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Relative amounts of sialic acid and fucose of amniotic fluid glycoconjugates in relation to pregnancy age

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The present knowledge concerning the glycan structures and role of glycoconjugates derived from amniotic fluid is fragmentary and mainly focuses on the individual glycoproteins. The question has arisen as whether the general glycosylation pattern of amniotic fluid glycoconjugates can change with the progression of a normal pregnancy. In the present work we have described the dynamic, quantitative alterations in relative amounts of sialic acid and fucose linked by a variety of anomeric linkages to subterminal oligosaccharide structures of amniotic fluid glycoconjugates in relation to pregnancy age. The analysis was performed in the following groups of amniotic fluids derived from normal pregnancy by lectin dotting method: "2nd trimester" (14–19 weeks), "3rd trimester" (29–37 weeks), "perinatal period" (38–40 weeks), "delivery at term" (39–41 weeks) and "post date pregnancy" (41–43 weeks). In the "3rd trimester" the amniotic fluid glycoconjugates contained higher relative amounts of glycans terminated by α2-6-linked sialic acid ($p < 0.00002$) and by α1-6 innermost fucose ($p < 0.000001$) than those in the 2nd trimester. In contrast, they showed the lower relative amount of fucose linked α1-3 ($p < 0.02$). At the perinatal period the relative amount of α2-6-linked sialic acid increased ($p < 0.03$), and it then decreased during delivery ($p < 0.02$) to the level found in the "3rd trimester" group. In the post date pregnancy all parameters studied increased. The sialyl- and fucosyl-glycotopes of the amniotic fluid glycoconjugates may play an critical role in growth and tissue remodeling of the foetus, as well as may might reflect maturation of a foetus. Additionally, a determination of the glycotope expressions might be helpful in prenatal diagnosis as predictor factors for well being of mother and child.
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Abbreviations: **AAA, Aleuria Aurantia Lectin; MAA, Maackia amurensis lectin; SNA, Sambucus nigra lectin; LTA, Tetragonolobus purpureus lectin; UEA, Ulex europaeus lectin.**

Introduction

Amniotic fluid contains many biochemical components, which originate from the foetus tissues and excretions, and the placental tissues, as well as from the maternal organism. The appearance of them in amniotic fluid depends on the stage of gestation [1]. The biochemical profile of many amniotic fluid substances can been used for prenatal diagnosis in order to establish foetal maturity, foetal well being or even a large number of foetal abnormalities as well as, in order to predict gestational age, and labour [2,3].

Most of amniotic fluid molecules present in amniotic fluid are structurally glycoconjugates i.e. glycoproteins including mucins, glycopeptides, oligosaccharides and glycolipids. This includes oncofoetal antigens [4], various cytokines [5], hormones [6]; and enzymes [7]. The carbohydrate structures of glycoconjugates are considered to be carrier of encoded biological information and they are essential for cell-cell and cell-substrate interactions [8,9]. Many authors have reported that carbohydrate antigens, particularly terminated by sialic acid and fucose, exhibit interesting biological properties, independent of their metabolic fate. They are thought to play diverse roles in development of the embryo, promote the growth and differentiation of the foetus tissues, and moreover

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they can mediate or modulate various adverse conditions [10,11].

Heterogeneity of the glycan part of glycoconjugates make them highly diverse and in many cases, this diversity has been shown to be species, tissue, and cell specific. Moreover, the glycan units on the same molecule can exhibit a high degree of subtle variations in their structure depending on dynamic physiological or pathological biochemical conditions that exist in the body at the time when the molecule is released. These alterations were found to be associated with the protein studied and with the disease. The majority of reported changes in glycosylation are related to the degree of glycan branching and the type of substitution of the N-linked glycans with sialic acid and/or fucose [12]. Also, the changes in glycoprotein glycosylation have been reported to take place during the normal menstrual cycle and during pregnancy, and they are regulated by steroid hormones [13].

Sialic acid (*N*-acetyl-5-neuraminic acid) and fucose (6-deoxy-L-galactose) expressed on a variety of vertebrate cells and tissue types are mostly at terminal position of most N- and O-glycans of glycoproteins as well as glycolipids. Both sugars can be attached by variable glycosidic linkages. Sialic acids linked by α2-3 and α2-6 glycosidic linkages are found on terminal galactose moieties, on terminal or subterminal moieties, or on an internal GalNAc moiety. Glycan modified by terminal α2-6 sialic acid is generally not modified further, except possibly by some member of the poly α2-8 sialyltransferase family [14]. The complex glycans of glycoconjugates are known to contain L-fucose in various α-glycosidic linkages: α1-2 to galactose as well as α1-3 and α1-4 to external *N*-acetylglucosamine and α1-6 to core glycan *N*-acetylglucosamine [15].

The present knowledge concerning the glycan structures and role of glycoconjugates derived from amniotic fluid is fragmentary and mainly concerns the individual glycoproteins. The question has arisen as whether the general glycosylation pattern of amniotic fluid glycoconjugates can change with the progression of a normal pregnancy. In the present work we have examined the degree of sialylation and fucosylation of glycoconjugates present in amniotic fluid in relation to progression of normal pregnancy. For this purpose, the relative degree of sialic acid linked to glycans by α2-3 and α2-6 glycosidic anomeric linkages, as well as fucose attached by three types of glycosidic linkages α1-6, α1-3 and α1-2 of amniotic fluid glycoconjugates were estimated in the amniotic fluid samples of: 2nd and the 3rd trimesters, perinatal period, delivery at term, as well as post date pregnancies. For these studies a convenient, and simple dotting method by the use of lectin (Table 1) with well-known sugar specificity was performed.

Table 1. The major binding specificities of used lectins

Origin and used abbreviation of lectin	*Binding preference*
Maackia amurensis (MAA)	Terminal sialic acid linked α2-3 to Gal of N- and O-glycans [35].
Sambucus nigra (SNA)	Terminal sialic acid linked α2-6 to Gal/GalNAc of N- and O-glycans [36].
Aleuria aurantia (AAA)	Fucose linked α1-6 to GlcNAc of N-glycans, <Fucα1-2Gal;<Fucα1-3GlcNAc linked to N- and O-glycans [37].
Tetragonolobus purpureus (LTA)	Fucose linked α1-3 to GlcNAc (in Lewis^x) of N- and O-glycans [38].
Ulex europaeus (UEA)	Fucose linked α1-2 to Gal <Fucα1-2Galβ1, <Fucα1-3 or 1-4GlcNAc (in Lewis^x or Lewis^y) of N- and O-glycans [39].

Materials and methods

Patients and the sampling

Samples of amniotic fluid (96) with gestational age between 14 and 43 weeks were taken from pregnant women receiving prenatal care in the Department and Clinic of Reproduction and Obstetrics at Wrocław Medical University (Poland) in the 1997–2000 years. Accurate gestational dating was defined as knowledge of the most recent menstrual period with confirmation by ultrasonographic evaluation. There were no signs of vaginal infection or chorioamnionitis. All samples were collected with the informed consent of the individual women and the study was approved by a local ethics committee. The samples used were the remaining fluid after, the routine diagnostic procedures were performed. Amniotic fluid was obtained by transabdominal amniocentesis under ultrasonographic guidance or by transvaginal amniotomy during delivery at term. Immediately after amniocentesis all amniotic fluid samples were centrifuged at 3000 g for 20 min., aliquoted and stored at -80°C until used. Frozen samples were thawed at 20°C before used. Amniotic fluid samples contaminated with blood or meconium were discarded.

The samples of amniotic fluid were divided into the following groups:

1. "2nd trimester", $n = 40$; the samples were from 14 to 19 weeks of gestation. The mean age of the women was 38 years, and ranged from 28–42 years. The samples were drawn in order to establish foetal karyotyping. These women delivered healthy infants at term without malformations or chromosomal abnormalities.
2. "3rd trimester", $n = 22$; from 29–37 weeks of pregnancy. The mean age of women was 28 years and ranged from 22–39 years.
3. "Perinatal period", $n = 6$; from 38–40 weeks of pregnancy. The mean age of women was 30 years and ranged from 24–34 years.

4. "Delivery at term", $n = 6$; from 39–41 weeks of pregnancy. The mean age of women was 30 years and ranged from 25–33 years.
5. "Post date pregnancy", $n = 22$; from 41–43 weeks of pregnancy. The mean age of women was 25 years and ranged from 18–33 years.

Methods

Lectin dotting for the analysis of sialylation and fucosylation of glycoconjugates

The components of amniotic fluid, including glycoconjugates, were immobilized to nitrocellulose paper (NC, 0,45 μm pore size, from SERVA GmbH, Heidelberg, Germany) and interaction of glycoconjugates with specific lectins (Table 1) was done according to the procedure given by Boehringer Mannheim in "Glycan Differentation Kit" with some modifications listed below.

The amount of an amniotic fluid protein necessary for a nitrocellulose coating, as well as dilutions of biotin labelled lectins (Vector Laboratories Ltd, Peterborough, UK) and Extr-Avidin-alkaline phosphatase (Sigma, St. Louis, MD, USA) were established in the preliminary experiments. The experimental details of the lectin dotting are described as below. The amniotic fluid samples were prediluted with 0.05 M Tris/HCl, 0.15 M NaCl, pH 7.5 (TBS) to the protein concentration 100 mg/l and then 100 μl of sample was added per well of Bio-Dot SF Microfiltration (BIORAD) apparatus assembled with a pre-soaked nitrocellulose sheet in the form of a slot. After 5 min. the nitrocellulose paper was washed once with TBS. The free binding sites on the nitrocellulose were blocked with 2% of Tween-20 in the TBS for 1 h at room temperature and next overnight at 4°C. After the blocking step the nitrocellulose was washed 3 times with washing buffer TBS-T (TBS containing 0.01% Tween-20), and the respective dots were incubated at room temperature for 1 h with biotin-labelled lectins diluted with TBS-T to the final concentration given in parentheses: SNA (1 μg/ml), MAA (5 μg/ml), AAA 5 μg/ml), LTA (10 μg/ml), UEA (10 μg/ml). After that, the paper was washed 3 times with TBS-T and the nitrocellulose was incubated at room temperature for 1 h with Extr-Avidin-alkaline phosphatase (1:200 000). Next, the plate was washed with washing buffer and the coloured reaction was developed by incubating the nitrocellulose in 10 ml of the following freshly prepared solution: 37.5 μl of X-phosphate (5-bromo-4-chloro-3-indolylphosphate, Sigma, St.Louis, MD, USA) and 50 μl of NBT-chloride (nitroblue tetrazolium chloride, Sigma, St.Louis, MD, USA) in 10 ml of 0.1 M Tris/HCl, pH 9.5, containing 0.05 M $MgCl_2$, 0.1 M NaCl at room temperature for 1 min. To stop the reaction H_2O was added. The nitrocellulose paper was dried. The intensity of the slots corresponding to the reactivity with lectins was quantified by scaning and then densitometric analysis. The analysis was performed with a computer equipped with the image analysis software package SigmaGel Spots Mode Measurements (Gel Analysis Software, Version 1.0, Jandel Scientific GmbH). Results are expressed in pixels, arbitrary units, corresponding to the respective lectin reactivity with 10 μg of amniotic fluid protein. Total protein concentration was determined by the bicinchoninic acid method according to Smith *et al*. [16], with bovine serum albumin used as a standard.

The control probes were performed to demonstrate the specificity of a lectin as well as an absence of detectable endogenous reactive materials. The positive controls were the following: asialo-haptoglobin preparation derived from ovarian cancer fluid [17] for AAA, LTA, UEA, transferrin for SNA (Boehringer Mannheim, from "Glycan Differentation Kit"), mouse glycophorin for MAA (a gift from dr hab. Hubert Krotkiewski, Institute of Immunology and Therapiae Experimantalis, PAN, Wrocław, Poland), respectively. The negative control was the human albumin preparation (Sigma, St.Louis, MD, USA) included into the test instead of amniotic fluid sample. The background intensity was undetectable when the TBS was included into the tests instead of reagents: (1) lectin, (2) ExtrAvidin-AP, and (3) amniotic fluid sample.

Statistical analysis

The statistical analysis was used the STATISTICA 5.0 computer program. For the statistical significance the U Mann-Whitney test was used and the correlations were estimated according to Sperman. A two-tailed p-value of less than 0.05 was considered significant.

Results

Glycoconjugates present in human amniotic fluid reacted with all used lectins, which are known to recognize the following sugar structures: (1) sialic acid attached by α2-3 (MAA: *Maackia amurensis* agglutinin) and by α2-6 (SNA: *Sambucus nigra* agglutinin) glycosidic linkages, (2) fucose linked through the following glycosidic linkages: α1-6 (AAA: *Aleuria aurantia* agglutinin), α1-3 (LTA: *Tetragonolobus purpureus* agglutinin), and α1-2 (UEA: *Ulex europaeus* agglutinin).

Sialylation

The analysis of the interaction of amniotic fluid glycoconjugates with appropriate specific MAA and SNA lectins (Table 2 and in Figure 1A–B) demonstrate a positive correlation between the pregnancy age ($r = 0.52$; $p < 0.001$ and $r = 0.75$, $p < 0.001$, respectively) and the expression of sialic acid α2-3 linked as well as α2-6 linked to glycoconjugates.

The relative amounts of formed complexes of amniotic fluid glycoconjugates with MAA were on this same level from the beginning of the 2nd trimester (694 ± 238 arbitrary units) to the 37th week of pregnancy (639 ± 346), and then increased to the value of arbitrary units 964 ± 251, 947 ± 190 and 1203 ± 284 in the groups of "perinatal period" ($p < 0.03$), "delivery at term" ($p < 0.015$), and "post date pregnancy" ($p < 0.000001$), respectively (Figure 1A, Table 2). However, the results of the group of "3rd trimester" significantly differed from the "post date pregnancy" ($p < 0.00002$), only.

Table 2. The expression of sialic acid and fucose on amniotic fluid glycoconiugates in relation to pregnancy age

	Glycoconjugates reactivity with lectins:*					
	MAA	*SNA*		*AAA*	*LTA*	*UEA*
Groups of amniotic fluids and number of samples	*specific for sialic acid attached by the linkages:*			*specific for fucose attached by the linkages:*		
	α2-3	*α2-6*	*Ratio** MAA/SNA*	*α1-6*	*α1-3*	*α1-2*
2nd trimester $n = 40$	694 ± 238^{3}	965 ± 299^{1}	0.79 ± 0.37^{5}	373 ± 192^{1}	515 ± 197^{2}	468 ± 317^{4}
3rd trimester $n = 22$	639 ± 346^{4}	1458 ± 328^{7}	0.42 ± 0.16^{6}	868 ± 347^{8}	390 ± 203^{4}	427 ± 255^{4}
Perinatal period $n = 6$	964 ± 251	1806 ± 292^{6}	0.53 ± 0.12	837 ± 62	252 ± 148^{4}	715 ± 352^{4}
Delivery at term $n = 6$	947 ± 190	1413 ± 193^{4}	0.68 ± 0.16	580 ± 206^{4}	413 ± 258	470 ± 228^{4}
Post date pregnancy $n = 22$	1203 ± 284	2099 ± 225	0.57 ± 0.11	1085 ± 344	661 ± 419	1251 ± 367

*The expression of sialic acid and fucose in amniotic fluid glycoconiugates was analysed based on the reactivity of the constant amount of amniotic fluid glycoconjugates with respective lectins by dotting as described in *Material and Methods.* The results are expressed in arbitrary units (pixels) and they are given as a mean ± standard deviation (mean ± SD).
**Ratio value reflects the proportion of the expression of sialic acid linked by α2-3 to those linked by α2-6 glycosidic bonds to the glycan of glycoconjugates.
The U Mann-Whitney test was used for the statistical calculations. A *p*-value of less than 0.05 was considered significant.
[1]Significantly different from 3rd trimester, perinatal period, delivery at term, and post date pregnancy.
[2]Significantly different from 3rd trimester, perinatal period.
[3]Significantly different from perinatal period, delivery at term and post date pregnancy.
[4]Significantly different from post date pregnancy.
[5]Significantly different from 3rd trimester, perinatal period and post date pregnancy.
[6]Significantly different from delivery at term and post date pregnancy.
[7]Significantly different from perinatal period and post date pregnancy.
[8]Significantly different from delivery at term.

The relative amounts of formed complexes of the amniotic fluid glycoconjugates with SNA (α2-6 linked sialoglycoconjugates) increased from the value of arbitrary units 965±299 observed in the "2nd trimester" to the value of 1458 ± 328 in the "3rd trimester" ($p < 0.00002$) and further to the value of 1806 ± 292 in the the "perinatal period" ($p < 0.0001$), (Figure 1B, Table 2). However, they decreased during the partus, e.i. in the group of "delivery at term" (1413 ± 193 arbitrary units; $p < 0.002$). The highest glycoconjugate reactivity with SNA showed the samples from "post date pregnancy" group (2099 ± 225 arbitrary units) and the statistical differences were found when the results were compared with those from "2nd trimester group" ($p < 0.000001$), 3rd trimester ($p < 0.00002$), "perinatal period" ($p < 0.02$), "delivery at term" ($p < 0.0003$).

Amniotic fluid glycoconjugates from the 2nd trimester of pregnancy showed a significantly higher MAA/SNA ratio value (0.79 ± 0.37) than those samples of groups of "3rd trimester" (0.42 ± 0.16; $p < 0.00002$), and "perinatal period" (0.53 ± 0.12; $p < 0.049$) and "post date pregnancy" (0.57 ± 0.11; $p < 0.007$).

Fucosylation

The results of fucose expression determinations on amniotic fluid glycoconjugates in relation to pregnancy age are shown in Table 2 and in Figure 2A–C.

The relative amount of glycoconjugates recognized by AAA ($r = 0.64$; $p < 0.0001$) showed a positive correlation with the pregnancy age (Figure 2A). The relative amounts of amniotic fluid α1-6 fucosylated glycoconjugates increased more than twice in the "3rd trimester" (868 ± 347 arbitrary units; $p < 0.000001$) and in the "perinatal period" (837 ± 62 arbitrary units; $p < 0.0002$), as well as nearly three times in "post date pregnancy" (1085 ± 344; $p < 0.000001$) in comparison with those of 2nd trimester of pregnancy (373 ± 192 arbitrary units). However, the reactivity of glycoconjugates with AAA slightly declined during the partus, e.i. in the group of "delivery at term" (580 ± 206 arbitrary units).

The relative amount of glycoconjugates recognized by UEA ($r = 0.47$; $p < 0.001$) showed a weak positive correlation with the pregnancy age (Figure 2C). The differences among the relative amounts of amniotic fluid α1-2 fucosylated glycoconjugates derived from 2nd (468 ± 317 arbitrary units) and 3rd trimesters (427 ± 255 arbitrary units), the "perinatal period" (715 ± 352 arbitrary units) and the "delivery at term" (470 ± 228 arbitrary units) groups were without statistical significance. Only, the relative amount of amniotic fluid α1-2 fucosylated glycoconjugates was significantly higher in "post date pregnancy" (1251 ± 367 arbitrary units) group, when they were compared with all studied groups ("2nd trimester": $p < 0.000001$; "3rd trimester": $p < 0.000001$; "perinatal period": $p < 0.01$; "delivery at term": $p < 0.0006$).

On the contrary, the relative amount of glycoconjugates recognized by LTA ($r = -0.05$) did not show any correlation with the pregnancy age (Figure 2B). However, the relative amount of amniotic fluid α1-3 fucosylated glycoconjugates decreased significantly, when the results from "3rd trimester"

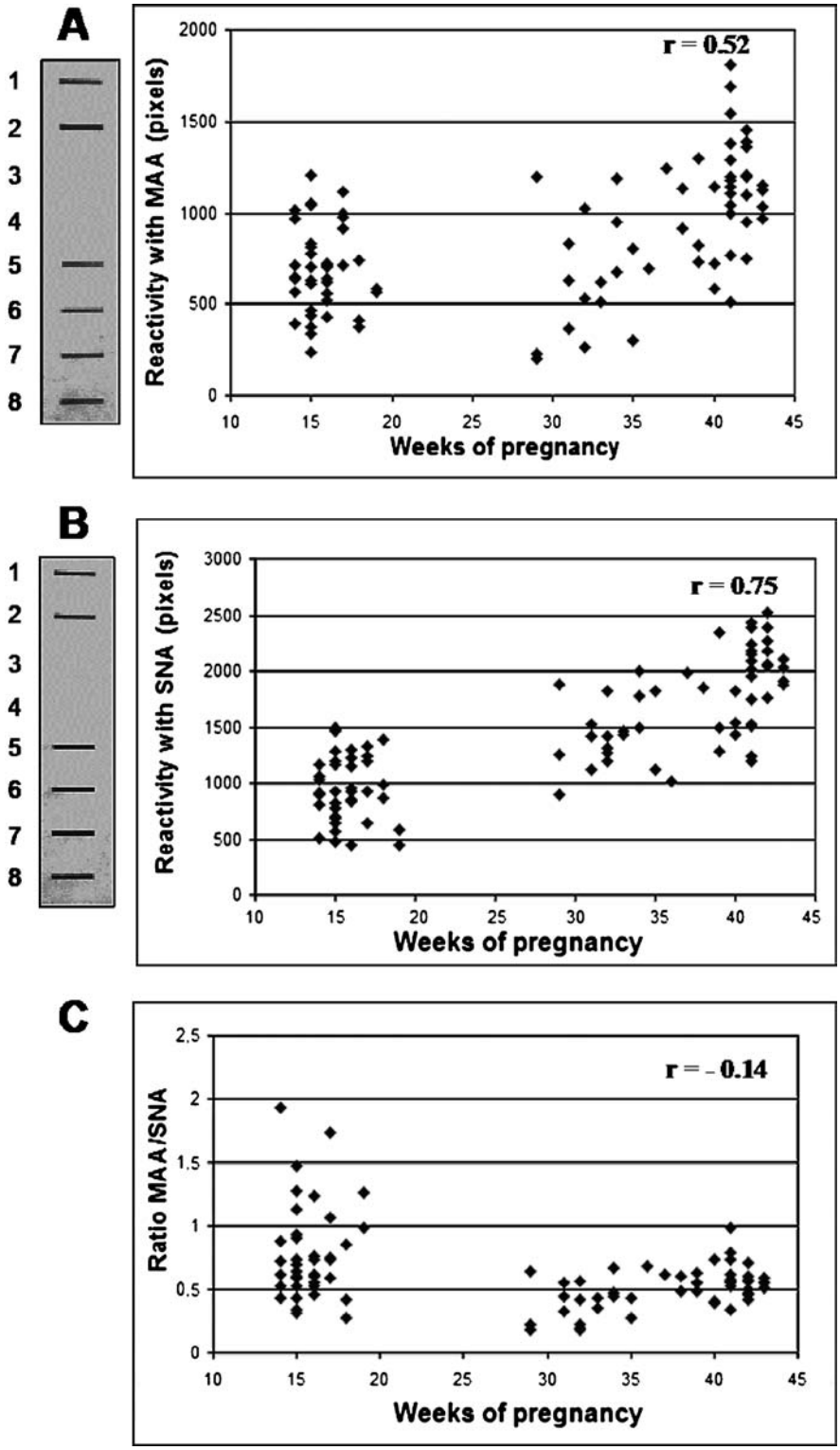

Figure 1. Reactivity of amniotic fluid glycoconiugates with sialic acid specific lectins in relation to pregnancy age. The constant amount of amniotic fluid glycoconjugates bound to the nitrocellulose sheets were analysed with α2-3-sialo- specific *Maackia amurensis* lectin MAA in (A) and with α2-6-sialo-specific *Sambucus nigra* lectin SNA in (B) by glycoconjugate-lectin dotting method as described in *Material and Methods* section. The results are expressed in arbitrary units (pixels) as well as the ratio of amniotic fluid glycoconjugates reactivity with MAA to the glycoconjugate reactivity with SNA in (C). On the left of the diagram the representative dots are shown. The dots were coated with the following samples: (1) and (2) the respective positive controls, (3) and (4) the negative controls (5–8) studied amniotic fluid samples.

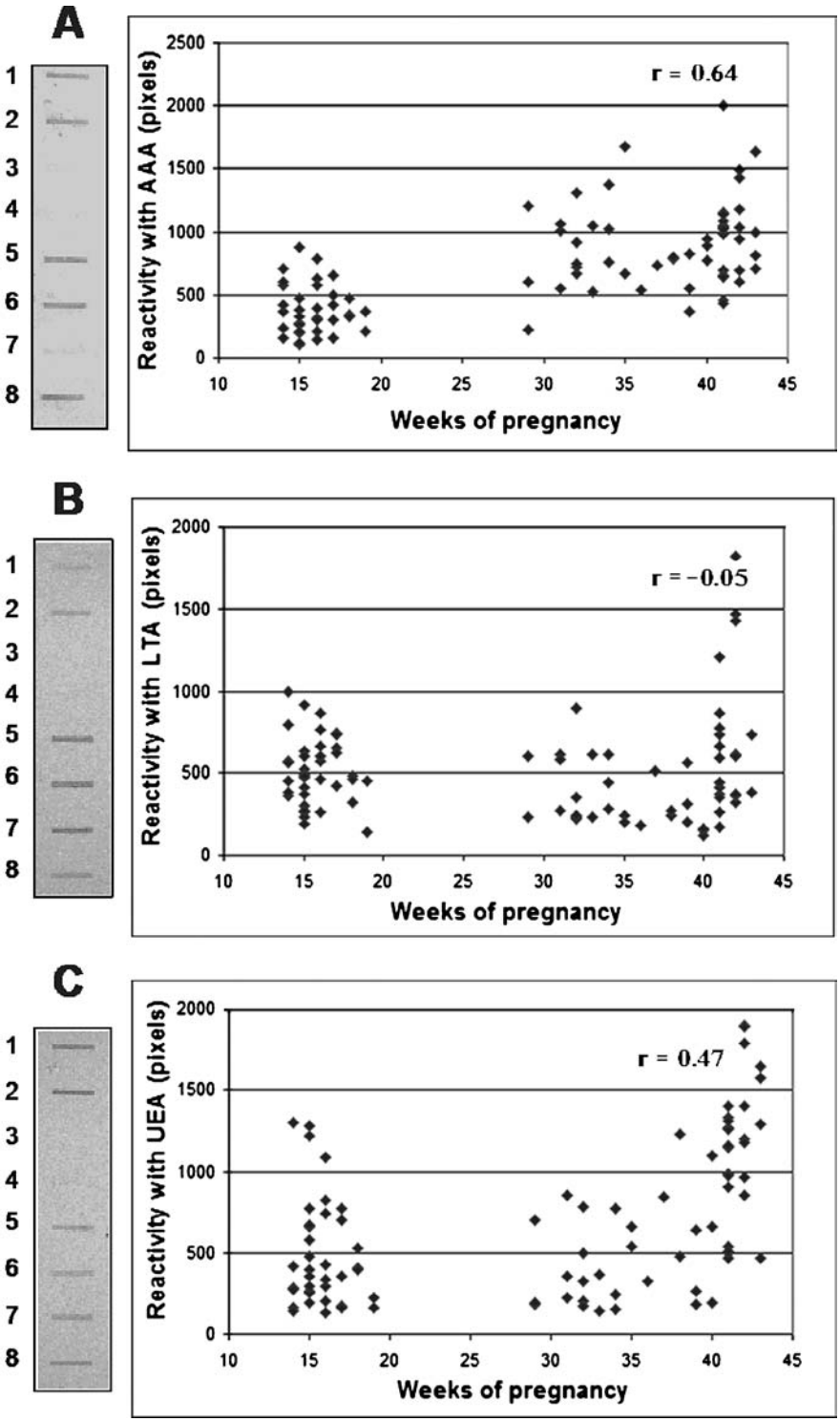

Figure 2. The reactivity of amniotic fluid glycoconiugates with fucose specific lectins in relation to pregnancy age. The constant amount of amniotic fluid glycoconjugates bound to the nitrocellulose sheets were analysed with α1-6-fucose specific lectin (AAA) in (A) and with α1-3-fucose-specific lectin (LTA) in (B) and with α1-2-fucose specific lectin (UEA) in (C) by the glycoconjugate-lectin dotting method as described in *Material and Methods* section. The results are expressed in arbitrary units (pixels). On the left of the diagram the representative dots are shown. The dots were coated with the following samples: (1) and (2) the respective positive controls, (3) and (4) the negative controls (5–8) studied amniotic fluid samples.

(390 ± 203 arbitrary units; $p < 0.02$) as well as from "perinatal period" (252 ± 148 arbitrary units; $p < 0.005$) were compared with those from 2nd trimester (515 ± 197 arbitrary units). Further, with the progression of gestation, the reactivity increased, particularly in the cases of "post date pregnancy" (661 ± 419; $p < 0{,}0025$).

Discussion

The present paper describes the alterations in relative amounts of sialic acid and fucose linked by variety of anomeric linkages to subterminal oligosaccharide structures of amniotic fluid glycoconjugates in relation to pregnancy age. The changes in the sialylation and fucosylation of the amniotic fluid glycoconjugates were dynamic, quantitative and they have been found to be associated with the progression of normal gestation. The most prominent glycan modifications observed during normal pregnancy are listed in Figure 3.

The data were obtained based on the lectin reactivity towards defined sugar structure specificity; however the lectin-dot method used is known to carry certain limitations. It does not measure the total sialic acid or fucose content, but only that ligand which is conformationally accessible for lectin binding. Moreover, because of the great amount and variability of glycoconjugates in amniotic fluid and the limited binding sites for them, mainly the sugar structures with high affinity to the lectin were determined. The glycan structures with low affinity were probably not recognized, since they could not resist the competition for binding sites on lectin, and this fact makes the lectin-test more specific. Despite the above mentioned limitations, the lectin-dot test permits, without harmful glycan preparation, to demonstrate the subtle glycan structure modifications of glycoconjugates present in biological samples during changeable physiopathological conditions of the living organism.

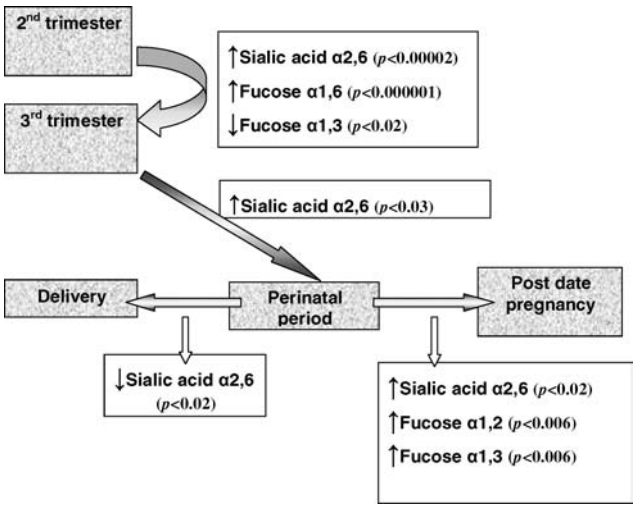

Figure 3. The main alteration in relative amounts of sialic acid and fucose linked by a variety of linkages to subterminal oligosaccharide structures of amniotic fluid glycoconjugates in relation to pregnancy age.

The composition of the amniotic fluid components is known to change throughout gestation because of its origination from different sources: from mothernal, foetal and placental tissues. Moreover, the presence of some components in amniotic fluid have been shown to be dependent on cytokines and changeable female hormones levels [18,19]. Maternal oestriol level increases throughout pregnancy with a surge in the last 4 to 6 weeks and finally decreases at delivery. Progesterone (inhibits uterine contractions) level rises also appropriately but drops off after the 40th week of gestation [2]. Also, there is some evidence that the N-glycosylation apparatus is selectively activated in utero by oestrogen [13,20]. Thus, as observed by us the patterns of sialylation and fucosylation of amniotic fluid glycoconjugates during the progression of gestation are the net results of several sites of its biosynthesis and exchanges among mother, foetus and placenta.

The highly α2-3-sialylated glycotope, called "oncofoetal", are known to be frequently expressed on amniotic fluid and foetal tissue glycoconjugates [4,21–23]. For example, the oncofetal domain in amniotic fluid fibronectin is created by O-glycans, which contain the characteristic sequence composed from N-acetyl-5-neuraminic acid linked by α2-3 glycosidic bond to galactose. This fibronectin glycotope appears to be among the most effective markers for preterm delivery [22,24]. Moreover, in early pregnancy increasing expression of sialic acid linked α2-3 in the deciduas surrounding the implantation site have been shown to be associated with the implantation of the embryo in the uterus of pregnant rats and mice [25]. The α2-3 (but not α2-6) linked sialic acid glycoconjugates, further modified by α1-3 fucosylation (a Lewis^x epitope) are essential ligands for E-, P-, and L-selectin binding in adhesive interactions [20,26,27]. Modifications in sialic acid expression have been shown to occur during development and differentiation of porcine tissues. The changes included a decrease or complete loss of sialic acid linked α2-3, which was replaced by sialic acid linked α2-6 during maturation of central nervous system [28]. In our observations the ratio value of amniotic fluid glycoconjugate reactivity with MAA to the glycoconjugate reactivity with SNA, which reflects the proportion of the expression of sialic acid linked to the glycans by α2-3 glycosidic bond to those linked by α2-6, changed dramatically from the value 0.79 in the 2nd trimester to the value 0.42 in the 3rd trimester. At this period of pregnancy, the changes in ratio value resulted from the increasing expression of α2-6-sialylated glycans, whereas the expression of α2-3-sialylated glycotope remains constant (Table 2). At the turn of 2nd and 3rd trimesters, simultaneously with physiological development of the foetus organs, the amniotic fluid glycoconjugates contained higher relative amounts of glycans terminated by α2-6-linked sialic acid. However, in the 3rd trimester the synthesized sialic acid by the mother, crosses the placenta to contribute to the foetal growth [19]. In contrast to foetal tissues, the glycoproteins derived from plasma of adult

persons, are known to contain larger amounts of glycans terminated by α2-6 linked sialic acid [17,22]. Moreover, at the turn of 2nd and 3rd trimesters relative amounts of glycans terminated by α1-6 innermost fucose attached to the core region of N-glycan increased. In contrast, they showed the lower relative amount of fucose linked α1-3 to external antennae (Table 2, Figures 1–2). The increased expression of core fucose (α1-6 fucosylation) in pregnancy has been demonstrated for hCG and transferrin [5,27]. According to Nemansky *et al*. [6] the increased core fucosylation of N-glycans may reflect the change from an invasive state to a more nurturing role for the placenta during pregnancy.

The other critical phase in glycosylation of the amniotic fluid glycoconjugates was found around the perinatal period (38–40 weeks of gestation) and during the delivery at term. This period was characterized by the significant alterations in expression of α2-6-linked sialic acid. Namely, the relative amount of α2-6-linked sialic acid increased at the "perinatal period" group ($p < 0.03$), but next it decreases during delivery to the level found in the "3rd trimester" group. The other alterations such as the increase of α2-3 sialylation, and α1-2 fucosylation in perinatal period as well as the decrease of α1-6 fucosylation during delivery were not statistically significant. Nevertheless, studies with a larger number of patients would be necessary to confirm our findings.

In the post date pregnancy group all studied parameters of glycosylation increased (Table 2 and Figures 1–3). The relative amounts of α2-6-linked sialic acid ($p < 0.02$), α1-2- ($p < 0.01$) as well as α1-3 linked fucose ($p < 0.0025$) were statistically higher than those from "perinatal period" group and α2-6-linked sialic acid ($p < 0.0003$), α1-2 ($p < 0.0006$) and α1-6 linked fucose ($p < 0.001$) than those from "delivery at term" group. The profound alterations observed by us in glycosylation in post date pregnancy (there is a risk for development of the post maturity syndrome and intrauterine growth retardation of foetus) could have the clinical implications.

The human foetus is surrounded by amniotic fluid containing a combination of glycoconjugates with potent biological activities. Pregnancy-induced changes in terminal glycosylation of glycoconjugates appear to be essential in some physiological functions of glycoconjugates. The carbohydrate part of glycoproteins protects a molecule against proteolytic attack. Sialylated and fucosylated oligosaccharides are involved in cell-cell interactions and are thought to participate in a variety of important processes, such as cellular recognition phenomena, a regulation of the biological activity of some glycoproteins, a cell maturation and in cellular antigenicity [15,29]. Surface expressed negatively charged sialoglycans contribute to the growth and differentiation of hematopoietic progenitor cells [30]. Structures bearing sialic acid may contribute to the homeostatic maintenance of circulating half-life of plasma glycoproteins by virtue of masking terminal galactose that would ordinarily contribute to the removal of such proteins from the circulation through the asialoglycoprotein receptor [14]. Sialylated α2-3 or α2-6 N-glycans of glycoproteins serve as ligands (counterreceptors) for sialic acid binding proteins (sialoadhesin and selectin families). However, the biological role of α2-3 to α2-6 linked sialic acids have been suggested to be quite different. The contribution of α2-3 linked sialic acid is important for the viability of peripheral CD8+ T cells- in cytotoxic-T-cell response [14,31]. The glycoconjugates bearing both sialyl Lewis^x and sialyl Lewis^a antigens could play a role in the prevention of leukocyte adhesion on the foetal syncytiotrophoblast [32]. A few definitive functions have been assigned to α2-6-linked sialic acid modifications. Sialic acid linked α2-6 serves as a receptor for human infectious strains of the influenza virus and it is ligand for CD22 on the surface of B-lymphocytes. The interaction involving CD22 and α2-6 linked sialic acid to Gal β1-4GlcNAc structures of ligands might have an effect in signal transduction events in the immune system [14].

Human amniotic fluid contains a variety of glycoproteins, several of which have been shown to exert immunomodulatory effects. There is some evidences that the biosyntesis of the sialyl Le^x structures takes place in the amniotic fluid. Glycodelin A and transferrin carry fucosylated LacdiNAc structures and sialylated Lewis^x antigen, respectively. Sialylated Lewis^x glycotope have been reported to act as inhibitors of E-selectin-mediated cell adhesion [33]. The glycan alterations can change the binding properties of the glycoconjugates. Increased expression of sialyl Lewis^x may abrogate the maternal immune/inflammatory response by blocking the primary adhesive interactions required for the expression of such activities [10]. This hypothesis is supported by the increased expression of sialyl Lewis^x determinants on the glycan of AGP during acute inflammation [34]. All above mentioned features can be important during pregnancy.

In conclusion, the human foetus is bathed in amniotic fluid containing the glycoconjugates, of which the glycans are found to be modified gradually in relation to the progression of pregnancy. The degree of sialylation and fucosylation of the amniotic fluid glycoconjugates could play a critical role in growth and tissue remodeling of the foetus, and might reflect maturation of a foetus as well as being implicated in the mechanism underlying the protection of the human foetus from the external harmful agents including maternal immune response. Detailed characteristics of sialic acid and fucose structures appearing during pregnancy may lead to their application as factors predictive for the well being of mother and child.

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