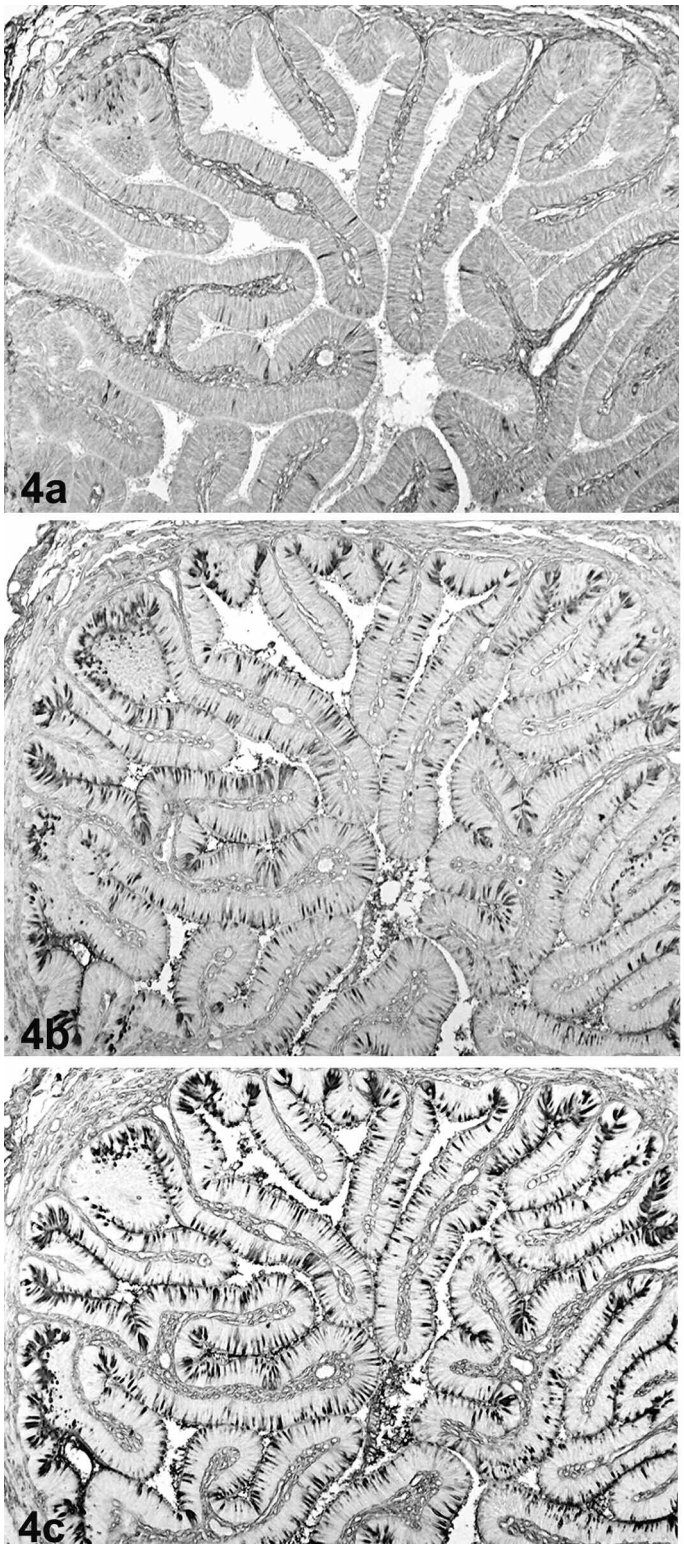

Fig. 4. The rabbit ampulla at 12 h after the hormone administration. **a.** PNA: the lectin weakly binds to a few secretory cells along and at the bottom of the mucosal plicae. **b.** Sialidase/PNA: enzyme digestion induces an increased expression of reactive secretory cells. **c.** KOH/sialidase/PNA: the reactive cells are more numerous and more strongly stained. x 80

moderate labelling at the apical cell portions in both the ampullary and isthmic epithelium, mainly at the cilia of the ciliated cells, with slight modifications at the various times after the hormone administration (Fig. 2).

Identification and characterization of sialic acid linked to D-Gal(ß1,3)-D-GalNAc

At most of the different stages of the HCG treatment, PNA lectin weakly labelled a few secretory cells of the ampullary epithelium (Figs. 3a, 4a, 5a), sometimes with preferential concentration of the affinity sites at the bottom of the mucosal plicae. No significant reactivity was detected in the isthmic epithelium (inset in Figs. 3c, 5c, inset in Fig. 6a). Modifications of these binding patterns, induced by enzymatic and chemical treatments, concerned the intensity of staining and/or the amount of the reactive secretory cells in the ampulla (Figs. 3b, 4b,c, 5b), and the appearance of new binding sites in secretory cells of the isthmus (Figs. 3c-f, 5d, 5e, 6a-c). Evaluation of PNA affinity in pretreated and untreated specimens, at different times after HCG administration, are summarized in Tables 1 and 2. By comparing the values of PNA reactivity with and without sialidase predigestion, the occurrence and distribution of Sia-D-Gal(ß1,3)-D-GalNAc sequences can be deduced. Additional information was provided about the presence and position of acetylated groups at the pyranose ring (acetyl substituents on C_4) and/or side chain (acetyl substituents in C_7 and/or C_8 and/or C_9) of sialic acid residues as well as about their linkage to the subterminal sugar. These results, referring to the ampulla and isthmus, are presented in the histograms of Figs. 7 and 8, respectively. For histogram construction, the differences between the numerical values attributed to the native PNA reactivity and to the PNA reactivity induced by the enzymatic and chemical treatments were reported in the Y axis. The various stages examined after HCG administration were reported in the X axis.

Controls

Sections incubated with unconjugated lectins or with lectins added with the appropriate hapten sugars were unstained. Immersion of sections in enzyme-free buffer resulted in a non-significant decrease of lectin binding. The results of controls for the efficacy of enzymatic digestion were as expected. The desulphation procedure proved to positively affect the subsequent MAL II staining.

Discussion

We have here presented both a direct visualization of sialoderivatives containing Sia(α2,3)Gal and Sia(α2,6)Gal groups, and an indirect demonstration of Sia-D-Gal(ß1,3)-D-GalNAc sequences which occur in the oviductal epithelium of pseudo-pregnant rabbits after human chorionic gonadotropin treatment (Bondi et al.,

1997). Application of two distinct histochemical approaches proved to be a useful tool in that it produced a wide range of data on distribution patterns and structural features of sialic acids present at different locations and accessibility conditions. The occurrence of sialoderivatives was visualized either at the cell surfaces, as components of the cell glycocalix or luminal content, or in the supranuclear cytoplasm of the secretory, non-

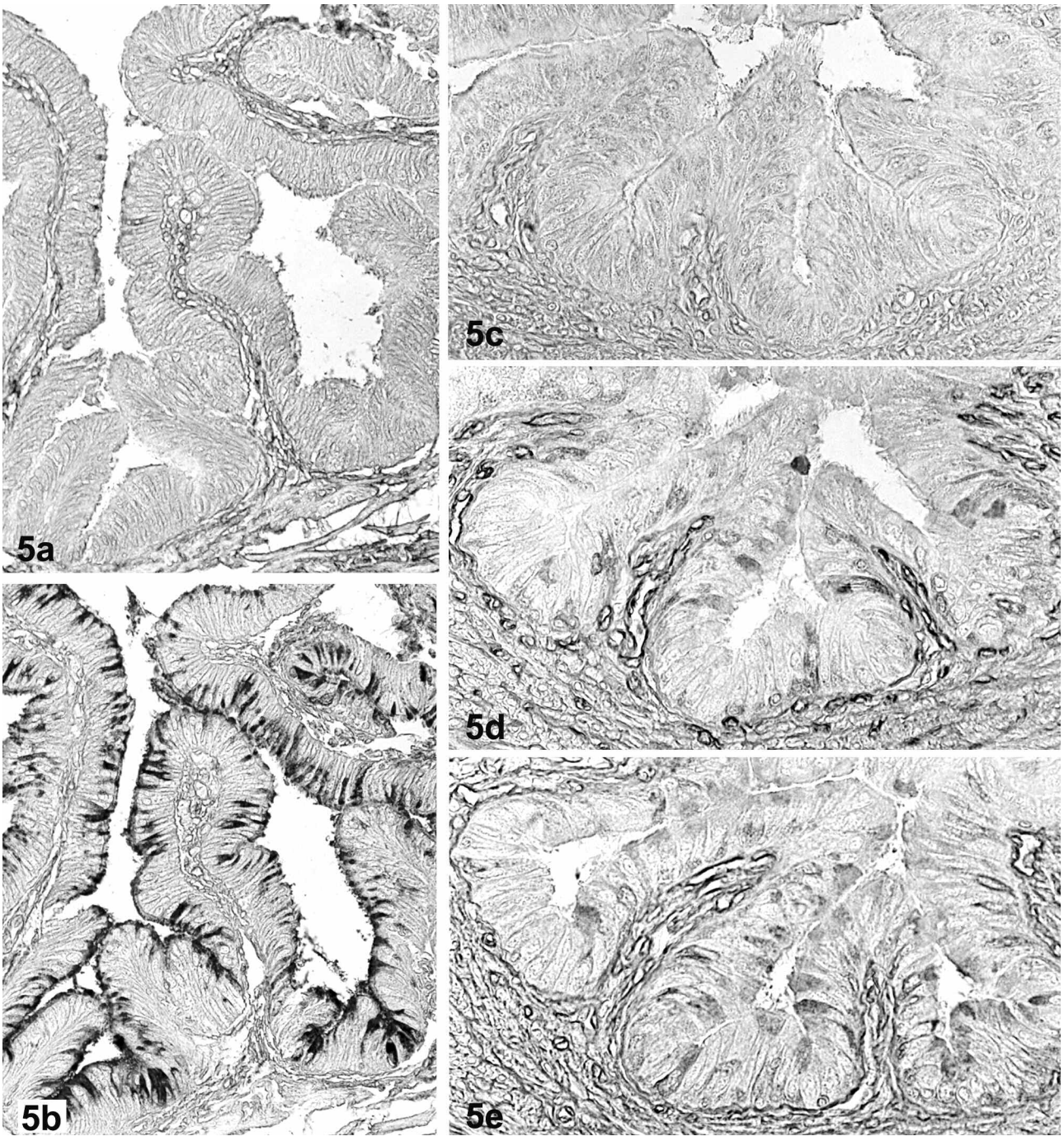

Fig. 5. The oviduct ampulla **(a, b)** and isthmus **(c-e)** at 18 h after HCG treatment. **a.** PNA does not stain the ampullary epithelium. x 250. **b.** Sialidase/PNA: a strong staining is widely distributed along and at the bottom of the mucosal plicae. x 250. **c.** PNA affinity sites are not present in the isthmus. x 400. **d.** Sialidase/PNA: a moderate labelling is induced in a few secretory cells. x 400. **e.** KOH/sialidase/PNA: a higher number of reactive cells can be detected. x 400

ciliated cells, probably in relation to the synthetic pathway of the secretory products. Moreover, application of both procedures allowed the limits of each of them to be partly overcome. Indeed, identification of sialic acid residues using sialidase digestion prior to lectin labelling depends strictly on terminalization and unmasking of the sialic acid acceptor sugar to which the lectin has to bind. It derives that sialic acid residues which are present as short side chains cannot be visualized. In turn, the direct labelling of sialic acids with specific lectins might be hampered by a high concentration of negative charges which can prevent the access of the lectin to its affinity sites (Menghi et al., 1985). This was probably the reason for our failure to detect MAL II labelling in the isthmic epithelium where a large occurrence of sulphated glycocomponents has been previously demonstrated (Menghi et al., 1984, 1992). Indeed, after desulphation pretreatment, few sites of MAL II reactivity became evident in the isthmic secretory cells.

Our results indicate that the expression patterns of sialylated components, containing Sia(α2,3)Gal groups, Sia(α2,6)Gal groups, or Sia-D-Gal(ß1,3)-D-GalNAc sequences, are region-specific in the rabbit oviduct and change at different times after HCG treatment. Coincident distribution patterns, produced by MAL II and PNA following sialidase digestion, accounted for a large occurrence of Sia(α2,3)-D-Gal(ß1,3)-D-GalNAc sequences in the secretory cells of the ampullary region. However, in samples from 10 to 18 hours after HCG administration, MAL II showed weak to no reactivity in the ampulla, probably due to difficult accessibility of the lectin to its affinity sites. Indeed, the negative binding patterns produced by MAL II were concomitant with the highest expression of sialic acid residues at the luminal surface of the ampullary epithelium, as indicated by SNA binding patterns.

Sialoglycoconjugates containing Sia(α2,6) groups proved to be more largely distributed in the isthmus than in the ampulla. In both epithelia, the SNA binding sites were mainly confined to the luminal cell surface. In line with the suggested role for sialoglycoconjugates in modulating the cell adhesion properties (Bazil and Strominger, 1993; Komatsu et al., 1999), sialylated components at the luminal sites might improve the correct progression of gametes and/or embryos along the oviductal lumen. It is also reasonable to suggest that sialic acid residues, belonging to glycoconjugates at the epithelium luminal surface, may be involved in cell-cell recognition and interaction processes, as documented in

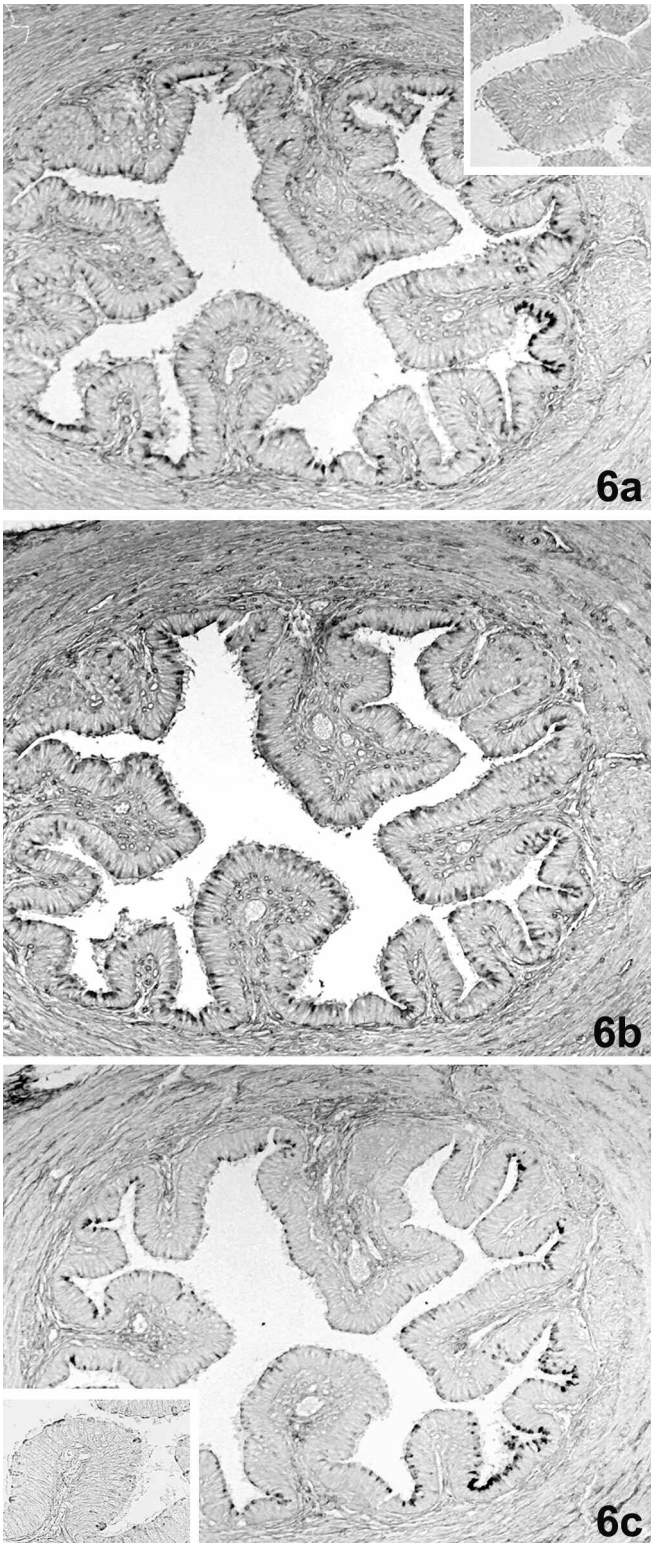

Fig. 6. Rabbit oviductal isthmus at 72 h after HCG administration. **a.** Sialidase/PNA: only after sialidase digestion, PNA binds to the apical portions of some secretory cells, mainly at the bottom of the mucosal plicae. **Inset in a**: no PNA reactivity is present in untreated samples. **b.** KOH/sialidase/PNA: the amount and intensity of PNA affinity sites increase, compared with sialidase/PNA binding pattern. **c.** 1mMPO/KOH/sialidase/PNA: mild oxidation results in reduced staining which partially resists even to strong oxidation (**inset**, 44mMPO/KOH/sialidase/PNA). x 80

several systems (Kemp, 1970; Fukuda, 2000). In the oviduct, glycoconjugate-mediated cell recognition can be fairly well related to the sperm-epithelial cell interactions which occur *in vivo* and *in vitro* in both the ampulla and isthmus of several species (Raychoudhury and Suarez, 1991; Suarez et al., 1991; Thomas et al., 1994). Contact with oviductal epithelium is considered to be beneficial for sperm viability as well as for maintenance of sperm motility and fertilizing capacity (Smith and Yanagimachi, 1990; Pollard et al., 1991; Chian and Sirad, 1994; Dobrinski et al., 1997). The binding of spermatozoa to the oviductal cells may also prevent polyspermic fertilization (Hunter and Leglise, 1971) and regulate capacitation and hyperactivation of sperm (Smith and Yanagimachi, 1991; Chian et al., 1995; Dobrinski et al., 1997). There is evidence that sperm binding is mediated by specific, but distinct, carbohydrate ligands in the species studied so far (DeMott et al., 1995; Lefevre et al., 1995). It has been demonstrated that sperm binding to bovine oviductal epithelium involves fucose recognition mediated by a Ca^{2+}-dependent lectin-like molecule associated with the sperm plasma membranes (Lefevre et al., 1997; Suarez et al., 1998; Ignotz et al., 2001).

The comparative analysis of PNA reactivity after sialidase digestion, deacetylation and oxidation pretreatments allowed the differential expression patterns of the sialoglycocomponents containing Sia-D-Gal(ß1,3)-D-GalNAc sequences to be further detailed. A prevalence of sialic acid residues lacking in acetyl groups was found in the ampulla, except for a transitory co-expression of non-acetylated and C_4-acetylated forms concomitant with ovulation time. This finding suggests a correlation with functional requirements associated with the rapid passage of the ovulated eggs which reach the ampullary-isthmic junction within a few minutes from ovulation (Weinberg and Pauerstein, 1980). Previous morphological studies suggested a modulation of secretory processes in the ampullary epithelium at ovulation time and they indicated marked changes in the ultrastructural features of the secretory granules (Bondi et al., 1997). The latter finding was correlated to a transition from mucous secretion to serous secretion processes. The type and timing of the sialic acid modifications, as evidenced here in the ampulla secretory cells, might support the conclusions of the ultrastructural studies. An additional correlation to morphological aspects may be proposed for the marked increase of sialoglycocomponents found in the ampulla 18 hours after the hormonal treatment. Under similar conditions, previous ultrastructural investigations pointed out structural features indicative of increased secretory activity in the ampullary epithelium which showed a large occurrence of electron-dense granules in distinct secretory cells, termed as dark and light cells, as well as in some ciliated cells (Bondi et al., 1997).

In the isthmus, a high and heterogeneous content in sialic acid residues linked to D-Gal(ß1,3)-D-GalNAc sequences was evidenced at the first hours after the hormonal treatment. At this time, while the ampulla is

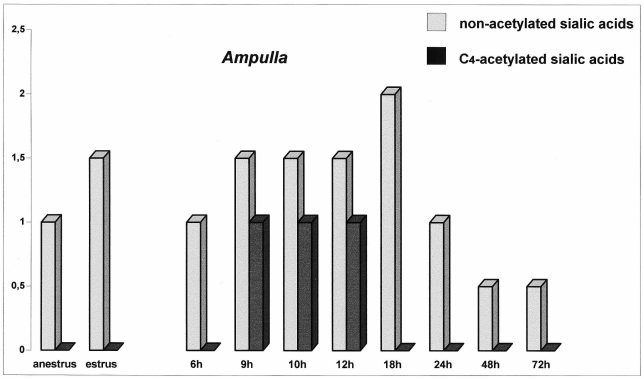

Fig. 7. Histogram representing presence and position of acetyl substituents in sialic acids belonging to Sia-D-Gal(ß1,3)-D-GalNAc sequences which are expressed in the ampulla of rabbit oviduct at different times after HCG administration.

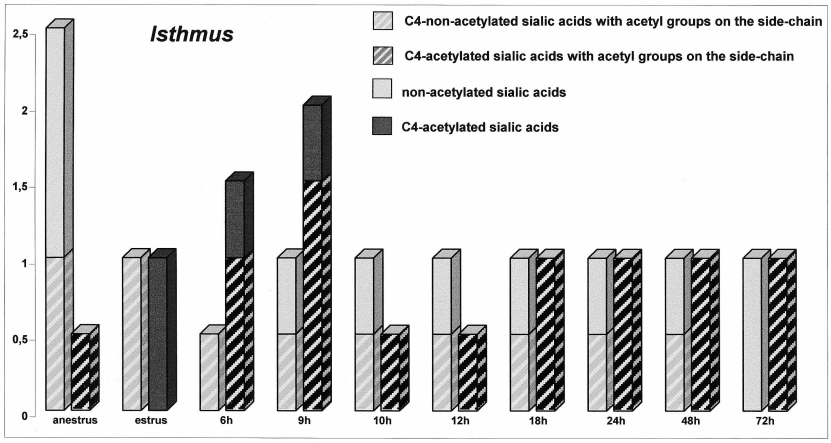

Fig. 8. Histogram representing presence and position of acetyl substituents in sialic acids belonging to Sia-D-Gal(ß1,3)-D-GalNAc sequences which are expressed in the isthmus of rabbit oviduct at different times after HCG administration.

still preparing to receive the ovulated eggs, the isthmus would be more directly engaged in promoting the sperm migration. Thereafter, the occurrence of sialoderivatives gradually stabilizes and a steady state in quantitative and qualitative sialic acid expression becomes evident from 18 to 72 hours after the hormonal treatment. When considering that the eggs reach the rabbit isthmic region 24 hours after the hormone administration and stay there for 48 hours, these sialylated components, mainly located at the apical portion of secretory cells, probably represent secretory products which might contribute to preparing and maintaining suitable conditions for the early events of embryo development. The analysis of their composition reveals that the sialoglycoconjugates expressed in the isthmic region are rich in highly acetylated sialic acid residues showing acetyl substituents at either the pyranose ring and/or the polyhydroxyl side-chain. In addition, in samples examined from 18 to 72 hours after the hormone administration, partial resistance of PNA reactivity to strong oxidation pretreatment also indicated a specific occurrence of C_4,C_9-acetylated sialic acids linked to subterminal galactose residues via α2,3 bonds. The physico-chemical properties deriving from both high acetylation degree of sialic acids and presence of α2,3 linkages, which stabilize the glycan structure more than α2,6 linkages, suggest that sialoglycoconjugates expressed in the isthmus might protect the fertilized eggs by enhancing the visco-elastic properties of mucus and the resistance to endogenous neuraminidase activities. In accordance with the functional correlates of highly acetylated sialic acids, a lubricant action on the oviductal epithelium may also be considered.

In conclusion, a great variety was evidenced in sialic acid residues linked to subterminal galactose, which were differently expressed in the ampulla and isthmus of the rabbit oviduct after HCG treatment. From this heterogeneity, which further specifies spatial and temporal specializations within the rabbit oviduct, a large spectrum of functions can be predicted, depending on distinct structural features of sialic acids present at different locations and times of the estrous cycle. Accordingly, the *in situ* characterization of both sialoglycoconjugates and their derivatives acquires special interest as does the choice of valuable tools for this purpose. We think that the present methodological approach, by integrated application of two distinct techniques and with a comprehensive series of resulting data, can be proposed as a reliable approach to fulfil these requirements.

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