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Lectin binding in the umbilical cord in altered glycemia

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Abstract

Objective: Content and distribution of the oligosaccharides in the umbilical cord from pregnancies with altered glycemia were investigated.
Study design: A prospective cohort study was conducted in the Florence Policlinic of Careggi, Italy. Samples of cord from physiological pregnancies ($n = 20$), from pregnancies with minor degree of glucose intolerance ($n = 20$) and from pregnancies with gestational diabetes mellitus (GDM) treated with insulin ($n = 20$) were collected. Eleven lectins were used (ConA, WGA, PNA, SBA, DBA, LTA, UEA I, OOA, GSL II, MAL II and SNA) in combination with chemical and enzymatic treatments.
Results: Increase of *N*-acetyl-D-glucosamine and a loss of sialic acid in the umbilical cord of the cases with minor degree of glucose intolerance with respect to the other study groups was observed. D-Galactose(β1 → 3)-*N*-acetyl-D-galactosamine, *N*-acetyl-D-galactosamine and L-fucose were in less amount in both the pathological groups with respect to the control one.
Conclusion: The increase of some glycoconjugates carbohydrates and the loss of others in the umbilical cord from pregnancies with minor degree of glucose intolerance might be related to its morphofunctional alterations in a not diabetic altered glycemia. Moreover, the treatment with insulin in the GDM might play a role in restoring partially the normal glycosilation in the cord components in the attempt to renew some their functions.

Keywords: Umbilical cord; Glycoconjugates oligosaccharides; Gestational diabetes; Minor degree of glucose intolerance; Lectins

1. Introduction

The umbilical cord is an important organ for the growth and the well-being of the fetus.

Some investigations have shown that altered composition and metabolism of this organ are observed in some diseases occurring in pregnancy such as fetal growth restriction [1], preeclampsia [2] and diabetes [3].

Scanty data are available in the literature concerning the glycoconjugates oligosaccharides in the umbilical cord from physiological pregnancies and from those complicated by various types of pathologies [4–7].

On the other hand, it is well known that in normal and pathological tissues the carbohydrates play an important role in biological processes such as enzymatic activities, cell-to-cell adhesion and cellular recognition and proliferation [8,9]. Besides, some oligosaccharides of the proteoglycans and the collagens might play an important role in the physical maintenance of tissue structure and integrity [10]. It is also seen that the 'coating' of the oligosaccharides on many glycoproteins may have the function to protect the protein chain from recognition by proteases or antibodies [10] and to form antithrombogenic barrier in the vascular lumen [11,12].

The aim of this study was to investigate content and distribution of the glycoconiugates oligosaccharides in human umbilical cord from pregnancies of women with minor degree of glucose intolerance than that defining gestational diabetes, and from pregnancies of women with gestational diabetes mellitus. For this purpose, a battery of eight horseradish peroxidase (HRP) conjugated lectins and three biotinilated lectins was used.

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2. Materials and methods

2.1. Group classification

Three study groups were considered: group 1—women with uncomplicated pregnancies (cases $n = 20$) (control group); group 2—women with pregnancies complicated by a minor degree of glucose intolerance (cases $n = 20$); group 3—women with pregnancies with gestational diabetes mellitus (GDM) treated with insulin (cases $n = 20$). The gestational age was comprised between 35 and 38 weeks.

No women with chronic hypertension, renal disease or pregestational diabetes were included. The women, in all the study groups, were delivered by cesarean route. Controls were women undergoing cesarean section on one of the following indications: breech presentation, cephalopelvic disproportion or psychological reasons.

Criteria for diagnosis of minor degree of glucose intolerance were: abnormal 50 g glucose challenge test (GCT) value (plasma glucose level $\geq$135 mg/dl after 1 h) and negative 100 g glucose tolerance test (GTT) [13]. Criteria for diagnosis of GDM were: abnormal GCT value and positive 100 g GTT [13].

Informed written consent was obtained from each patient.

2.2. Umbilical cord collection

One to two days before delivery, ultrasonographic measurements of the umbilical cords diameter were performed. At delivery, five blocks of cords cross-sections for each case were taken at random and immersed in 4% neutral buffered formalin for 48 h. The specimens were then processed routinely, embedded in paraffin and 5 μm thick sections were prepared. Following deparaffinization, some sections were stained with Haematoxilyn–Eosin (HE) for conventional histological examination while the other sections were treated according to the lectin histochemistry.

2.3. Lectin histochemistry

The lectins and their carbohydrate specificity are listed in Table 1. The lectins DBA, SBA, PNA, ConA, WGA, LTA, UEA I and OOA were HRP-conjugated. They were obtained from Sigma Chemicals Co. (St. Louis, MO, USA). GSL II, MAL II and SNA were biotinylated lectins and were purchased from Vector Laboratories Inc. (Burlingame, CA, USA).

2.3.1. HRP-conjugated lectins

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) 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 solutions (DBA 25 μg/ml, SBA 20 μg/ml, PNA 25 μg/ml, ConA 50 μg/ml, WGA 20 μg/ml, LTA 25 μg/ml, UEA I 25 μg/ml, OOA 50 μg/ml) and then rinsed three times in PBS. Staining of the sites containing bound lectin-HRP was obtained by incubating the slides with 0.7 mg/ml 3,3′-diaminobenzidine and 0.7 mg/ml hydrogen peroxide in 0.06 M TBS (SIGMA FAST DAB tablet set, Sigma Chemical Co., St. Louis, MO, USA) for 10 min at room temperature.

2.3.2. Biotinylated lectins

After endogenous peroxidase inactivation and wash in BSA solution, the sections were rinsed in 0.05 M Tris buffered saline (TBS), pH 7.6. The endogenous avidin-binding activity was blocked using the avidin–biotin blocking kit (Vector Lab.). After three washes in TBS the sections were incubated for 30 min at room temperature with biotinylated lectins solutions (GSL II 20 μg/ml, MAL II 15 μg/ml, SNA 15 μg/ml). After other three washes in TBS the sections were then incubated with avidin–biotin–peroxidase complex (ABC, Vector Lab.) at 1:100 dilution with TBS for 30 min. After three washes in TBS, the peroxidase activity sites were visualized, as above described for the HRP-lectins.

All the specimens were then rinsed in distilled water, dehydrated using graded ethanol solutions, cleared in xylene and mounted in Permount.

Controls for lectin staining included: (1) substitution of conjugated lectins with unconjugated lectins; (2) substitution

Table 1
Carbohydrate binding specificity of lectin

Lectin	Abbrevation	Carbohydrate binding specificity
Dolichos biflorus agglutinin	DBA	α-D-GalNAc
Soybean agglutinin	SBA	α/β-D-GalNAc > D-Gal
Peanut agglutinin	PNA	D-Gal(β1 → 3)-D-GalNAc
Canavalia ensiformis agglutinin	ConA	α-D-Man > α-D-Glc
Wheat germ agglutinin	WGA	$(D\text{-GlcNAc})_n$, Neu5Ac
Lotus tetragonolobus agglutinin	LTA	α-L-Fuc
Ulex europaeus agglutinin I	UEA I	α-L-Fuc
Osage orange agglutinin	OOA	D-Gal > α-D-GalNAc
Griffonia (*Bandeiraea*) *simplicifolia* lectin II	GSL II	D-GlcNAc
Maackia amurensis lectin II	MAL II	Neu5Ac(α2 → 3)Gal
Sambucus nigra lectin	SNA	Neu5Ac(α2 → 6)Gal/GalNAc

of the substrate medium with buffer without lectin; (3) incubation with each lectin in the presence of its hapten sugar (0.1–0.5 M in PBS and TBS).

2.4. Enzyme and chemical treatments

2.4.1. Glucose oxidase

Glucose oxidase converts glucose residues into gluconic acid. Since ConA labels both glucose and mannose, after glucose oxidase pre-treatment, the staining is only due to mannose. Sections were washed in acetone buffer at pH 5.0 and incubated overnight with 200 U/ml type VII glucose oxidase from *Aspergillus niger* at 37 °C, in a moist chamber, prior to staining with ConA [14].

2.4.2. Neuraminidase digestion

In some experiments sialic acid was removed by pre-treating the sections for 18 h at 37 °C in a solution of sodium acetate buffer 0.25 M, pH 5.5, containing 0.1 U/ml neuraminidase (neuraminidase Type X from *Clostridium perfringens* (Sigma Chemical Co., St. Louis, MO, USA)), 5.0 mM $CaCl_2$ and 154 mM NaCl, prior to staining with lectin-HRP conjugates [15]. Prior to the neuraminidase treatment, a deacetylation technique was performed to render the enzyme digestion effective, with 0.5% KOH in 70% ethanol for 15 min at room temperature [16]. Controls containing the neuraminidase buffer without the enzyme were also prepared.

2.4.3. Acid hydrolysis

In other experiments sialic acid was removed by pre-treating the sections for 2–3 h at 82 °C with 0.1 M HCl [17].

2.4.4. Evaluation of staining location

Twenty fields, selected at random, were examined for each section by an investigator blinded to the tissue identity and scored for location of staining. The amniotic epithelial cells, the stromal cells, the fibres and the ground substance in the peripheral portion and in the adventitial portion of the Wharton jelly and the endothelial cells, the smooth muscle cells and the intercellular spaces of the walls of the vessels were examined. All the slides were stained with the same batch to eliminate inter-batch variation.

2.4.5. Statistical analysis

To compare clinical data between the group 1 and the groups 2 and 3 the *t*-test for paired samples was used. Probability of less than 0.05 ($P < 0.05$) was considered statistically significant.

For each section stained with each lectin, with or without treatments, a quantitative analysis was performed to evaluate the intensity of reactivity in the following fields: the amniotic epithelial cells, the peripheral portion and the adventitial one of the Wharton's jelly, the endothelial cells and the wall of the vein and the arteries. For this purpose the optical density was measured using a computerized image analyzer program (Image-Pro Plus v. 4.5, Media Cybernetics). The staining intensity was measured and expressed in arbitrary units standardized from 0 to 250, being 0 the maximum of the staining and 250 no staining. Five measurements for each field were made. Statistical analysis was performed using the *t*-test for paired samples ($P < 0.05$ was considered significant) to evaluate: (1) the difference in lectin reactivity, in specimens of the same group, without and with chemical or enzymatic treatments (ConA, WGA, PNA) and the difference between MAL II and SNA reactivity; (2) the difference in lectin reactivity between specimens of the group 1 and of the groups 2, 3 and between those of the group 2 and of the group 3. All sections were examined by a single observer blinded to the origin of the cord under study. Reproducibility of the measuring analysis was assessed comparing the measurements made by one observer at different times and the measurements of two observers. Intra-observer coefficient of variation was 1.5% and inter-observer coefficient of variation was 5.2%.

3. Results

3.1. Clinical results

The clinical details of the women whose umbilical cords were used for this study are reported in Table 2. Mean birth weight, placenta weight and umbilical cord diameter were significantly higher in the groups 2 and 3 with respect to the group 1 ($P < 0.05$). The other clinical data did not show significant differences between the pathological groups (groups 2 and 3) and the control group (group 1).

Table 2
Clinical details of the study groups

	Group 1 ($n = 20$)	Group 2 ($n = 20$)	Group 3 ($n = 20$)
(1) Maternal age (years)	31.0 ± 7.5	31.3 ± 8.0	32.0 ± 5.8
(2) Gestation at delivery (week)	38.0 ± 2.0	37.8 ± 2.0	37.2 ± 5.7
(3) Birth weight (g)	3360.2 ± 348.2	3540.2 ± 348.1*	3570.1 ± 350.0*
(4) Placental weight (g)	618.8 ± 80.1	650.1 ± 70.5*	660.7 ± 85.1*
(5) Umbilical cord diameter (mm)[a]	16.1 ± 3.0	19.5 ± 2.3*	20.1 ± 3.1*
(6) Parity (*n*)	1 (0–2)	1 (0–2)	1 (0–2)

Values shown are mean ± S.E.M. (3)–(5) group 2, group 3 vs. group 1.
[a] Ultrasonographic measurements.
* $P < 0.05$.

3.2. Morphological remarks

The umbilical cord from physiological pregnancies is constituted by Wharton's jelly, two arteries and one vein. The Wharton's jelly is a particular connective tissue, surrounded by an amniotic epithelium, constituted by stromal cells, fibres and extracellular matrix called also ground substance. In the jelly a peripheral and an adventitial portion (around the vessels), more compact, are distinguishable. The arteries and the vein present a wall formed by smooth muscle cells and an endothelium, higher in the vein.

Our histological data showed that in the umbilical cord from minor degree of glucose intolerance and diabetes the stromal cells and the fibres of the jelly were more spaced, the arteries appeared hypoplastic, often one closed and the vein dilated when compared to the cord of the control group (Fig. 1A–C).

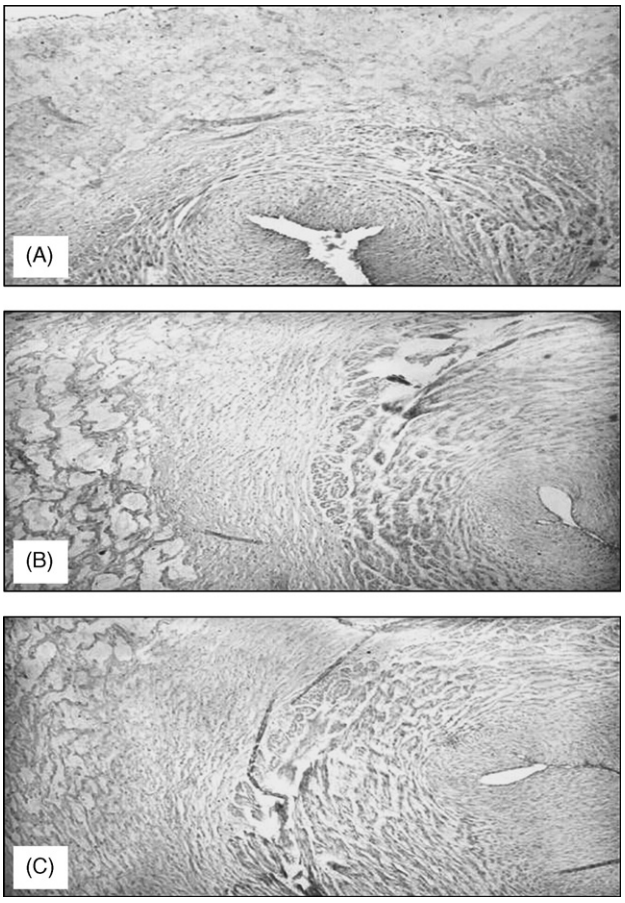

Fig. 1. Haematoxilyn/Eosin, Wharton's jelly and artery, 37 weeks. The stromal cells and the fibres of the jelly appear more spaced and the artery partially closed in the cord of the minor degree of glucose intolerance (B) and of the diabetes (C) cases when compared with the cord of the control case (A) (original magnification ×40).

3.3. Lectin histochemistry

3.3.1. Lectin binding location

3.3.1.1. Group 1 (Table 3).

ConA:

Wharton's jelly: Reactivity was observed in the amniotic epithelial cells, in the perinuclear cytoplasm of the stromal cells and in the fibres.

Vessels: The endothelial cells and the perinuclear cytoplasm of the smooth muscle cells of the vessels reacted with this lectin.

Glucose oxidase/ConA: After glucose oxidase treatment, no reactivity was observed in the fibres of the jelly.

WGA and deacetylation-neuraminidase-WGA/acid hydrolysis-WGA:

Table 3
Lectin binding in the Group 1

	Wharton's jelly				Vein		Artery	
	Epithelial cells	Stromal cells	Fibres	Ground substance	Endothelial cells	Wall	Endothelial cells	Wall
ConA	+	+perin	+	−	+	+perin	+	+perin
Gluc ox/ConA	+	+perin	−	−	+	+perin	+	+perin
WGA	+	+	+	−	+	+sp	+	+sp
KOH/N/WGA Ac hyd/WGA	+	+	+	−	+	+sp	+	+sp
PNA	+	+peri	+peri	+	+	+sp	+	+sp
KOH/N/PNA Ac hyd/PNA	+	+	+	+	+	+sp	+	+sp
SBA	+	−	−	−	+	−	+	−
DBA	−	−	−	−	−	−	−	−
LTA	+	+	+	−	+	+sp	+	+sp
UEA I	+	+	+	−	+	+sp	+	+sp
OOA	+	+	+	+	+	+sp	+	+sp
GSL II	+	+	+	−	+	+sp	+	+sp
MAL II	+	+	+	+	+	+sp	+	+sp
KOH/N/MAL II Ac hyd/MAL II	−	−	−	−	−	−	−	−
SNA	+	+	+	+	+	+sp	+	+sp
KOH/N/SNA Ac hyd/SNA	−	−	−	−	−	−	−	−

+: Reactivity; −: no reactivity; perin: perinuclear cytoplasm; peri: peripheral portion of the Wharton's jelly; sp: intercellular spaces.

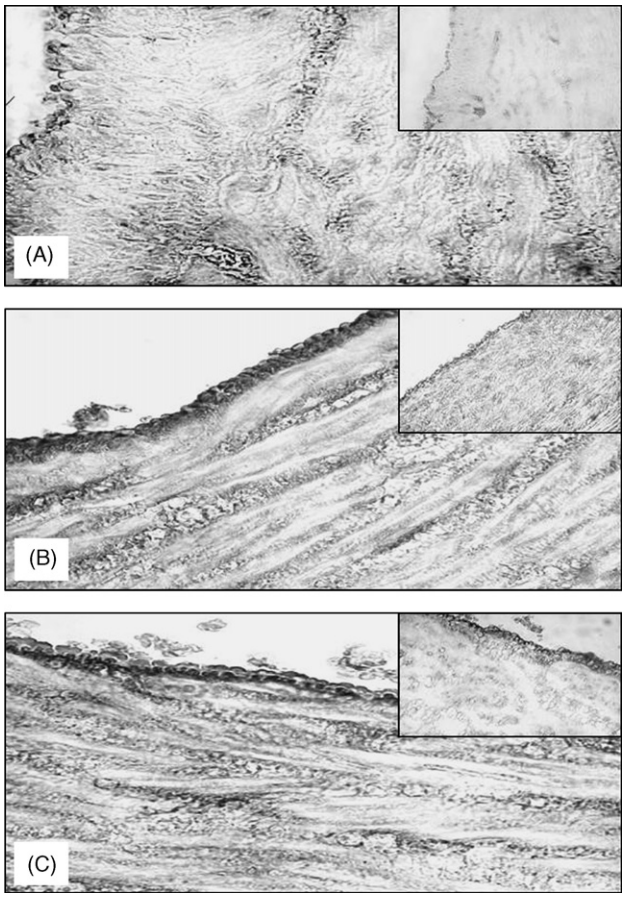

Fig. 2. WGA, vein, 37 weeks. Reactivity is observed in the endothelial cells and in the intercellular spaces of the vein in the groups 1 (A), 2 (B) and 3 (C). The reactivity of the endothelial cells appears stronger in the group 2 (B) and 3 (C) with respect to the group 1 (A). (Original magnification ×400). In the insets, the WGA reactivity after deacetylation-neuraminidase treatment is observable. In the groups 1 (inset A) and 3 (inset C), reactivity after treatment appears weaker in the endothelial cells and in the intercellular spaces, and in the group 2 (inset B) only in the endothelial cells when compared with reactivity without treatment. Reactivity appears stronger in the endothelial cells and in the intercellular spaces of the group 2 (inset B) and in the endothelial cells of the group 3 (inset C) with respect to the group 1 (inset A). The intercellular spaces of the group 2 (inset B) show stronger reactivity with respect to the group 3 (inset C) (original magnification ×60).

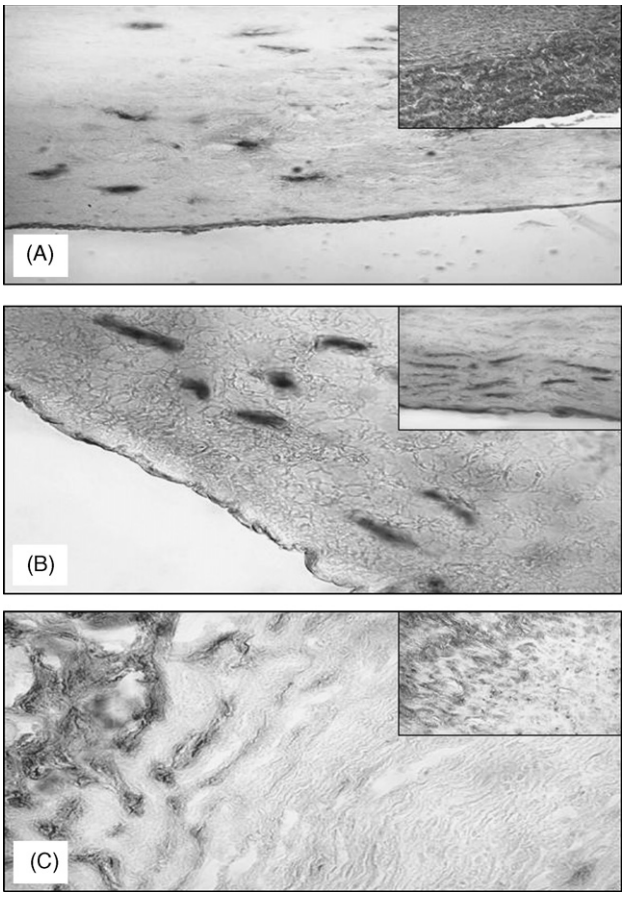

Fig. 3. PNA, Wharton's jelly, 37 weeks. Reactivity is detectable in the amniotic epithelial cells of the groups 1 (A) and 2 (B), in the stromal cells, the fibres and the ground substance of the peripheral portion of the groups 1 (A), 2 (B) and 3 (C) and in the ground substance of the adventitial portion of the group 1 (A) (original magnification ×200). In the insets, the PNA reactivity after deacetylation-neuraminidase treatment is observable. Reactivity after treatment is stronger in the amniotic cells of the group 1 (inset A), in the stromal cells and the fibres of the peripheral portion of the groups 1 (inset A) and 3 (inset C) and appears in the adventitial one with respect to the reactivity without treatment; in the group 3 (inset C), also reactivity of the ground substance of the adventitial portion appears. In the group 2 (inset B) the reactivity after treatment is stronger in the amniotic cells and appears in the ground substance of the adventitial portion. Reactivity of the jelly appears weaker in the groups 2 (inset B) and 3 (inset C) with respect to the group 1 (inset A). The stromal cells and the fibres are more reactive in the group 3 (inset C) when compared with the group 2 (inset B) (original magnification ×30).

Wharton's jelly: The amniotic epithelial cells, the stromal cells and the fibres showed reactivity.

Vessels: The endothelial cells and the intercellular spaces of the wall in the vessels reacted with this lectin (Fig. 2A).

PNA:

Wharton's jelly: PNA reacted with the amniotic epithelial cells, the stromal cells and the fibres of the peripheral portion of the jelly and the ground substance of the peripheral and the adventitial portions (Fig. 3A).

Vessels: The endothelial cells and the intercellular spaces of the wall of the vessels reacted with this lectin.

Deacetylation-neuraminidase-PNA/acid hydrolysis-PN-A: After deacetylation-neuraminidase digestion or acid hydrolysis, reactivity appeared also in the stromal cells and in the fibres of the adventitial portion of the jelly (inset, Fig. 3A).

SBA: The amniotic cells and the endothelial cells of the vessels reacted with SBA.

DBA: No reactivity was observed in the cord components.

LTA and UEA I:

Wharton's jelly: The amniotic cells, the stromal cells and the fibres reacted with this lectin.

Vessels: Reactivity was observed in the endothelial cells and in the intercellular spaces of the wall (Fig. 4A).

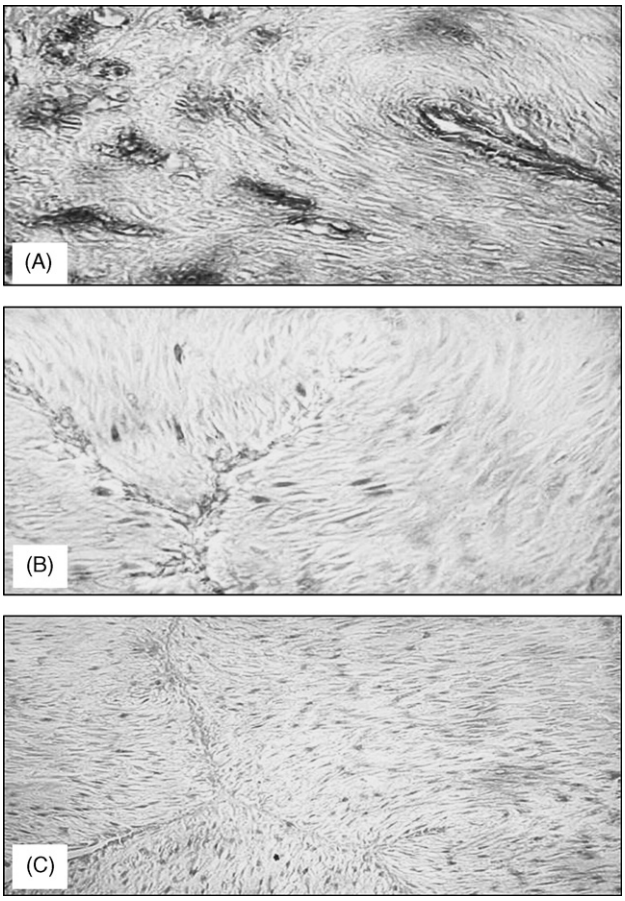

Fig. 4. LTA, artery, 38 weeks. In the group 1 (A) reactivity is observable in the endothelial cells and in the intercellular spaces. In the group 2 (B) the perinuclear cytoplasm of some smooth muscle cells and in the group 3 (C) of all the muscle cells is reactive (original magnification A and B ×400, C ×200).

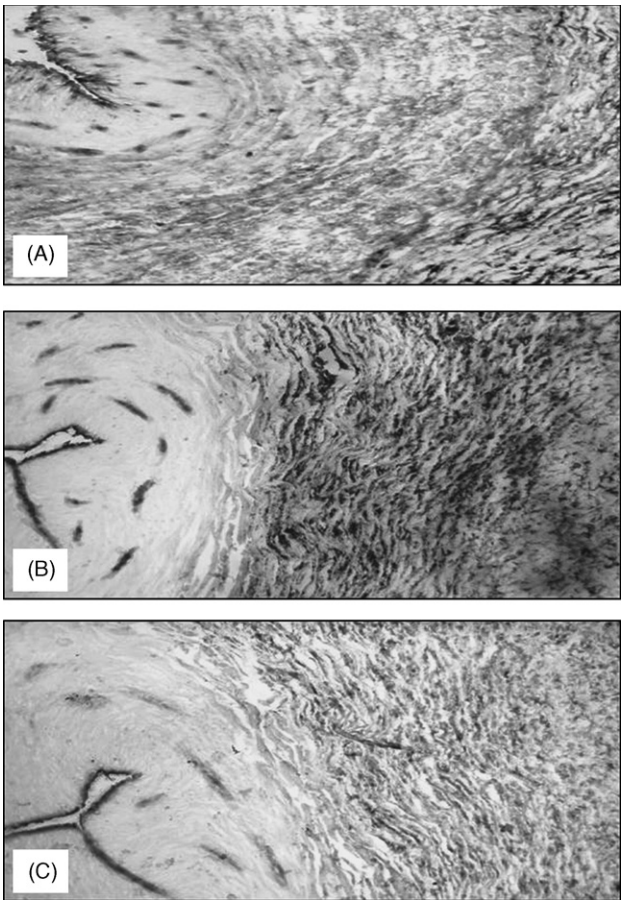

Fig. 5. GSL II, Wharton's jelly and artery, 37 weeks. Reactivity is observable in the stromal cells and the fibres of the jelly, in the endothelial cells and the intercellular spaces of the artery of the groups 1 (A), 2 (B) and 3 (C). In the group 2 (B) also the ground substance of the jelly appears reactive. Reactivity of the stromal cells, of the fibres of the jelly, of the endothelial cells and of the intercellular spaces of the artery appears stronger in the group 2 (B) with respect to the group 1 (A); in the group 3 (C) only the endothelial cells appear more reactive. Reactivity of the stromal cells and of the fibres of the jelly and of the intercellular spaces appears more intense in the group 2 (B) with respect to the group 3 (C) (original magnification ×100).

OOA:

Wharton's jelly: Reactivity was detected in the amniotic epithelial cells, in the stromal cells, in the fibres and in the ground substance.

Vessels: In the vein and in the arteries, reactivity was observed in the endothelial cells and in the intercellular spaces of the wall.

GSL II:

Wharton's jelly: The amniotic epithelial cells, the stromal cells and the fibres showed reactivity (Fig. 5A).

Vessels: The endothelial cells and the intercellular spaces of the wall in the vessels reacted with this lectin (Fig. 5A).

MAL II and SNA:

Wharton's jelly: In the amniotic epithelial cells, in the stromal cells, in the fibres and in the ground substance reactivity was detected (Fig. 7A).

Vessels: In the vein and in the arteries, reactivity was observed in the endothelial cells and in the intercellular spaces of the wall (Fig. 6A).

Deacetylation-neuraminidase-MAL II and SNA/acid hydrolysis-MAL II and SNA: After deacetylation-neuraminidase digestion or acid hydrolysis, no reactivity was observed in the cord components.

3.3.1.2. Groups 2 and 3 (Tables 4 and 5).

ConA and glucose oxidase/ConA, WGA and deacetylation-neuraminidase-WGA/acid hydrolysis-WGA: In both the groups the reactivity location of these lectins, without and after treatments, was the same of that observed in the group 1 (Fig. 2B and C).

PNA:

Wharton's jelly: In both the groups the amniotic epithelial cells and the stromal cells, the fibres and the ground substance of the peripheral portion of the jelly reacted with PNA (Fig. 3B and C).

Vessels: In the two groups the endothelial cells and the intercellular spaces of the wall of the vein reacted with lectin. In the arteries only the intercellular spaces of the wall reacted.

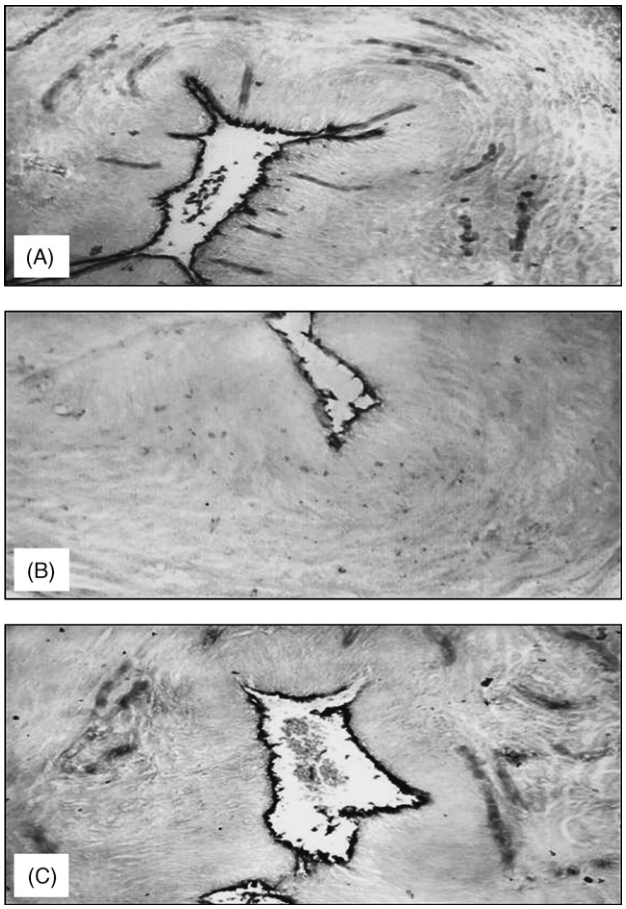

Fig. 6. MAL II, artery, 37 weeks. Reactivity is observable in the endothelial cells and in the intercellular spaces in the groups 1 (A) and 3 (C). In the group 2 (B) only the endothelial cells appear reactive and show weaker reactivity when compared with those in the groups 1 (A) and 3 (C) (original magnification X 150).

Deacetylation-neuraminidase-PNA/acid hydrolysis-PN-A: After deacetylation-neuraminidase digestion or acid hydrolysis, in the group 2 reactivity appeared also in the ground substance of the adventitial portion of the jelly and in the endothelial cells of the arteries (inset, Fig. 3B). In the group 3 also the components of the adventitial portion of the jelly and the endothelial cells of the arteries, not reactive with PNA without treatment, reacted with the lectin (inset, Fig. 3C).

SBA: In both the groups only the amniotic epithelial cells and the endothelial cells of the vein reacted with SBA.

DBA: In both the groups only the amniotic epithelial cells reacted with this lectin.

LTA: In the group 2 reactivity was observed in the amniotic epithelial cells and in the perinuclear cytoplasm of some smooth muscle cells of the vein and the arteries wall (Fig. 4B). In the group 3 the amniotic epithelial cells, the perinuclear cytoplasm of the stromal cells and the perinuclear cytoplasm of all the smooth muscle cells of the vessels wall reacted (Fig. 4C).

UEA I:

Wharton's jelly: In the two groups the amniotic epithelial cells, the stromal cells and the fibres reacted with this lectin.

Vessels: In the group 2 reactivity was observed in the endothelial cells and in the intercellular spaces of the vessels wall. In the group 3 the endothelial cells and the perinuclear cytoplasm of the smooth muscle cells of the vessels reacted.

OOA: In both the groups reactivity location of this lectin was the same of that observed in the group 1.

GSL II: In the group 3 the reactivity location of this lectin was the same of that observed in the group 1 (Fig. 5C).

Table 4
Lectin binding in the Group 2

	Wharton's jelly				Vein		Artery	
	Epithelial cells	Stromal cells	Fibres	Ground substance	Endothelial cells	Wall	Endothelial cells	Wall
ConA	+	+perin	+	−	+	+perin	+	+perin
Gluc ox/ConA	+	+perin	−	−	+	+perin	+	+perin
WGA	+	+	+	−	+	+sp	+	+sp
KOH/N/WGA Ac hyd/WGA	+	+	+	−	+	+sp	+	+sp
PNA	+	+peri	+peri	+peri	+	+sp	−	+sp
KOH/N/PNA Ac hyd/PNA	+	+peri	+peri	+	+	+sp	+	+sp
SBA	+	−	−	−	+	−	−	−
DBA	+	−	−	−	−	−	−	−
LTA	+	−	−	−	−	+perin[a]	−	+perin[a]
UEA I	+	+	+	−	+	+sp	+	+sp
OOA	+	+	+	+	+	+sp	+	+sp
GSL II	+	+	+	+	+	+sp	+	+sp
MAL II	+	−	−	+adv	+	−	+	−
KOH/N/MAL II Ac hyd/MAL II	−	−	−	−	−	−	−	−
SNA	+	−	−	+adv	+	+sp	+	+sp
KOH/N/SNA Ac hyd/SNA	−	−	−	−	−	−	−	−

+: Reactivity; −: no reactivity; perin: perinuclear cytoplasm; peri: peripheral portion of the Wharton's jelly; adv: adventitial portion of the Wharton's jelly; sp: intercellular spaces.

[a] Some cells.

Table 5
Lectin binding in the Group 3

	Wharton's jelly				Vein		Artery	
	Epithelial cells	Stromal cells	Fibres	Ground substance	Endothelial cells	Wall	Endothelial cells	Wall
ConA	+	+perin	+	−	+	+perin	+	+perin
Gluc ox/ConA	+	+perin	−	−	+	+perin	+	+perin
WGA	+	+	+	−	+	+sp	+	+sp
KOH/N/WGA Ac hyd/WGA	+	+	+	−	+	+sp	+	+sp
PNA	+	+peri	+peri	+peri	+	+sp	−	+sp
KOH/N/PNA Ac hyd/PNA	+	+	+	+	+	+sp	+	+sp
SBA	+	−	−	−	+	−	−	−
DBA	+	−	−	−	−	−	−	−
LTA	+	+perin	−	−	−	+perin	−	+perin
UEA I	+	+	+	−	+	+perin	+	+perin
OOA	+	+	+	+	+	+sp	+	+sp
GSL II	+	+	+	−	+	+sp	+	+sp
MAL II	+	+	+	+adv	+	+sp	+	+sp
KOH/N/MAL II Ac hyd/MAL II	−	−	−	−	−	−	−	−
SNA	+	+	+	+adv	+	+sp	+	+sp
KOH/N/SNA Ac hyd/SNA	−	−	−	−	−	−	−	−

+: Reactivity; −: no reactivity; perin: perinuclear cytoplasm; peri: peripheral portion of the Wharton's jelly; adv: adventitial portion of the Wharton's jelly; sp: intercellular spaces.

In the group 2 also the ground substance of the jelly was reactive (Fig. 5B).

MAL II:

Wharton's jelly: In the group 2, reactivity was observed in the amniotic epithelial cells and in the ground substance of the jelly adventitial portion. In the group 3, reactivity was detected in the amniotic epithelial cells, in the stromal cells, in the fibres of both the jelly portions and in the ground substance of the jelly adventitial portion.

Vessels: In both the groups, the endothelial cells of the vessels were reactive. In the group 3 also the wall intercellular spaces reacted with lectin (Fig. 6B and C).

SNA:

Wharton's jelly: In the group 2, the amniotic epithelial cells and the ground substance of the jelly adventitial portion were reactive (Fig. 7B). In the group 3, reactivity was detected in the amniotic epithelial cells, in the stromal cells, in the fibres of both the jelly portions and in the ground substance of the jelly adventitial portion (Fig. 7C).

Vessels: In both the groups, reactivity was observed in the endothelial cells and in the intercellular spaces of the vessels wall.

Deacetylation-neuraminidase-MAL II and SNA/acid hydrolysis-MAL II and SNA: After deacetylation-neuraminidase digestion or acid hydrolysis, no reactivity was observed in the cord components.

3.4. Statistical analysis

Comparing the reactivity intensity measurements, in each group, with ConA, WGA and PNA, without and after treatments, the statistical analysis showed that the reactivity, after treatments, with ConA, in all the groups, and with WGA, in the groups 1 and 3 of all the cord components was significantly lower with respect to that with ConA and WGA without treatments ($P < 0.05$). In the group 2, the reactivity intensity with WGA, after treatments, of the amniotic epithelial cells and of the endothelial cells of the vessels was significantly lower ($P < 0.05$). In the groups 1 and 3 the PNA reactivity, after treatments, was higher in the amniotic epithelial cells (group 1: $P < 0.001$; group 3: $P < 0.05$), in the jelly (group 1: $P < 0.001$; group 3: $P < 0.05$), in the endothelial cells of the vein ($P < 0.01$), in the endothelial cells of the arteries (group 1: $P < 0.01$; group 3: $P < 0.001$) and in the vessels wall ($P < 0.05$) with respect to reactivity with PNA without treatments. PNA reactivity, after treatments, in the group 2, was higher in the amniotic epithelial cells, in the jelly adventitial portion and in the endothelial cells of the vessels ($P < 0.05$) when compared with reactivity without treatments. SNA reactivity, in all the groups, was higher in the intercellular spaces of the vessels wall with respect to MAL II reactivity (groups 1 and 3: $P < 0.01$; group 2: $P < 0.05$). The measurements in the two portions of the jelly, in the three groups, showed that the intensity of reactivity with WGA and PNA, without and after treatments, UEA I, OOA and GSL II was statistically higher in the peripheral portion with respect to that in the adventitial one ($P < 0.05$); in the group 1, also reactivity with LTA, MAL II and SNA and in the group 3 only with MAL II and SNA was higher ($P < 0.05$). In the group 2, MAL II and SNA reactivity was higher in the adventitial portion when compared with the peripheral one ($P < 0.05$).

Comparing the measurements of the lectin reactivity of the group 1 with those of the groups 2 and 3, the statistical analysis showed that the reactivity intensity with WGA in the endothelial cells of the vessel was significantly higher in

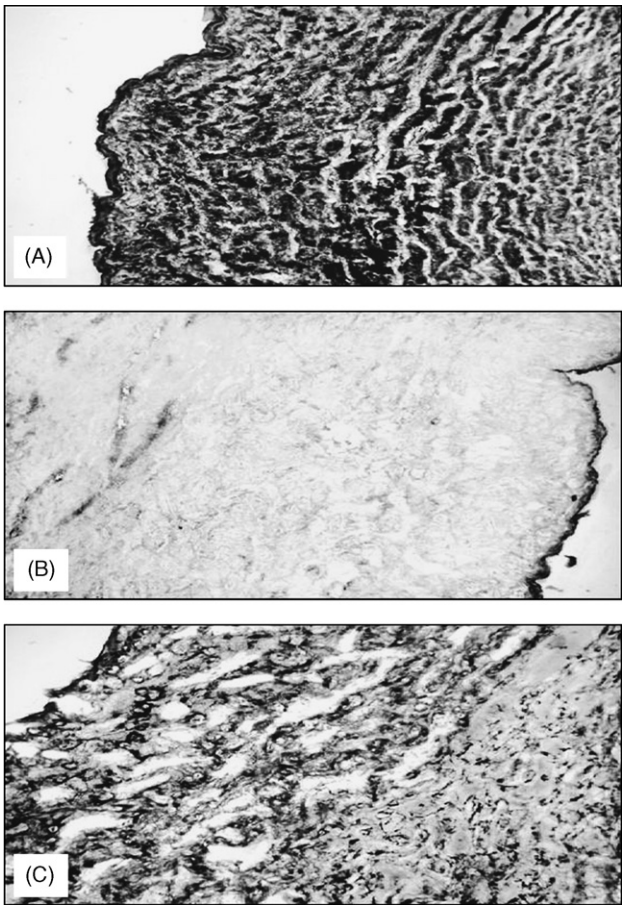

Fig. 7. SNA, Wharton's jelly, 36 weeks. Reactivity is detectable in the amniotic epithelial cells, in the stromal cells and in the fibres of the peripheral and adventitial portions of the groups 1 (A) and 3 (C). The ground substance of the two portions are reactive in the group 1 (A) and only of the adventitial one in the group 3 (C). In the group 2 (B) the amniotic cells and the ground substance of the adventitial portion appear reactive. Reactivity of the jelly in the group 1 (A) is stronger with respect to the groups 2 (B) and 3 (C). In the group 2 (B) the amniotic cells show weaker reactivity with respect to the group 3 (C) (original magnification ×200).

the groups 2 and 3 with respect to the group 1 ($P < 0.05$); WGA reactivity, after treatments, and GSL II reactivity were higher in the jelly, in the endothelial cells and the wall of the vessels of the group 2, and in the endothelial cells of the group 3 ($P < 0.05$). In the groups 2 and 3, PNA reactivity was significantly lower in the adventitial portion of the jelly and in the endothelial cells of the arteries when compared with the group 1 ($P < 0.05$); after treatments, PNA reactivity was lower in the amniotic epithelial cells of the groups 2 and 3 ($P < 0.01$), in the jelly of the groups 2 ($P < 0.001$) and 3 ($P < 0.01$), in the vessels endothelial cells (vein: $P < 0.05$; arteries: $P < 0.01$) and in the wall ($P < 0.05$) of the group 2. In the groups 2 and 3, reactivity with SBA was lower in the endothelial cells of the arteries and with DBA was higher in the amniotic epithelial cells with respect to the group 1 ($P < 0.05$). LTA reactivity, in the groups 2 and 3, was lower in the jelly (peripheral portion: $P < 0.01$; adventitial portion: $P < 0.05$), and in the vessels endothelial cells and in the wall ($P < 0.05$) when compared with the group 1; UEA I reactivity was lower in the jelly of the two groups ($P < 0.05$). MAL II and SNA reactivity was lower in the amniotic cells of the group 2 ($P < 0.01$) and of the group 3 ($P < 0.05$), in the jelly of the groups 2 ($P < 0.001$) and 3 ($P < 0.01$), and in the vessels endothelial cells ($P < 0.01$) and in the wall (MAL II: $P < 0.05$; SNA: $P < 0.01$) of the group 2 with respect to the group 1.

The comparison of the measurements of the group 2 with those of the group 3, showed that the reactivity intensity with WGA, after treatments, and with GSL II was statistically higher in the jelly and in the vessels wall of the group 2 ($P < 0.05$). PNA reactivity, after treatments, was lower in the amniotic epithelial cells, in the jelly ($P < 0.05$), in the vessels endothelial cells (vein: $P < 0.05$; arteries: $P < 0.01$) and in the wall ($P < 0.05$) of the group 2 with respect to the group 3. The LTA reactivity was lower in the jelly and in the vessels wall of the group 2 ($P < 0.05$). MAL II and SNA reactivity was statistically lower in the amniotic epithelial cells and in the jelly ($P < 0.05$), in the vessels endothelial cells ($P < 0.01$) and in the wall (MAL II: $P < 0.05$: SNA $P < 0.01$) of the group 2 with respect to the group 3.

4. Discussion

Our data showed that many carbohydrates investigated in this study were present in the various components of the umbilical cord of all three the study groups, although some in different amount; other carbohydrates were present or lacking in some groups.

It is to be noted that *N*-acetyl-D-glucosamine was more abundant in the Wharton's jelly of the group 2 with respect to that of the groups 1 and 3, while the D-galactose-(β1 → 3)-*N*-acetyl-D-galactosamine, in subterminal position, was in less amount in the jelly of the groups 2 and 3 with respect to the group 1.

In the amniotic epithelial cells of the group 1 *N*-acetyl-D-galactosamine was revealed by SBA, indicating the presence of β anomer of this carbohydrate. In the pathological groups reactivity with SBA and DBA revealed the presence of the α anomer or α and β anomer of this carbohydrate. Therefore, the α anomer of *N*-acetyl-D-galactosamine seems to be a marker of the umbilical amniotic epithelial cells in these pathologies.

α-L-Fucose, revealed by LTA, characterized the amniotic epithelial cells, the stromal cells and the fibres of the jelly in the group 1; only the amniotic epithelial cells in the group 2 and the amniotic epithelial cells and the stromal cells in the group 3.

Therefore, α-L-fucose was present with two types of linkage: reactivity with LTA suggests the presence of reactive sites containing α-L-fucose which bind via α(1–6) linkage to penultimate glucosaminyl residues and/or difucosilated oligosaccharides, while reactivity with UEA I indicates the presence of α-L-fucose bound via β1,2

linkage to penultimate D-galactose-(β1–4)-*N*-acetyl-D-glucosamine residues [18–21]. However, it is to be observed that in the pathological groups α-L-fucose bound via α(1–6) linkage to penultimate glucosaminyl residues and/or difucosilated oligosaccharides was lacking in some components of the jelly.

The localization and the intensity of reactivity with WGA and PNA after treatments, and with MAL II and SNA demonstrated that sialic acid linked α(2–3) to galactose and linked α(2–6) to galactose or *N*-acetylgalactosamine was present in all the components of the jelly of the groups 1 and 3, although in the group 3 in less amount. In the group 2 sialic acid was absent in the stromal cells and in the fibres.

A correct distribution and amount of these carbohydrate moieties might have an important role in maintaining the integrity of the components of the jelly as well as in metabolic processes, thus permitting the jelly to play its physiological functions, as the protection of the vessels from inelasticity and compression and the diffusion of substances in particular from the vein to the arteries [22,23]. Moreover, the stromal cells seem to play a role in regulating of the umbilical cord blood flow, since these cells have characteristics of fibroblasts and smooth muscle cells [24]. The loss of some carbohydrates and/or the increase of others in the jelly of the umbilical cord in cases of diabetic or not diabetic altered glycemia might play a role in impairing the integrity of its components and then their functions. On the other hand, our morphological remarks showed that in the cord both in the diabetes and in the minor degree of glucose intolerance cases the stromal cells and the fibres were more spaced with respect to the control group. Morphological data on the Wharton's jelly, in literature, showed alteration in the pattern of distribution of its fibres, suggesting a deleterious effect by diabetes on the tissue components of the jelly [25]. In other studies it has been also seen that in gestational diabetes the umbilical cord was significantly larger with respect to that in the physiological gestation and that the main increase was due to an increase of jelly content [26,27]. It is to suppose that this increase might be due to an increase of production of mucopolysaccharides rich in glucosamine, which could account for water retention and increased thickness of the umbilical cord [5]. According our data only in the minor degree of glucose intolerance group *N*-acetyl-D-glucosamine was more abundant in the jelly of the umbilical cord. However, the most important finding was the loss of sialic acid, known to play an important role in the strengthening the membrane stability [28], and the achievement of a certain degree of cell surface rigidity [29]. This suggests that even in these cases of unclaimed diabetes an impaired functionality of the umbilical cord jelly might begin. On the other hand, it is seen that also a simple alteration of maternal glycemia represents an impaired milieu of growth and development for the fetus [30–33].

It is noteworthy that, according to our findings, in the treated GDM group the distribution and content of the oligosaccharides is almost like that in the control group, suggesting a role of the insulin and probably of other correlated factors in the restoring the normal glycosylation in the jelly components.

In the group 1, *N*-acetyl-D-glucosamine, sialic acid, D-galactose-(β1 → 3)-*N*-acetyl-D-galactosamine and α-L-fucose (LTA and UEA I) were more abundant in the peripheral portion of the jelly with respect to the adventitial one in according to the data observed in a previous study [7]. Even in the groups 2 and 3 *N*-acetyl-D-glucosamine, D-galactose-(β1 → 3)-*N*-acetyl-D-galactosamine and α-L-fucose (UEA I) had the same distribution in the two portions; in the group 3 also sialic acid showed this distribution, while in the group 2 it was present only in the adventitial portion. As suggested by a previous study [7], the different amount of carbohydrates in the two portions might contribute to exert their different functions: protection of the vessels by the peripheral portion and diffusion of substances between the vessels by the adventitial one. In the pathological groups, in particular in the minor degree of glucose intolerance, the lacking of some carbohydrates in both the portions might contribute to impair their functions.

In the endothelial cells of the vessels *N*-acetyl-D-glucosamine was more abundant in the groups 2 and 3 with respect to the group 1. Sialic acid linked α(2–3) to galactose and linked α(2–6) to galactose or *N*-acetylgalactosamine was present in the endothelial cells in all the groups, although in less amount in the group 2. D-Galactose-(β1 → 3)-*N*-acetyl-D-galactosamine, in terminal position, and β anomer of the *N*-acetyl-D-galactosamine were observed in the endothelial cells of the vein in all the study groups and in the endothelial cells of the arteries only in the group 1.

In the intercellular spaces of the vessels α-L-fucose, revealed by LTA, was present only in the group1, while (-L-fucose, revealed by UEA I, was present in the intercellular spaces of the vessels in the groups 1 and 2. In the groups 2 and 3 α-L-fucose (LTA) characterized the perinuclear cytoplasm of the muscle cells of the vessels, while α-L-fucose (UEA I) was present in this site in the group 3. In the intercellular spaces of the groups 1 and 3 sialic acid linked α(2–6) to galactose or *N*-acetylgalactosamine was more abundant with respect to sialic acid linked α(2–3) to galactose. In the group 2 sialic acid linked α(2–3) to galactose was absent in the intercellular spaces, while sialic acid linked α(2–6) to galactose or *N*-acetylgalactosamine was in less amount when compared with the groups 1 and 3.

As it occurred in the jelly, the oligosaccharides present in the vessels might have a role in maintaining the structure and the metabolic processes of their components and thus permitting to play their physiological functions as the diffusion of substances and the regulation of the blood flow, due to the smooth muscle cells [23]. Moreover, the endothelial cells seem to play a role in transmitting signals coming from the blood flow to the muscle cells, for the regulation of the contractions and the remodelling of the vessels [34]. The lack of some carbohydrates and the increase of others in the

umbilical vessels, in particular in the arteries, of cases with diabetic or not diabetic altered glycemia, might indicate a morphofunctional alteration of the vessels components. On the other hand, our morphological data of the cord in the pathological groups showed hypolplastic and oblitered arteries and dilated vein. In literature, some studies have showed that in the diabetes pregnancies the vessels show damage (in particular the arteries), alteration of permeability of the endothelium and alteration of the composition of their wall [25,35,36]. It has been supposed that the high concentration of glucose would induce various biological processes that might led to morphofunctional alteration of the endothelial cells [37–41]. In a study on the umbilical vein has been seen that the glucose would stimulate the synthesis of glucosamine in the endothelial cells that would carry out to a dysfunction of the endothelium [42]. In the vessels of the minor degree of glucose intolerance group a loss of some carbohydrates, in particular sialic acid, and an increase of *N*-acetyl-D-glucosamine might suggest that already in these cases a dysfunction of the endothelium and of the vessel wall might begin to establish. In the umbilical vessels of the treated GDM group was observed a oligosaccharides distribution and content almost like that in the control group, in particular in the vein. Probably, the treatment with insulin might influence the re-establishment the normal glycosilation in the umbilical vein. In the arteries, the fact that with treatment the glycosilation appeared less restored might be due to more serious morphofunctional damage.

In conclusion, our data seem to demonstrate that the loss of some carbohydrates and the increase of others in the umbilical cord coming from minor degree of glucose intolerance cases could be related to an alteration of the structure and functionality of this organ also with a simple alteration of maternal glycemia. On the other hand, the treatment with insulin in GDM cases might play a role in re-establishing partially the normal glycosilation in the constituents of the cord in attempting to restore some their normal functions.

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