Histol Histopathol (2003) 18: 735-740
DOI: 10.14670/HH-18.735

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Lectin binding in the human foetal testis

G. Gheri[1], G.B. Vannelli[1], M. Marini[1], G.D. Zappoli Thyrion[2] and E. Sgambati[1]

[1]Department of Anatomy, Histology and Forensic Medicine and

[2]Department of Medicine, University of Florence, Policlinico di Careggi, Florence, Italy

Summary. In the present study we have investigated the oligosaccharidic content of the glycoconjugates within the human foetal testis starting from its earliest differentiation phase (8, 10 and 12 weeks of gestation). To this purpose we have used a battery of six horseradish peroxidase-labelled lectins (SBA, PNA, WGA, UEAI, LTA and ConA). We have obtained a complete distributional map of the sugar residues of the glycoconjugates in the coelomic mesothelium, tunica albuginea, pre-Sertoli cells, pre-gonocytes, Leydig cells, basement membrane of the sex cords, interstitial tissue, mastocytes and endothelial cells of the capillary vessels. Since the beginning of the testis differentiation phase the cells of the coelomic mesothelium showed a large amount of sugar residues. In the pre-Sertoli cells and in the pre-gonocytes a role played as structural molecules by some oligosaccharides could be hypothesized. D-galactose-(ß1→3)-N-acetyl-D-galactosamine, sialic acid, N-acetyl-D-glucosamine and α-D-mannose could be involved in inducing and maintaining the cellular activity of the Leydig cells.

Key words: Oligosaccharides, Foetal human testis

Introduction

The oligosaccharidic content of the glycoconjugates of the human testis has been widely investigated in healthy adult subjects (Lee and Damjanov, 1985; Malmi et al., 1987; Arenas et al., 1998).

To our knowledge, no data are available in the literature on the sugar residues of the glycoconjugates in the testis of the human embryo and/or foetus. On the other hand, it is well known that in developing tissues the sugar residues of the glycoconjugates play a key role in biological processes such as enzymatic activities (Firestone and Heath, 1981), as receptor molecules, in determining cell-to-cell adhesion, cellular recognition and proliferation (Damjanov, 1987; Zanetta et al., 1994).

Offprint requests to: Prof. G. Gheri, Department of Anatomy, Histology and Forensic Medicine, Policlinico di Careggi, Viale Morgagni, 85, Florence, Italy. e-mail: gheri@unifi.it

The aim of the present study was to investigate the sugar residues of the glycoconjugates in foetal testis during its earliest differentiation phases (8, 10 and 12 weeks of gestation) using a battery of six horseradish-peroxidase lectins.

Materials and methods

Six human foetuses at different gestational ages (8, 10 and 12 weeks of gestation (w.g.)) were dissected 3-5 hr after spontaneous or therapeutic abortion (legal abortions were performed in authorized hospitals and certificates of approval were obtained from patients. The study protocols were approved by the University Ethical Committee). Fertilization ages were confirmed according to the crown-rump and the crown-heel measurements (Falin, 1969; Pelliniemi and Niemi, 1969). The testes, in a number of two for each w.g., were removed and fixed in Carnoy's fluid and routinely processed for light microscopy to obtain 5 mm-thick paraffin sections. Some sections were stained with haematoxylin-eosin to get a general overview.

Lectin histochemistry

After hydration, the sections were treated with 0.3% hydrogen peroxide for 10 min to inhibit the endogenous peroxidase, rinsed in distilled water and washed with 1% bovine serum albumin (BSA) (Murata et al., 1983) in 0.1 M phosphate-buffered saline (PBS) pH 7.2. The sections were then incubated for 30 min at room temperature in horseradish peroxidase-conjugated lectins (HRP-conjugated lectins) dissolved in phosphate-buffered saline (0.1 M PBS pH 7.2, 0.1 M NaCl, 0.1 mM $CaCl_2$, $MgCl_2$ and $MnCl_2$) and then rinsed three times in PBS. The optimal concentration for each lectin (Sigma Chemical Co., St. Louis, MO) which allowed maximum staining with minimum background was as follows: SBA (Glycine max, binding specificity α/ß-D-GalNAc > D-Gal) 20 mg/ml; PNA (Arachis hypogaea, binding specificity D-Gal (ß1→3)-D-GalNAc) 25 mg/ml; ConA (*Canavalia ensiformis*, binding specificity α-D-Man > α-D-Glc) 50 μg/ml; WGA (*Triticum vulgare*, binding specificity (α-D-GlcNAc)n and sialic acid) 20 mg/ml,

LTA (*Lotus tetragonolobus*, binding specificity α-L-Fuc) 25 mg/ml; and UEA I (*Ulex europaeus*, binding specificity α-L-Fuc) 25 μg/ml. Staining of the sites containing bound HRP-lectins was obtained by incubating the slides with PBS (pH 7.0), containing 3,3' diaminobenzidine (DAB) (25 mg/100 ml) and 0.003% hydrogen peroxide, for 10 min at room temperature. Specimens were rinsed in distilled water; dehydrated using graded ethanol solutions, cleared in xylene and mounted in Permount.

Controls for lectin staining included: 1) substitution of unconjugated lectins for HRP-lectin conjugates; 2) exposure to HRP and substrate medium without lectin; 3) oxidation with 1% periodic acid for 10 min prior to lectin staining; and 4) exposure of sections to 10/12 mg/ml of each HRP- lectinconjugate containing 0.1 M D-galactose, D-glucose, D-mannose, L-fucose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine and methyl-D-mannopyranoside.

Sialidase digestion

In some experiments sialic acid was removed by pretreating the sections for 18 hr at 37 °C in a solution of 0.25 M sodium acetate buffer, pH 5.5, containing 0.1 unit/ml sialidase (neuraminidase Type X from *Clostridium perfrigens* (Sigma Chemical Co., St. Louis, MO)), 5.0 mM $CaCl_2$ and 154 mM NaC_1, prior to staining with HRP-lectin conjugates. Controls containing the sialidase buffer without the enzyme were also prepared.

Glucose oxidase digestion

Hydrolysis of terminal glucose residues was made by using glucose oxidase from Aspergillus niger (Sigma Chemical Co., St. Louis, MO). The sections were incubated in 0.2 M sodium acetate buffer (pH 5) for 5 min and afterwards with the enzyme, at a dilution of 50 U/ml for 12 h at 37 °C.

Results

SBA

The apical portion of the cells of the coelomic mesothelium and the granules of the mastocytes reacted in the testes of the 8-w.g. foetuses.

In the testes of 10-w.g. foetuses reactivity was observed in the apical portion of the coelomic mesothelium cells, in the tunica albuginea, the cytoplasmic granules of the Leydig cells (Fig. 1) and of the mastocytes.

In the testes of the 12-w.g. foetuses reactivity was detected in the apical portion of the coelomic mesothelium and at the granules of the mastocytes.

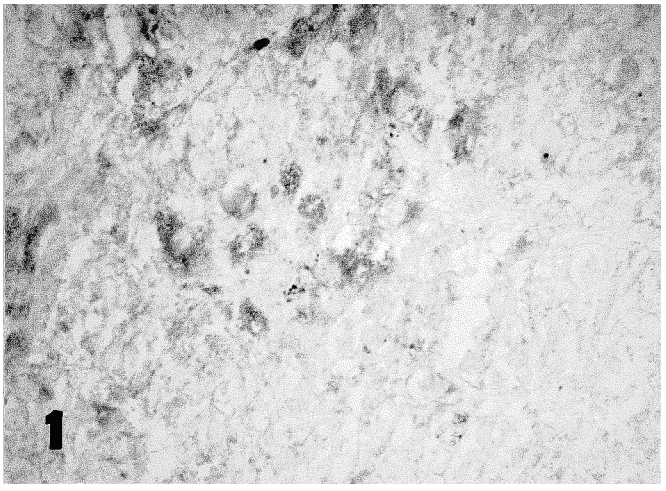
Following neuraminidase digestion, no increase of the reaction was observed.

PNA

In the testes of 8-w.g. foetuses the apical portion of the cells of the coelomic mesothelium, the Golgi region of the PGCs (pre-gonocytes), the basement membrane of the sex cords and the endothelial cells of the capillary vessels reacted with PNA.

In the testes of 10-w.g. foetuses reactivity was detected in the apical portion of the cells of the coelomic mesothelium, in the tunica albuginea, in the pre-Sertoli cells, in some PGCs, in the Leydig cells, in the fibres of the interstitial tissue, in the basement membrane of the sex cords, in the mastocytes and in the endothelial cells of few capillary vessels.

The same reactivity was seen in the testes of 12-w.g.



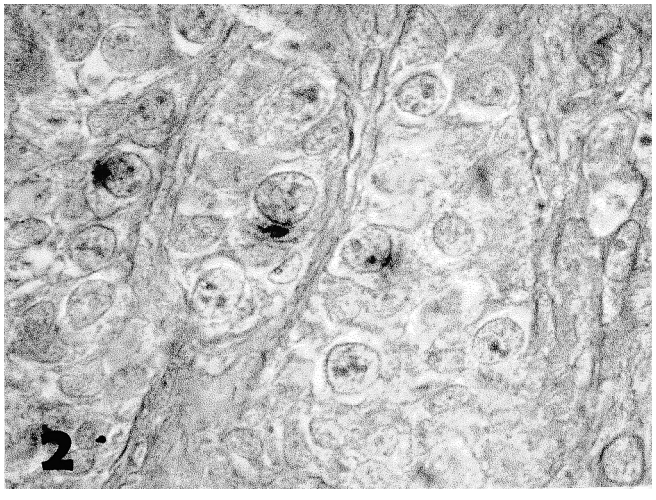


Fig. 1. HRP-SBA. 10-w.g foetuses. Reactivity is observable within the cytoplasm of the Leydig cells. x 470

Fig. 2. HRP-PNA. 12-w.g. foetuses. The Golgi region of some PGCs shows reactivity. x 1280

foetuses (Fig. 2). In this case, anyway, PGC reactivity was detected at the Golgi region and at the surface of the Leydig cells.

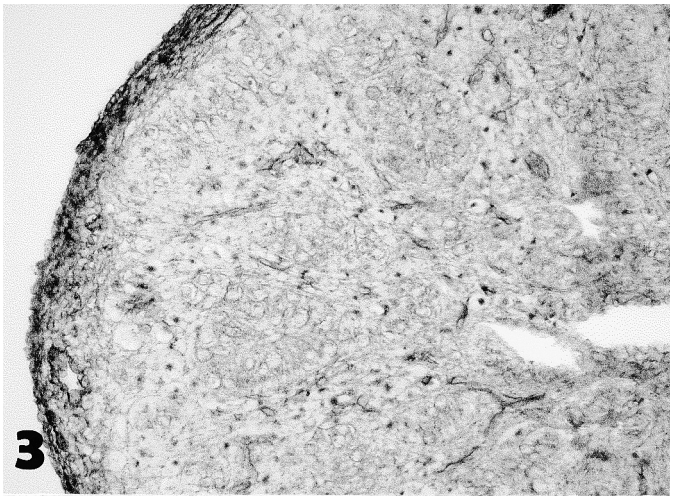
Following neuraminidase digestion, in the 8-w.g. testes reactivity was enhanced in the apical portion and appeared in the cytoplasm of the cells of the coelomic mesothelium, in the granules of the pre-Sertoli and the Leydig cells, in the interstitial stroma and at the granules of the mastocytes.

In the 10-w.g. foetuses an increase in reactivity was

Table 1. Lectin binding in foetal testis.

	COELOMIC MESOTHELIUM	TUNICA ALBUGINEA	PRE-SERTOLI CELLS	PGCs (pre-gonocytes)	LEYDIG CELLS	BASEMENT MEMBRANE OF THE SEX CORDS	INTERSTITIAL TISSUE	MASTOCYTES	CAPILLARY VESSELS (endo-thelium)
SBA	**1a**							**1gr**	
	1a	*1*			*1gr*			*3gr*	
	1a							3gr	
PNA	**2a**			**2G**		**2**			**2#**
	2a	*2**	*1c*	*2c**	*1c*	*1*	*1*	*2gr*	*2#*
	2a	2	1c	2G	2gr2G	1s	2	2	3gr
Neuraminidase - PNA	**3a2c**		**2gr**	**2gr**	**2gr**	**2**	**2**	**3gr**	**2#**
	2a	*2*	*2c**	*2G*	*2gr*	*2*	*2*		*3*
	2a1gr	1	2G	2gr2G	1s	2	2	3gr	3
WGA	**3s**		**2gr3s**	**2gr3s**	**2c**	**3**	**2**	**3gr**	**3**
	3c	*3*	*2gr*	*2gr2s2G*	*3G*		*2*	*3gr*	*3*
	3c	2	2gr	3G2s	2	2	3gr	3	
UEAI	**2c**								**2**
	1c	*2*			*1gr*				*3*
					1gr				3
LTA									
					1gr				1#
ConA	**1c**		**1c**	**1c**	**1c**		**1**	**3gr**	
	1c	*1*	*1c*	*1c*	*1c*		*1*	*3gr*	
			1gr		3gr		1	3gr	

In bold type, 8 w.g.; in italic type, 10 w.g.; in underlined type, 12 w. g.; *, some cells; #, only few vessels; a, apical portion of the cell; gr, cytoplasmic granules; G, Golgi region; s, cell surface; 1, weak reaction; 2, moderate reaction; 3, strong reaction.



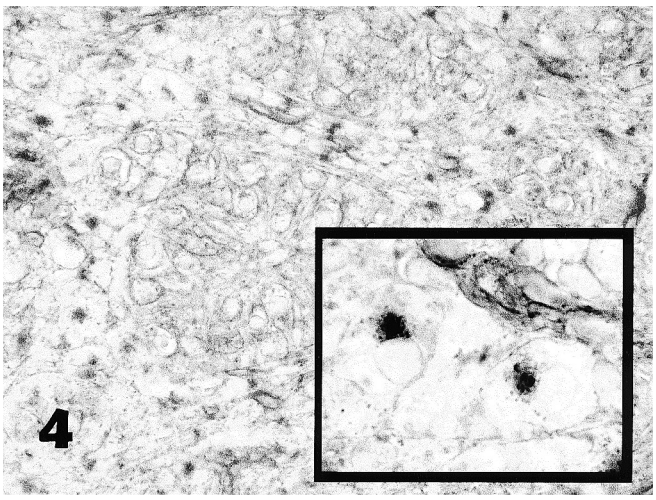


Fig. 3. HRP-WGA- 10-w.g. foetuses. Strong reactivity is observable in the tunica albuginea. x 270

Fig. 4. HRP-WGA- 10-w.g. foetuses. Cytoplasmic granules within the Sertoli cells and the Golgi region of the Leydig cells show reactivity. Inset: a higher magnification of two Leydig cells (x 1,280). x 470

seen in the pre-Sertoli cells, in the basement membrane of the sex cords and in the interstitial tissue.

Following neuraminidase treatment, in the testes of 12-w.g. reactivity appeared in the cytoplasmic granules of the cells of the coelomic mesothelium, at the Golgi region of the pre-Sertoli cells and of the PGCs and at the surface of the Leydig cells. Reactivity was enhanced in the fibres of the interstitial tissue.

WGA

In the testes of 8-w.g. reactivity was detected at the surface of the cells of the coelomic mesothelium, the surface and cytoplasmic granules of the pre-Sertoli cells and of PGCs, the Leydig cells, the basement membrane of the sex cords, in the fibres of the interstitial tissue, in the granules of the mastocytes and in the endothelial cells of the capillary vessels.

In the 10-w.g. foetuses reactivity was detected in the cells of the coelomic mesothelium, in the tunica albuginea (Fig. 3), in the cytoplasmic granules of the pre-Sertoli cells, in the cytoplasmic granules, the cell surface and the Golgi region of PGCs and the Golgi region of the Leydig cells (Fig. 4). Reactivity was also seen in the interstitial fibres, in the granules of the mastocytes and in the endothelial cells of the capillary vessels.

In the 12-w.g. foetuses WGA reactivity was detected in the cells of the coelomic mesothelium, in the surface and the cytoplasm of the cells of the tunica albuginea, in

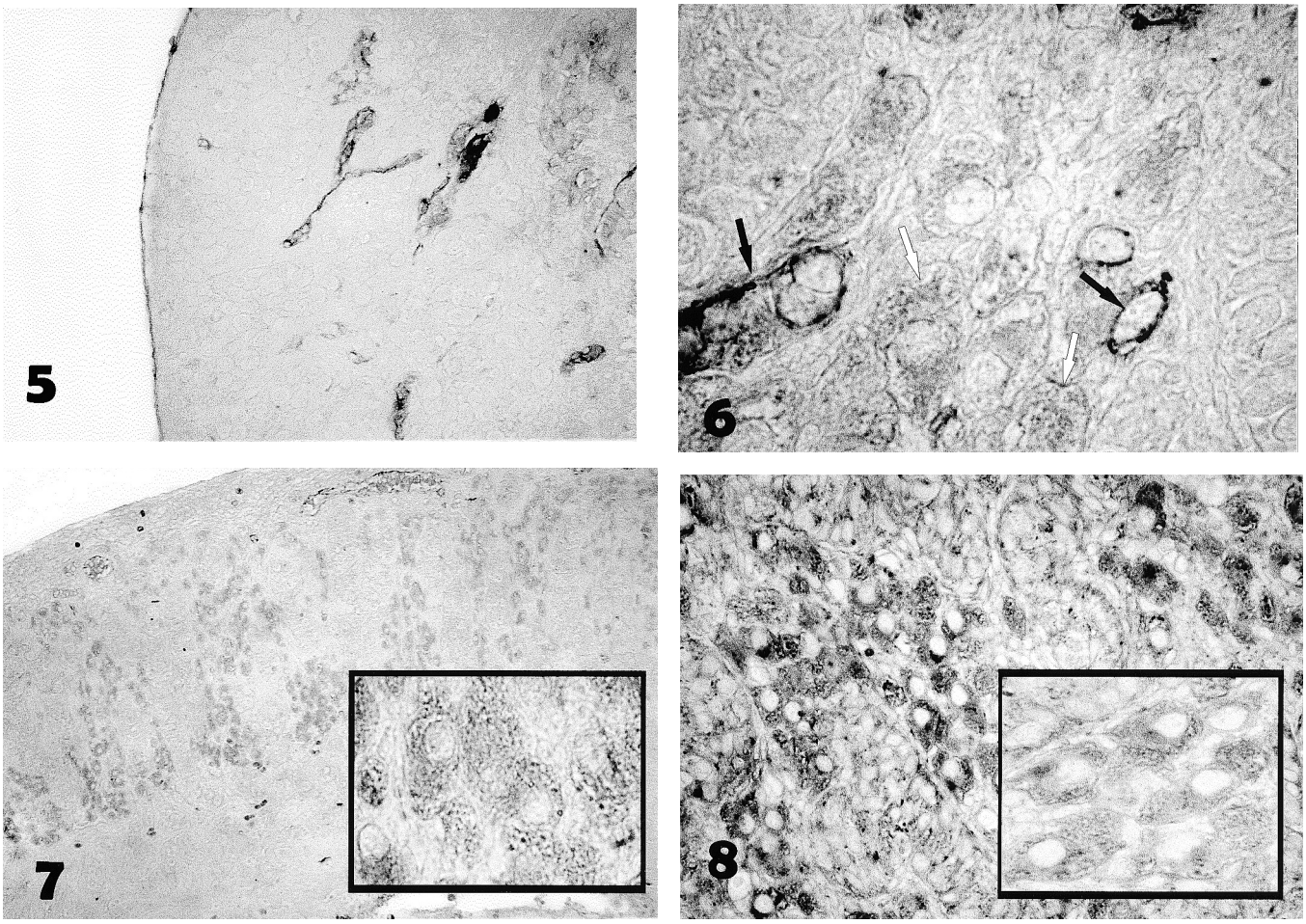


Fig. 5. HRP-UEAI 8-w.g. foetuses. The coelomic mesothelium and the endothelial cells of the capillary vessels react with this lectin. x 120

Fig. 6. HRP-UEAI 12-w.g. foetuses. Reactivity is seen in the cytoplasmic granules of the Leydig cells (white arrows) and in endothelial cells of the capillary vessels (black arrows). x 470

Fig. 7. HRP-LTA12-w.g. foetuses. Reactive Leydig cells are observable. Inset: a higher magnification of some Leydig cells (x 1280). x 120

Fig. 8. HRP-ConA 12-w.g. foetuses. Strong reactivity is observable within the Leydig cells. The Sertoli cells show weaker reactivity. Inset: a higher magnification of some Leydig cells (x 1280). x 470

the cytoplasmic granules of the pre-Sertoli cells, in the surface and the cytoplasm of the PCGs and in the surface and the Golgi region of the Leydig cells.

Following sialidase digestion, no appreciable decrease of the reaction was observed.

UEA I

In the testes of the 8-w.g. foetuses reactivity was seen in the cytoplasm of the cells of the coelomic mesothelium and in the endothelial cells of the capillary vessels (Fig. 5).

The testes of 10-w.g. foetuses showed UEA I reactivity in the cytoplasm of the cells of the coelomic mesothelium, in the tunica albuginea, in the granules of the mastocytes and in the endothelial cells of the capillary vessels.

In the testes of 12-w.g. foetuses the granules of the Leydig cells and the endothelial cells of the capillary vessels reacted with UEA I (Fig. 6).

LTA

In the 12-w.g. foetuses reactivity was seen in the Leydig cells and in the endothelial cells of few capillary vessels (Fig. 7).

ConA

In the testes of 8- and 10-w.g. foetuses ConA reactivity was observed in the cells of the coelomic mesothelium, in the pre-Sertoli cells, in the PGCs, in the Leydig cells, in the interstitial fibres and in the granules of the mastocytes. In the 10-w.g. foetus reactivity was also seen within the tunica albuginea.

In the testes of 12-w.g. foetuses ConA binding was observed in the apical granules of the pre-Sertoli cells, in the outermost sex cords, in the cytoplasmic granules of the Leydig cells (Fig. 8), in the interstitial fibres and in the granules of the mastocytes. Following glucose oxidase treatment no decrease in reactivity was detected.

Discussion

Since from the beginning of the testis differentiation phase (8-w.g.) the cells of the coelomic mesothelium appeared characterized by a large amount of sugar residues. In fact, N-acetyl-D-galactosamine, D-galactose-(ß1→3)-N-acetyl-D-galactosamine in subterminal and terminal position, sialic acid, N-acetyl-D-glucosamine, α-L-fucose and α-D-mannose were detected. It is to be noted, anyway, that N-acetyl-D-galactosamine, D-galactose-(ß1→3)-N-acetyl-D-galactosamine and sialic acid were seen at the free border of the cells of the coelomic mesothelium, while sialic acid, N-acetyl-D-glucosamine, α-L-fucose and a-D-mannose were observed within the cytoplasm and/or at the surface of the cells (N-acetyl-D-glucosamine). In the previously mentioned sites α-L-fucose only reacted with UEAI lectin. Reactivity with UEAI indicates the presence of α-L-fucose bound via ß1,2 linkage to penultimate D-galactose (ß1→4)- N-acetyl- D-glucosamine residues (Debray et al., 1981; Schulte and Spicer, 1983; Foster et al., 1991). The sugar residues detected within the cytosol could be considered as structural molecules, whereas it appeared difficult to state the role played by the sugar residues located in the apical surface. According to our previous observations in other developing epithelia (Gheri Bryk and Gheri, 2002), the oligosaccharides located in the apical portion of the cells were a typical feature of the bathyprismatic cells, probably playing a role in determining and/or maintaining the cellular polarity. This was the first evidence, in our experience, of this type of location in flattened (8-w.g.) and in cubical (10- and 12-w.g.) cells. A role of receptors molecules could be speculated for these oligosaccharides.

Lectin binding in the tunica albuginea was firstly observed in the 10-w.g. foetus. N-acetyl-D-galactosamine, α-L-fucose and α-D-mannose were detected in this tunica only in the 10-w.g. foetuses and were absent in the 12-w.g. foetuses. In this regard it is to be noted that only in the 10-w.g. did the incoming sex cords appear clearly separated by coelomic mesothelium.

The pre-Sertoli cells, the PGCs and the Leydig cells were characterized by the presence of D-galactose-(ß1→3)-N-acetyl-D-galactosamine in terminal and/or in subterminal positions, sialic acid, N-acetyl-D-glucosamine and α-D-mannose since from the 8-w.g. a-D-mannose was absent in the PGCs in the testis at 12 w.g. The cellular localization of the previously mentioned sugar residues changed within the considered weeks of gestation. The Leydig cells also showed the presence of N-acetyl-D-galactosamine at 10 w.g. and of α-L-fucose bound via ß1,2 linkage to penultimate D-galactose (ß1→4)- N-acetyl- D-glucosamine residues at 10 and 12 w.g. In the 12-w.g. testes α-L-fucose also binding via an a-(1-6) linkage to penultimate glusaminyl residues or by difucosilated oligosaccharides was observed. Despite the cellular localization, the same sugar residues were observed in the Sertoli cells, in the spermatogonia and in the Leydig cells in the testis of adult healthy subjects (Lee and Damjanov, 1985; Malmi et al., 1987; Arenas et al., 1998).

This fact suggests that these oligosaccharides could assume a significance of structural molecules in the pre-Sertoli cells. It could also be hypothesized, by the presence of small gap junctions between the pre-Sertoli cells and the PGCs (Larsen, 1996), that these sugar residues could also play a role in male sexual differentiation. Since it is reported in the literature that the differentiation of the PGCs in spermatogonia occurs late in the gestational period (Falin, 1969), in this case a structural role for these oligosaccharides also could be postulated. With regard to the Leydig cells, it is to be noted that the presence of cells exhibiting morphological features of steroidogenic activity has been reported from

the beginning of 8-w.g. (Makabe et al., 1995). The Golgi region of the Leydig cells might be related to the assembly and secretion of glycoproteins from their surface to the interstitial spaces and then into the lymphatic vessels; in addition, this secretion may also help to build up the constituents of the cell coat (Familiari et al., 1979; Motta, 1983). As far as the presence of the sugar residues of the glycoconjugates in the Leydig cells is concerned, a possible role played by D-galactose-(ß1→3)-N-acetyl-D-galactosamine, sialic acid, N-acetyl-D-glucosamine and α-D-mannose in inducing and maintaining the cellular activity could be hypothesized. The appearance in the Leydig cells of α-L-fucose, bound via α(1-6) linkage to penultimate glucosaminyl residues and/or difucosilated residues at 12 w.g., could suggest that this sugar residue is involved in attaining a maturity and then in inducing a full functional activity in these cells.

D-galactose-(ß1→3)-N-acetyl-D-galactosamine, sialic acid, N-acetyl-D-glucosamine and α-L-fucose were constantly detected in the endothelial cells of the capillary vessels. These sugar residues could play a role in the uptake and in regulating the transporting of substances between the blood flow and the interstitial tissue. At 12 w.g. α-L-fucose, revealed by LTA, was detected within the endothelial cells of a few capillaries. LTA binding suggests the presence of reactive sites containing α-L-fucose bound via α(1-6) linkage to penultimate glucosamynil residues and/or difucosilated residues. The appearance at 12-w.g. of this α-L-fucose characterised by this type of linkage could suggest the achievement of a more selective activity by the endothelial cells of the capillary vessels in filtering substances toward the interstitial tissue.

References

Arenas M.I., Madrid J.F., Bethencourt F.R., Fraile B. and Paniagua R. (1998). Lectin binding of the human testis. Intern. J. Androl. 21, 332-342.

Damjanov I. (1987). Biology of disease. Lectin cytochemistry and histochemistry. Lab. Invest. 57, 5-20.

Debray H., Decout D., Strecker G., Spik G. and Montreuil J. (1981). Specificity of twelve lectins towards oligosaccharides and glycopeptides related to N-glycosyl proteins. Eur. J. Biochem. 117, 41-55.

Falin L.I. (1969). The development of genital glands and the origin of germ cells in human embryogenesis. Acta Anat. 72, 195-232.

Familiari G., Renda T. and Motta P. (1979). Surface coat in steroid – secreting cells of the mouse ovary: interstitial, thecal and luteal cells. Experentia 35, 3-5.

Firestone G.L. and Heath E.C. (1981). Role of protein glycosylation in the cAM-mediated induction of alkaline phosphatase in mouse L-cells. J. Biol. Chem. 256, 1404-1411.

Foster J.D., Getchell M.L. and Getchell T.V. (1991). Identification of sugar residues in secretory of olfactory mucosae using lectin histochemistry. Anat. Rec. 229, 525-544.

Gheri Bryk S. and Gheri G. (2002). Lectin histochemistry of enterocytes sugar residues in the gut of the chick embryo and of the newborn. It. J. Anat. Embryol. 107, 37-49.

Larsen W.J. (1996. Développement du système urogénital. In : Embryologie humaine. De Boeck Université. Bruxelles, pp.235-279.

Lee M.C. and Damjanov I. (1985). Lectin binding sites on human sperm and spermatogenic cells. Anat. Rec. 212, 282-287.

Makabe S., Naguro T., Heyn R. and Motta M. (1995). Ultrastructure of human Leydig cells at hearly gonadal embryogenesis. It. J. Anat. Embryol. 100, 525-533.

Malmi R., Kallajoki M. and Suominen J. (1987). Distribution of glycoconjugates in human testis. A histochemical study using fluorescein-and rhodamine- conjugated lectins. Andrologia 19, 322-332.

Motta P.M. (1983). Scanning electron microscopy of adrenal gland in mammals. In: Ultrastructure of endocrine cells and tissues. Martinus Nijhoff Publishers. Morta P.M. (ed). Martinus Nijhoff Publishers. Boston. pp 216-224.

Murata F., Tzuyama S., Suzuky S., Hamada H., Ozaka M. and Muramatzu T. (1983). Distribution of glycoconjugates in the kidney studied by use of labelled lectins. J. Histochem. Cytochem. 31(1a suppl.), 139-144

Pelliniemi L.J. and Niemi M. (1969). Fine structure of the human foetal testis. I. The interstitial tissue. Z. Zellforsch. 99, 507-522.

Schulte B.A. and Spicer S.S. (1983). Histochemical evaluation of mouse and rat kidneys with lectin horseradish peroxidase conjugates. Am. J. Anat. 168, 345-362.

Zanetta J.P., Badache A., Maschke S., Marschal P. and Kuchler S. (1994). Carbohydrates and soluble lectins in the regulation of cell adhesion and proliferation. Histol. Histopathol. 9, 385-412.

Accepted March 5, 2002