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Fucose and sialic acid expressions in human seminal fibronectin and α_1 -acid glycoprotein associated with leukocytospermia of infertile men

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Abstract. *Introduction*: The aim of this study was to compare fucose and sialic acid residue expression on fibronectin and α_1-acid glycoprotein in the seminal plasma of men suspected of infertility and suffering from leukocytospermia.
Subjects and methods: Seminal ejaculates were collected from 27 leukocytospermic and 18 healthy, normozoospermic men. The relative degree of fucosylation and sialylation of fibronectin and α_1-acid glycoprotein was estimated by ELISA using fucose and sialic acid specific lectins from *Aleuria aurantia*, *Lotus tetragonolobus*, and *Ulex europaeus* as well as *Maackia amurensis* and *Sambucus nigra*, respectively.
Results: Leukocytospermic seminal fibronectin, in comparison with fibronectin of normal fertile group, showed lower relative reactivity with AAL, LTA and UEA, and higher reactivity with MAA and SNA, while the AGP of the leukocytospermic group was less reactive with AAL, and the relative reactivity with LTA and MAA was significantly higher. Fibronectin and α_1-acid glycoprotein reactivity with UEA and MAA showed high positive correlations.
Discussion: Leukocytospermia was associated with the alterations of terminal monosaccharide expression in human seminal fibronectin and α_1-acid glycoprotein. The increase of sialyl-Lewis^x antigen in α_1-acid glycoprotein can be used as a marker of genital tract inflammation manifested by leukocytospermia.

Keywords: Fibronectin, α_1-acid glycoprotein, fucosylation, sialylation, leukocytospermic human seminal plasma

1. Introduction

Leukocytes are normally present in male reproductive tract but their significance in the human ejaculate is controversial. Some authors have shown that leukocytes attribute a favourable effect on sperm functions and have limited influence on sperm fertilizing capacity *in vitro* [12,16]. They believed that leukocytes may play a positive role in semen immune surveillance [16] and elimination of morphologically abnormal spermatozoa via phagocytosis [36]. On the other hand, the presence of excess leukocytes in the ejaculate higher than 1×10^6/ml, defined by World Health Organization as leukocytospermia [40], is reported to be associated with poor semen parameters. Activated leukocytes released bioactive molecules such as cytokines and enzymes [7], as well as stimulated the production of highly toxic free radicals and anti-sperm antibodies, what influenced on sperm metabolism and semen quality, as well as decreased sperm motility, acrosome reaction and fusiogenic ability [6,24,39,45]. Increased number

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of leukocytes in the ejaculate has been repeatedly associated with about 10–20% subfertility and infertility in men [27,33,39,40].

Many authors have reported that expression of glycotopes on glycoproteins through their sugar-based interactions is essential for interactions of biological systems, such as cell-cell and cell-substrate communications, receptor-mediated intracellular signaling [reviewed in [3,4]]. The oligosaccharides of glycoconjugates, particularly those terminated by sialic acid and fucose, can modulate protein function and lifespan [reviewed in [3,4]]. They can be modified during disease, thus the determination of monosaccharide expression is becoming useful in clinical biochemistry helping the diagnosis of some diseases [3,4,37].

In the present paper we were interested if any differences exist in the expression of fucose and sialic acid in leukocytospermic seminal plasma fibronectin (FN) and α_1-acid glycoprotein (AGP) of infertile men. The glycoproteins chosen for analysis are extremely microheterogenic and modifications of their glycan structures have been reported to be associated with some semen abnormalities [14,18,32]. However, FN and AGP have quite different overall structures, oligosaccharide patterns, and play different biological roles.

Fibronectin is multidomain and multifunctional adhesive glycoprotein. It contains 5–9% N- and O-oligosaccharides located in the collagen and cell binding domains. Through binding many ligands FN is reported to play a variety of roles in cellular adhesion, migration and differentiation, cellular proliferation and development, and wound repair processes [25, 38]. FN is believed to take part in fertilization capacity of human spermatozoa [41], activation of the proteasome and induction of the acrosome reaction in human sperm [5]. It is sensitive to proteolysis by exogenous and endogenous proteinases. Proteolysis can lead to the release of cryptic activities and/or loss of FN functions [42]. Fibronectin of an ejaculate is produced by peritubular myoid cells of testis [31]. In seminal plasma, FN is present as a set of FN fragments [15] released into the seminal fluid during lysis of the gel structure [21]. Seminal plasma totally lacks the intact fibronectin form and consists of FN fragments derived from those FN domains which are known to be glycosylated, i.e. the cell-binding domain [15,35] and collagen-domain [17]. Distribution of hypo- and a-sialylated FN glycoforms has been found to be associated with abnormal semen parameters and with high concentrations of fibronectin [14].

Human blood plasma AGP is one of the positive acute phase proteins which hepatic synthesis, regulated by some cytokines and steroid hormones, is known to increase due to systemic response of inflammation provoked by various stressful stimuli, such as trauma, wounding, bacterial infections. AGP has an ability to bind and transport small hydrophobic molecules [3, 37]. Blood plasma AGP (40 kDa) is heavily glycosylated (40–45% of sugars) by five complex type N-linked glycans [26]. During inflammation, AGP undergoes structural modifications of its oligosaccharide moiety, resulting in alterations of the degree of branching and fucosylation. AGP glycoforms are known to exert significant immunomodulatory effects [reviewed in [3,22,37]]. Seminal plasma AGP, synthesized locally by prostatic epithelial cells, is more heavily glycosylated [32]. The N-linked complex type glycans (di-, tri- and tetra-antennary glycans) of seminal plasma AGP, are terminated by α2,3- and α2,6-linked sialic acid, and additionally can be decorated by Lewis^x and Lewis^a structures, but do not contain core α1,6-linked fucose [18,32].

The relative amounts of terminal monosaccharide residues of FN and AGP in normal and leukocytospermic seminal plasma samples were analysed by fucose specific lectins from *Aleuria aurantia* (AAL: fucose α1,6< α1,2< α1,3-linked), *Lotus tetragonolobus* (LTA: fucose α1,3-linked) and *Ulex europaeus* (UEA: fucose α1,2-linked) and sialo-specific lectins from *Maackia amurensis* (MAA: sialic acid α2,3-linked) and *Sambucus nigra* (SNA: sialic acid α2,6-linked). However, our intention was not to determine the "true" structure of the carbohydrate units on human leukocytospermic seminal plasma FN and AGP, but alterations in the relative amounts of accessible glycotopes for reaction with specific lectins. Such an observation mimics a similar type of interaction which could occur between sialyl- and fucosyl-glycoconjugates and their specific receptors *in vivo*.

2. Pateints and methods

2.1. Samples

Seminal ejaculates were collected from 27 leukocytospermic male partners (20–45 years old) from couples visiting the andrologist for infertility and from 18 healthy donors (26–45 years old) apparently fertile men (all men had fathered at least one child). The ejaculates were collected by masturbation into sterile containers after 3–7 days of sexual abstinence. The ejaculates were allowed to stand at 37°C until liquefaction was

complete (no longer than 1 h) and standard semen analysis (volume, pH, morphology, concentration, motility, viability) was carried out at the semen analysis laboratory InviMed in Warsaw according to WHO [40] directives. Semen samples were centrifuged at 3500 $\times g$ for 10 min. at room temperature to obtain plasma. Seminal plasma was divided into small aliquots and frozen at -76°C until use.

Seminal plasma samples were divided into normal ($n = 18$) and leukocytospermic ($n = 27$) groups. The normal group was formed by normozoospermic samples given by healthy donors with proven fertility. The amount of spermatozoa was higher than 2×10^7/mL and more than 30% expressed the correct sperm morphology with a motility of $\geqslant 50\%$ or progressive motility $> 25\%$ at 1 h after ejaculation. The FN and AGP concentrations (354.2 $\pm$ 141 mg/l and 42.9 $\pm$ 33 mg/l, respectively) corresponded to normal seminal plasma FN and AGP values [14,18]. The leukocytospermic group was formed from samples, in which, according to WHO [40] directives, the leukocyte concentration was higher than 1×10^6/mL. In leukocytospermic group 17.2% of samples were cryptozoospermic, 34.5% asthenozoospermic and 48.3% oligoasthenozoospermic.

2.2. *Fibronectin and α_1 -acid glycoprotein concentrations*

The concentration of fibronectin was determined by sandwich ELISA [15], using mouse monoclonal antibody directed to cell-binding domain of human FN (FN30-8; TAKARA, Japan; 1:10 000) and human plasma FN preparation (0.4–50 ng/100 μl; Sigma Chemical Co, St Louis, MO, USA) as a standard.

The AGP concentration was determined by radial immunodiffusion [23] using goat anti-human AGP polyclonal antibodies (kindly prepared by Prof. T. Stefaniak, Wrocław University of Environmental and Life Sciences) and human plasma AGP preparation (10–200 μg/ml; Sigma Chemical Co, St Louis, MO, USA) as a standard.

2.3. *Determination of terminal monosaccharide exposition*

Three biotinylated fucose-specific lectins (Vector Laboratories Inc., Burlingame, CA, USA): *Aleuria aurantia* lectin (AAL), *Lotus tetragonolobus* agglutinin (LTA) and *Ulex europaeus* agglutinin (UEA), and two biotinylated sialic acid-specific lectins (Vector Laboratories Inc., Burlingame, CA, USA), *Maackia amurensis* agglutinin (MAA) and *Sambucus nigra* agglutinin (SNA) were used to determine exposition of fucose and sialic acid in FN and AGP by lectin-ELISA according to the procedure described earlier [9,18].

The lectins do not have an absolute specificity and are able to react with accessible exposed terminal sugars on glycoproteins. The *Aleuria aurantia* lectin mainly reacts with the innermost α1,6-linked fucose to a core N-acetylglucosamine of N-glycans and with lower affinity with α1,2- and α1,3-linked fucoses of the outer arms [43]. *Lotus tetragonolobus* agglutinin [44] and *Ulex europaeus* agglutinin [1] are known to recognize α1,3-linked and α1,2-linked fucoses to the galactose or N-acetylglucosamine of the antennas, respectively. However, the terminal α2,3-sialic acid limits the binding of LTA to α1,3-linked fucose of Lewis^x structure [44] and the appearance of a α1,2-fucosylated structure reduces the attachment of α2,3-sialic acid to glycans [46].

2.4. *Removal of terminal sugars of antibodies*

The anti-human FN and anti-human AGP antibodies had to be defucosylated and desialylated before using them in lectin-ELISA to avoid lectin binding to coated antibodies [14,18]. Shortly, one volume of polyclonal rabbit anti-human FN and polyclonal goat anti-human AGP antibodies (200 μl, pH = 8.1) was mixed with an equal volume of 100 mmol/l $NaIO_4$ in 100 mmol/l $NaHCO_3$, 0.2% Tween 20, pH 8.1. The mixture was incubated for 90 min. at room temperature in the dark and subsequently was dialysed against 100 mmol/l $NaHCO_3$, pH 9.2, for 3 h at 4°C. Such treatment resulted in elimination of immunoglobulin reactivity with fucose-specific lectins but not with sialic acid-specific lectins. Therefore, the antibodies were additionally treated with neuraminidase from *Vibrio cholerae* (0.4 U/20 μg protein) [8] to remove the rest of α2,6- and α2,3-sialic acids accessible for lectins.

2.5. *The lectin-ELISA procedure*

In the lectin-ELISA the plate was coated with deglycosylated antibodies which are able to bind and separate a glycoprotein from a biological sample. The expression of exposed fucosyl- and sialyl-residues of a glycoprotein was determined by specific lectin.

2.5.1. ELISA plate capture

Defucosylated and desialylated polyclonal rabbit anti-human FN or polyclonal goat anti-human AGP antibodies were diluted in 10 mM TBS pH 8.5 (1:2000 for FN fucoses and 1:4000 for AGP fucoses, respectively; 1:1000 for FN sialic acid and 1:2000 for AGP sialic acid, respectively), coupled to a polystyrene microtiter ELISA plate and incubated for 2 h at 37°C.

2.5.2. Sample dilution

Seminal plasma samples were diluted in 10 mM TBS, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.05% Tween 20, and 0.5% glycerine, pH 7.5, to obtain a glycoprotein solution containing in 100 μl: 100 ng of FN and 100 ng of AGP for reaction with AAL, 500 ng of FN and 500 ng of AGP for reaction with LTA and UEA, 100 ng of FN and 100 ng of AGP for reaction with MAA and SNA. The plate with seminal plasma samples was incubated 2 h at 37°C. All samples were analysed in duplicate. To demonstrate the specificity of lectin-glycoprotein interaction and to check the absence of detectable endogenous reactive materials, control probes were included for the test. The background absorbance was measured for samples in which seminal plasma was replaced by buffer, but with all other reagents. The following glycoproteins were used as the positive controls: haptoglobin and asialo-haptoglobin preparations derived from ovarian cancer fluid [13], transferrin for SNA (Glycan Differentiation Kit, Boehringer Mannheim, Germany), mouse glycophorin for MAA (a gift from Prof. H. Krotkiewski, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland). Human albumin preparation (Sigma Chemical Co, St Louis, MO, USA) was used as a negative control.

2.5.3. Reaction with lectin

The α1,6-, α1,3- and α1,2-linked fucose residues in FN and AGP were detected by biotinylated AAL, LTA and UEA, and α2,3- or α2,6-linked sialic acid residues were detected by biotinylated MAA or SNA, respectively. The lectin dilutions were established on the basis of series preliminary experiments. All lectins were diluted in 10 mM TBS containing 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.05% Tween 20, and 0.5% glycerine, pH 7.5 and the plate was incubated 1 h at 37°C.

2.5.4. The glycoprotein-lectin complex detection

The formed FN-biotinylated lectin and AGP-biotinylated lectin complexes were quantified using phosphatase-labeled ExtrAvidin (1 h, 37°C; 1:20 000 for FN and AGP fucosylation, and 1:10 000 for FN and 1:20 000 for AGP sialylation; Sigma Chemical Co, St Louis, MO, USA) and detected by the reaction with disodium 4-nitrophenyl phosphate (Merck, Darmstadt, Germany). The absorbances were measured in a Stat Fax 2100 Microplate Reader (Awareness Technology INC, USA) at 405 nm with a reference filter at 630 nm. The results were expressed in absorbance units (AU) after subtraction the background absorbances.

To remove any protein excess the plate was washed with a 10 mM TBS, 0.05% Tween 20, pH = 7.5 between each ELISA-step.

The background absorbance ranges were for FN: 0.081–0.082 AU (AAL), 0.071–0.127 AU (LTA), 0.065–0.077 AU (UEA), 0.093–0.096 AU (MAA), 0.059–0.063 AU (SNA), and for AGP: 0.064–0.081 AU (AAL), 0.08–0.123 AU (LTA), 0.077–0.081 AU (UEA), 0.065–0.079 AU (MAA), 0.053–0.058 AU (SNA), depending on the microtiter plate and day of experiment.

2.6. Statistical analysis

Statistical analysis was done using STATISTICA 6.0 computer program (StatSoft Inc., Tulsa, OK, USA). To determine the statistical significant differences, the Mann-Whitney test was used and correlations were estimated according to Spearman test. A two-tiled p-value of less than 0.05 was considered significant.

3. Results

3.1. FN and AGP concentrations

The differences of mean FN concentration were non-significant in normal and leukocytospermic seminal plasma groups (354.2 $\pm$ 141 mg/l and 417.7 $\pm$ 313 mg/l, respectively) (Table 1). In contrast, the mean concentration of AGP in leukocytospermic group (217.6 $\pm$ 457 mg/l) was 4-times higher ($p < 0.03$) than that in normal seminal plasma group (42.9 $\pm$ 33 mg/l) (Table 2).

3.2. Seminal FN fucosylation and sialylation

As shown in Table 1, the relative reactivity of seminal FN with fucose-specific lectins, such as AAL (0.54 $\pm$ 0.2 AU), LTA (0.09 $\pm$ 0.09 AU) and UEA (0.3 $\pm$ 0.3 AU) was significantly lower in the leukocytospermic group ($p < 0.006$, $p < 0.004$ and $p < 0.02$, respec-

Table 1
Relative reactivity of seminal FN with fucose- and sialo-specific lectins

Groups	FN (mg/l)	FN reactivity with lectins (AU)				
		fucose-specific			sialo-specific	
		AAL Fucα1,6GlcNAc (core) > Fucα1,2Gal > Fucα1,3GlcNAc	LTA Fucα1,3GlcNAc	UEA Fucα1,2Gal	MAA SA α2,3	SNA SA α2,6
Leukocytospermic $n = 27$	417.7 ± 313	0.54 ± 0.2	0.09 ± 0.09 11*	0.3 ± 0.3 4*	0.99 ± 0.8 1*	0.44 ± 0.3
		$p < 0.006$	$p < 0.004$	$p < 0.02$	$p < 0.00009$	$p < 0.0002$
Normal $n = 18$	354.2 ± 141	0.72 ± 0.2	0.18 ± 0.1 1*	0.49 ± 0.3	0.24 ± 0.1 1*	0.24 ± 0.1 1*

Concentration of FN was determined by ELISA [15] using mouse monoclonal antibody anti-human cell-binding domain of FN (TAKARA, Japan). The relative reactivity of constant amount of FN with biotinylated fucose-specific lectins (AAL, LTA, UEA) and sialo-specific lectins (MAA, SNA) was determined using lectin-ELISA [9,14], and expressed in absorbance units (AU) after subtraction the background absorbances.
Results are given as a mean values ± standard deviation. Statistical differences ($p < 0.05$) were calculated using Mann-Whitney test.
*The number of samples which reactivity with lectin were < 0.05 AU.

Table 2
Relative reactivity of seminal AGP with fucose- and sialo-specific lectins

Groups	AGP (mg/l)	AGP reactivity with lectins (AU)				
		fucose-specific			sialo-specific	
		AAL Fucα1,6GlcNAc (core) > Fucα1,2Gal > Fucα1,3GlcNAc	LTA Fucα1,3GlcNAc	UEA Fucα1,2Gal	MAA SA α2,3	SNA SA α2,6
Leukocytospermic $n = 27$	217.6 ± 457	0.76 ± 0.2	0.56 ± 0.5 10*	0.64 ± 0.5 1*	0.54 ± 0.5 2*	0.8 ± 0.2
	$p < 0.03$	$p < 0.0002$	$p < 0.04$		$p < 0.04$	
Normal $n = 18$	42.9 ± 33	1.21 ± 0.4	0.33 ± 0.3 1*	0.66 ± 0.4	0.27 ± 0.1	0.78 ± 0.3

Concentration of AGP was determined by radial immunodiffusion according to Mancini et al. [23] using goat anti-human AGP polyclonal antibodies. Reactivity of AGP constant amount with biotinylated fucose-specific (AAL, LTA, UEA) and sialo-specific lectins (MAA, SNA) was determined using lectin-ELISA [18], and expressed in absorbance units (AU) after subtraction the background absorbances.
Results are given as a mean values ± standard deviation. Statistical differences ($p < 0.05$) were calculated using Mann-Whitney test.
*The number of samples which reactivity with lectin were < 0.05 AU.

tively) than those in the normal group (0.72 ± 0.2 AU; 0.18 ± 0.1 AU and 0.49 ± 0.3 AU, respectively). In contrast, relative reactivity of seminal FN with MAA (0.99 ± 0.8 AU) and SNA (0.44 ± 0.3 AU) was significantly higher ($p < 0.00009$ and $p < 0.0002$, respectively) in the leukocytospermic group than those in the normal group (0.24 ± 0.1 AU and 0.24 ± 0.1 AU, respectively).

3.3. Seminal AGP fucosylation and sialylation

In the leukocytospermic group relative reactivity of seminal AGP with AAL (0.76 ± 0.2 AU) was significantly lower ($p < 0.0002$), while with LTA (0.56 ± 0.5 AU) significantly higher ($p < 0.04$) than those found for the normal group (1.21 ± 0.4 AU and 0.33 ± 0.3 AU, respectively). The seminal AGP relative reactivity with UEA was similar in leukocytospermic (0.64 ± 0.5 AU) and normal (0.66 ± 0.4 AU) groups (Table 2).

In the leukocytospermic group AGP relative reactivity with MAA (0.54 ± 0.5 AU) was significantly higher ($p < 0.04$) than that in the normal group (0.27 ± 0.1 AU), while with SNA there were no significant differences between seminal leukocytospermic (0.8 ± 0.2 AU) and normal (0.78 ± 0.3 AU) groups (Table 2).

3.4. Correlation between glycotope expositions in FN and AGP

Among analysed α1,6-, α1,3, and α1,2-linked fucosyl- and sialyl- α2,3- and α2,6-linked glycotopes of FN and AGP based on their reactivities with the respective lectins, the high positive correlations be-

tween the expressions of α1,2-linked fucose ($r = 0.61$, $p < 0.000013$) and α2,3-linked sialic acid ($r = 0.73$, $p < 0.000001$) in FN and AGP were exclusively found (Fig. 1).

4. Discussion

Two main findings emerge from our studies. The first is that seminal fibronectin and α_1-acid glycoprotein are reactive with UEA suggesting the presence of glycoform decorated by the α1,2-linked fucose. The second shows that low expression of α1,6-, α1,3, and α1,2-linked fucoses (AAL-, LTA- and UEA-reactive, respectively), high expression of α2,3- and α2,6-linked sialic acid (MAA- and SNA-reactive, respectively) in fibronectin, and the presence of α2,3-linked MAA-reactive sialic acid and α1,3-linked LTA-reactive fucose in AGP were associated with leukocytospermia.

The observed alterations of terminal monosaccharide residue expression should be related to FN fragments while to non-degraded molecule of AGP. Despite the above, in this article we use the term seminal fibronectin, instead of fibronectin fragments. Fucosylation and sialylation patterns of normal seminal fibronectin and α_1-acid glycoprotein differed remarkably from that described for their blood plasma counterparts [14,18,35]. Blood plasma fibronectin is reported to be weakly fucosylated through the α1,6-, and α1,3-linkages, and lacks the α1,2-linked fucose, and heavily sialylated, mainly through α2,6-type of linkage [14,35]. The reactivities of normal seminal plasma fibronectin with fucose-specific AAL and UEA, and sialo-specific MAA and SNA suggest that FN is heavily fucosylated through the α1,6- and α1,2-types of linkages, but weakly by α1,3-linked fucose, and is poorly sialylated (Table 1). The presence of fucose α1,6-linked in seminal fibronectin has been proved by Kosanović and Janković [17]. Human blood plasma α_1-acid glycoprotein does not contain α1,6- and α1,2-linked fucoses, but contains variable amount of fucose α1,3-linked, and is heavily sialylated, mainly through α2,6-type of linkage [18,34,37]. In contrast, the α_1-acid glycoprotein of normal seminal plasma showed low expression of fucose α1,3 (recognized by LTA), high expression of fucose α1,2 (reactive with UEA), and it was weakly sialylated through α2,3-type of linkage (Table 2). Although the evident AGP reactivity with broad specificity for AAL may suggest the presence of fucose α1,6, the seminal plasma α_1-acid glycoprotein is known to lack the core fucose α1,6. AGP reactivity with AAL may correspond to the presence of α1,2-linked fucose (Table 2). Poland et al. [32] have shown that seminal AGP reactivity with AAL negatively correlated with the appearance of Lewis^a determinant.

Here we show for the first time that seminal fibronectin and α_1-acid glycoprotein can be decorated by α1,2- fucosylated glycotope. The α1,2-fucosylated glycans can be found on the blood group antigen H (Fucα (1,2)-Galβ) and related antigens, including difucosylated Lewis^b [Fucα1,2Galβ1,3] [Fucα1,4GlcNAc β1-R] and Lewis^y [Fucα1,2Galβ1,4] [Fucα1,3GlcNAc β1-R] determinants. These determinants are absent on soluble glycoproteins synthesized by hepatocytes but they can appear on human erythrocytes and a variety of epithelial cells, e.g. gastrointestinal cells, lower genitourinary tract [2], and on the surface of embryo and uterine endometrial cells [20]. The α1,2-linked fucose may occur on some glycoconjugates of secretory individuals, in saliva [11] and amniotic fluid [9,28]. The recent analysis of the human seminal plasma glycome has revealed the presence of glycoproteins whose glycan antennae were terminated with Lewis^x and/or Lewis^y sequences [29]. Moreover, the difucosylated Lewis^y oligosaccharide structure (containing α1,2- and α1,3-linked fucoses) has been found in seminal glycodelin S [19,30]. It seems most probable that other seminal glycoproteins might also be decorated by α1,2-fucosylated glycotope.

Our work also shows the changes in the expression of terminal residues of fucose and sialic acid in seminal fibronectin and α_1-acid glycoprotein in relation to leukocytospermia. The glycopatterns of these two glycoproteins showed some similarities and contrasts. Alterations connected with expression of UEA-reactive α1,2-linked fucose and MAA-reactive α2,3-linked sialic acid in FN and AGP (Fig.1) showed considerable positive correlations (for fucose α1,2 $r = 0.61$, $p < 0.000013$ and for sialic acid α2,3 $r = 0.73$, $p < 0.000001$). This fact might be associated with a common stimulation agent leading to activation of α1,2 fucosylation and α2,3 sialylation processes and engagement of sialic acid in modulation of immune response [10,29]. The opposite differences were associated with expression of LTA-reactive α1,3 fucose in FN and AGP. The significantly higher relative amounts of the LTA-reactive fucose and MAA-reactive sialic acid of AGP, but not of FN, may correspond to the presence the sialyl-Lewis^x antigen in acute phase AGP. The sialyl-Lewis^x antigen (SA2,3Fucα1,3GlcNAcβ1-R) is a well accepted marker of inflammation [3,37]. In our

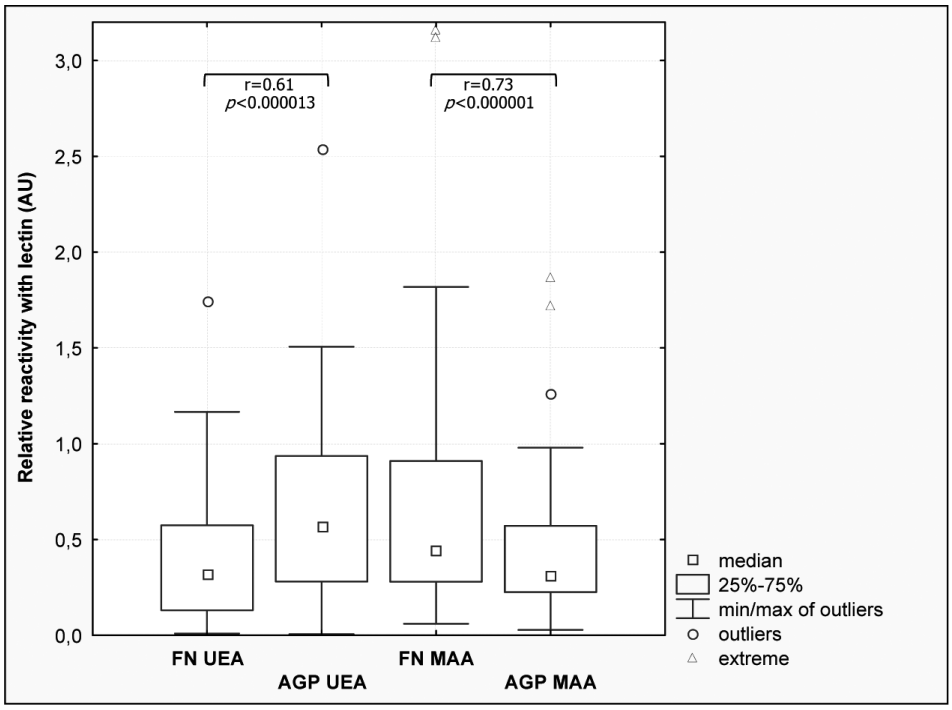

Fig. 1. Correlation between FN and AGP reactivity with lectins. FN UEA and FN MAA – fibronectin relative reactivity with UEA and MAA, respectively; AGP UEA and AGP MAA – AGP relative reactivity with UEA and MAA, respectively.

study 66.7% of samples showed the presence of LTA-reactive and MAA-reactive glycotopes in seminal AGP of patients suspected of infertility. These patients (18 from 27) may have had an infection of male genital tract or other type of inflammatory disease. On the other hand the high amount of leukocytes in sperm of the remaining 37.5% of patient samples with concomitant lack of LTA-reactive glycotope of AGP, can exclude the inflammatory etiology of leukocytospermia.

In conclusion, the appearance of UEA-reactive α1,2-fucosylated glycotope of seminal FN and AGP is not associated with leukocytospermia and probably reflects local tissue-derived synthesis. The leukocytospermia associated with increased expression of MAA-reactive α2,3-linked sialic acid in FN and AGP and increased of LTA-reactive α1,3-linked fucose in AGP can be related to inflammation of genital tracts. Determination of such glycotopes in AGP may help to separate patients suffering from male genital tract inflammation manifested by leukocytospermia from those with non-inflammatory condition. It seems very important because the incidence of leukocytospermia is reported to be high among infertile patients [39]. Thus, an early decision about appropriate treatment may improve fertility.

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