



Review

Anti-fibrillin-1 autoantibodies in normal pregnancy and recurrent pregnancy loss

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ABSTRACT

Problem: The aim of this study was to investigate anti-fibrillin-1 autoantibody in patients with a history of recurrent pregnancy loss (RPL) and during normal pregnancy.

Method of Study: Anti-fibrillin-1 IgG and IgM antibodies were measured by a home made ELISA in serum samples of 48 medically and obstetrically normal pregnant women, classified to three trimester groups, 15 female patients with RPL and 26 healthy non-pregnant women classified to two control subgroups: (a) women who had already had at least one previous successful pregnancy and (b) women who had never been pregnant. Differences in anti-fibrillin-1 autoantibodies between the groups were analyzed for statistical significance ($P < 0.05$) with one-way analysis of variance (ANOVA) and multiple comparison test — Post Hoc test, Least Significant Difference method.

Results: Anti-fibrillin-1 IgM autoantibodies were significantly decreased in the second and third trimester pregnant women compared to the nulligravida controls. RPL patients had significantly increased anti-fibrillin-1 IgM antibody compared to control group (a).

Conclusion: Fibrillin-1 degradation seems to be decreased during the second and third trimester of normal pregnancy. Increased anti-fibrillin-1 IgM antibodies in RPL patients may be a secondary phenomenon of increased fibrillin-1 degradation and contribute to the pathogenesis of pregnancy losses.

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Abbreviation: RPL, recurrent pregnancy loss.

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1. Introduction

Fibrillins are the principal structural components of elastic-fibre-associated microfibrils. Elastic fibers are made of an insoluble amorphous core of cross-linked elastin and a surrounding lattice of microfibrils [1]. Microfibrils are heterogeneous in composition and can also form macroaggregates without elastin. Integral and associated components of the microfibrils include the superfamily of fibrillins and latent TGF β -binding proteins (LTBPs), as well as the families of microfibrillar-associated glycoproteins (MAGPs), microfibril-associated proteins (MFAPs), fibulins and emilins [2]. Fibrillins-1 and -2 are large glycoproteins (350 kDa) [3], synthesized prior to tropoelastin deposition and polymerize into a characteristic “beads-on-a-string” structure, which gives rise to the microfibril lattice by lateral association of the polymers and by inclusion of other structural components.

The extracellular matrix plays a key role in organ formation and tissue homeostasis. Growth factors, potent regulators of cell differentiation, tissue morphogenesis and tissue homeostasis, reside in the extracellular matrix. Microfibrils probably have dual roles – in conferring mechanical stability and limited elasticity to tissues and in modulating the activity of growth factors of the transforming growth factor beta (TGF- β) superfamily (a family of 25-kDa homodimeric proteins with multifunctional effects on cellular growth and differentiation) [4]. Recent studies have shown that a functional relationship exists between fibrillin-1 and the activity of TGF β – a powerful cytokine that modulates cell survival and phenotype [5,6]. Pathological fibrillin-1-mediated regulation of TGF β bioavailability may be induced by microfibril degradation products [7].

Autoantibodies against short recombinant fragments of fibrillin-1 have been found in tight-skin mouse (an animal model of scleroderma), systemic sclerosis (SSc), mixed connective tissue disease, and primary pulmonary hypertension syndrome [8]. The reported frequency of anti-fibrillin-1 antibodies in Caucasian patients with scleroderma was 42% [9]. Sera from patients with diffuse SSc, calcinosis, Raynaud's, esophageal dysmotility, sclerodactyly, telangiectasias syndrome and mixed connective tissue disease also had significantly higher frequencies of anti-fibrillin-1 antibodies compared to control sera or to sera of patients with other non-SSc connective tissue diseases [8].

In pregnancy, remodeling of the extracellular matrix begins at an early stage. Blastocyst reception and implantation require various adaptations of the uterine microenvironment, including pregnancy stage-dependent expression of glycosaminoglycans and proteoglycans as well as synthesis, degradation and alteration of collagen fibrillogenesis. Fibrillin-1 was found among endometrial fibroblasts and decidual cells, respectively, in the pre- and postimplantation periods, and its expression in the endometrial stroma was found to be dependent on the pregnancy stage [10]. Fibrillin-1 is also abundant in the basement membrane regions of blood vessels, as well as in the luminal and glandular epithelia [10]. At the maternal–fetal interface and in Reichert's membrane of embryos at up to 6 days of development, fibrillin-1 appears to facilitate embryo expansion and fixation [10]. Changes in the fibrillin-1 organization and distribution during the peri-implantation period suggest that fibrillin-1 has a role in preparing the endometrium for embryo implantation.

The existence and distribution of fibrillin-1 within the decidua were studied by immunocytochemistry in formalin-fixed, paraffin-embedded tissues of different trimesters [11]. In first trimester, second trimester and term decidua, staining for fibrillin-1 was detected as a thick layer encapsulating decidual cells and in fibrillar form interconnecting these cells, being more intense than that during the menstrual cycle. Elastin was absent in all tissues examined. Fibrillin-1 appears to exist within the uterus in association with basal lamina components rather than with elastin-containing fibrils. Its presence in the endometrium and decidua suggests functional significance.

The site of fibrillin-1 in terms of human placenta was visualized by immunohistochemical staining, using a monoclonal antibody to human fibrillin-1 [12]. Fibrillin-1 was distributed extensively in the villous stroma, probably contributing for the elastic properties of the placenta and basal plate. Malak and Bell studied the distribution of elastin and fibrillin-containing microfibrils in fetal membrane specimens by histochemical, immunohistochemical, immunofluorescence, and electron microscopic techniques [13]. Fibrillin-containing microfibrils formed abundant longitudinal bundles that were primarily found in the fibroblast and reticular layers. The orientation of the bundles was parallel to the direction of membrane stretch. They also formed bundles that extended from the amniotic, chorionic, and decidual basement membranes to the adjacent tissues.

It could be suggested that during pregnancy, the homeostasis of both fibrillin-1 synthesis and degradation according to the gestation is critical for the uterine remodeling. Alterations of fibrillin-1 metabolism during pregnancy could induce increased production of anti-fibrillin-1 autoantibodies which could further enhance the metabolism alterations, influence the modulating activity of fibrillin-1 on the growth factors or cause autoimmune reactions which are potentially harmful for the pregnancy. In our previous studies on elastin turnover and autoantibody in normal pregnancy and recurrent pregnancy loss (RPL), we reported decreased elastin turnover during the third trimester of pregnancy and increased anti-elastin IgG autoantibody in RPL patients compared to the controls [14,15].

Still, anti-fibrillin-1 autoantibodies have not been an object of investigation in normal pregnancy and RPL. The aim of this study was to investigate the role of fibrillin-1 autoimmunity for the occurrence of RPL by determination of serum anti-fibrillin-1 IgG and IgM autoantibodies in patients with a history of RPL and healthy non-pregnant controls. Additionally, we aimed to investigate fibrillin-1 autoimmunity during normal pregnancy by determination of the serum levels of IgG and IgM autoantibodies in the first, second and third trimester pregnant women.

2. Material and methods

2.1. Subjects

This is a prospective study carried out on three subject groups including 48 healthy pregnant women, 15 consecutive non-pregnant women with a history of RPL and 26 healthy non-pregnant women as a control group. All individuals included in the study groups were Caucasian women from Bulgaria. Ethical permission was obtained from the local ethical committee, and informed consent was obtained from all women prior to entry into the study. Venous blood samples were obtained from the following groups and their subgroups.

Group 1 comprised of 48 pregnant women of a mean age of 27.5 (range 18–38) years attending prenatal care unit prior to termination of pregnancy. The inclusion criteria were: medically and obstetrically healthy pregnant women, first singleton pregnancy conceived normally, no history of miscarriage, or autoimmune disease. At the time of the blood sample obtaining, there were three subgroups of women, according to the gestational age: Group 1a – first trimester pregnancy ($n = 15$), Group 1b – second trimester pregnancy ($n = 16$) and Group 1c – third trimester pregnancy ($n = 17$).

Group 2 comprised of 15 non-pregnant women of mean age 33.5 (range 24–39) years with a previous history of recurrent pregnancy loss, defined as two or more consecutive pregnancy losses before 10 weeks' gestation. All women had suffered at least two unexplained previous miscarriages, with the group having suffered a mean of 2.16 (2–4) previous miscarriages and no live births. Only cases with postembryonic loss after a disappearance of fetal pulse on the ultrasound were included in the study. Documented first trimester preclinical and blighted ovum miscarriages were excluded. There were no karyotype abnormalities.

All women included in Group 2 were patients, attending the Center for Reproductive Health, Medical University of Pleven, during the 6-month period, since January 2009 through June 2009. They were referred by their obstetrician for laboratory evaluation after the other presumptive etiological factors (anatomic, hormonal or chromosomal defects, glucose tolerance test, toxoplasmosis serology, and antinuclear antibodies) had been ruled out. At the time of sampling, it had been at least 1 month since any of the women had suffered a miscarriage.

Group 3 (control group) comprised 26 healthy non-pregnant women with no history of miscarriage, or autoimmune disease. At the time of sampling, there were two subgroups of women, according to their birth status: Controls (a) ($n=11$) – women who had already had at least one previous successful pregnancy, mean age of 33 (range 25–42) and Controls (b) ($n=15$) – women who had never been pregnant, mean age of 24.6 (range 21–29).

At the time of the subject inclusion, a 10 ml blood sample was obtained by peripheral venipuncture, into a dry tube, for later serum anti-fibrillin-1 IgG and IgM antibody assaying. Serum was stored at -70°C until analysed using home made enzyme-linked immunosorbent assay (ELISA).

2.2. Competitive ELISA for testing the specificity of serum anti-fibrillin-1 antibodies

The specificity of serum anti-fibrillin-1 antibodies was tested via competitive ELISA, using human recombinant fibrillin-1 (Abnova Corporation, Taiwan), as a homologous inhibitor and α -elastin (Elastin Product Company, USA) and porcine tropoelastin (kindly presented by Lawrence B Sandberg, JL Pettis Memorial Veterans Medical Center, Loma Linda Medical School, Loma Linda, CA, USA) as heterologous inhibitors. Serum samples of 5 RPL patients, diluted 1:10 in PBS-Tween, were preincubated with different concentrations of each inhibitor (0.5, 2, 10 and 20 $\mu\text{g/ml}$) for 2 h at 37°C and overnight at 4°C . The reactivity of the treated sera was tested using ELISA for determination of anti-fibrillin-1 IgG and IgM antibodies and compared to the reactivity of the same, untreated sera, which was assumed to be 100%.

Inhibition (in %) of serum anti-fibrillin-1 IgG or IgM autoantibody after preincubation with inhibitors was established according to the following calculation:

$$\begin{aligned} \text{O.D. of serum anti-fibrillin-1 IgG (IgM) antibody (A)} &= 100\% \\ \text{O.D. of serum anti-fibrillin-1 IgG (IgM) antibody after} \\ \text{preincubation (B)} &= \text{X}\% \\ \text{Inhibition (X\%)} &= (B \times 100) : A. \end{aligned}$$

The means of X% of the 5th sera for each inhibitor's concentration were calculated.

2.3. ELISA for determination of serum anti-fibrillin-1 IgG and IgM antibodies

The serum levels of anti-fibrillin-1 (IgG and IgM) antibodies were measured by a home made ELISA. Microtiter 96-well plates (MICROLON, U-bottom, high binding, Greiner Bio One, Germany) were coated with fibrillin-1 by adding 100 μl of a solution of human recombinant fibrillin-1 (0.2 $\mu\text{g/ml}$ dissolved in 0.05 M carbonate buffer, pH 9.6) to each well and incubating for 2 h at 37°C and overnight at 4°C . Wells were washed with a solution of PBS, containing 0.05% Tween 20 (PBS-Tween) and then blocked by incubation for 1 h with 1% bovine serum albumin in PBS-Tween. After washing with PBS-Tween, 100 μl of patient sera, diluted 1:10 in PBS-Tween were added. The plates were incubated for 1 h at 37°C . The wells were then washed with PBS-Tween, incubated with a peroxidase-linked anti-human IgG and IgM (Sigma) diluted 1:25 000 for IgG and 1:50 000 for IgM in 0.1% human

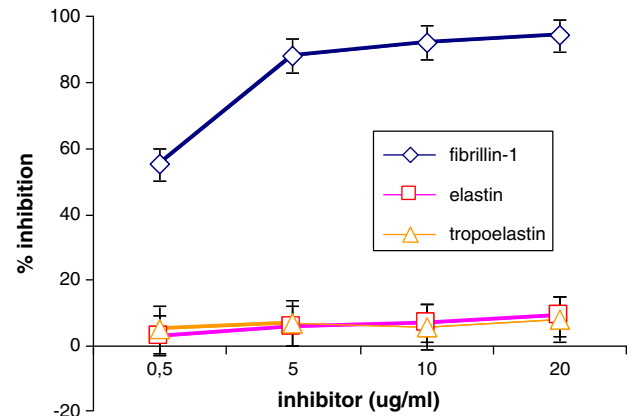


Fig. 1. Dose response inhibition of anti-fibrillin-1 IgG reactivity in 5 RPL patients' sera preincubated with fibrillin-1, elastin and tropoelastin. The inhibition of anti-fibrillin-1 IgG reactivity was assessed as described in [Material and methods](#). The results are presented as mean values of the percentages of inhibition \pm standard error (SE).

serum albumin in PBS-Tween and after washings reacted with o-phenylenediamine plus 0.1% H_2O_2 as colorimetric substrate. The reaction was terminated by 50 μl 8 N H_2SO_4 and the absorbance was read at 492 nm on automatic micro-ELISA plate reader. All experiments were run in parallel triplicates. The coefficient of variation was 7.2% ($n=10$).

2.4. Statistical analysis

Differences in anti-fibrillin-1 IgG and IgM autoantibodies between the groups were analyzed for statistical significance ($P<0.05$) with one-way analysis of variance (ANOVA) and multiple comparison test – Post Hoc test, Least Significant Difference (LSD method) using the statistical package SPSS, v. 15.

3. Results

3.1. Competitive ELISA for testing the specificity of serum anti-fibrillin-1 antibodies

Results from testing the specificity of anti-fibrillin-1 IgG and IgM antibody by competitive ELISA in 5 patients' sera preincubated with three inhibitors are presented on [Fig. 1](#) – for IgG and [Fig. 2](#) for IgM. The means of the percentages of inhibition of the fifth sera for each inhibitor's concentration (0.5, 5, 10 and 20 $\mu\text{g/ml}$) are shown. The inhibition of anti-fibrillin-1 reactivity in the samples preincubated with heterologous antigens was low – up to 9%, whereas homologous

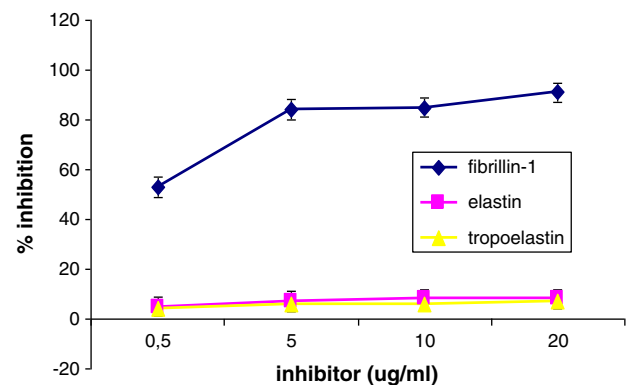


Fig. 2. Dose response inhibition of anti-fibrillin-1 IgM reactivity in 5 RPL patients' sera preincubated with fibrillin-1, elastin and tropoelastin. The inhibition of anti-fibrillin-1 IgG reactivity was assessed as described in [Material and methods](#). The results are presented as mean values of the percentages of inhibition \pm standard error (SE).

preincubation resulted in dose-dependent inhibition up to 94% in 20 µg/ml fibrillin-1 concentration of the preincubation solution.

3.2. Anti-fibrillin-1 antibodies in normal pregnancy

Results from determination of anti-fibrillin-1 IgG and IgM autoantibodies in the three trimester pregnant women and healthy non-pregnant controls are presented in Table 1. Mean values of optical density and standard errors for each study group are shown. The differences between pregnant women groups and the two non-pregnant control groups were examined by one-way analysis of variance (ANOVA). P value vs. each control group is presented for each pregnancy group.

Comparison of anti-fibrillin-1 IgG antibodies between the tree trimesters and control groups does not show significant differences. Significant differences of anti-fibrillin-1 IgM antibodies were established between second ($p=0.004$) and third trimester pregnant women ($p=0.047$) and the control group of non-pregnant nulligravida – IgM was significantly decreased in both trimesters compared to the controls ($p=0.004$, $p=0.047$) (Fig. 3).

3.3. Anti-fibrillin-1 antibodies in RPL patients

Comparison of anti-fibrillin-1 IgG antibodies between the study group of non-pregnant RPL patients and the two control groups does not show significant differences.

Serum levels of anti-fibrillin-1 IgM antibody in RPL patients were significantly increased compared to the first control group (Controls (a)) ($p=0.004$). Results are shown on Fig. 4.

4. Discussion

In the recent years much attention was given to extracellular matrix and its extensive remodeling during pregnancy, requiring both breakdown and resynthesis of its components. It is known, that improper turnover of endometrial extracellular matrix is associated with disturbances of implantation, unexplained infertility and recurrent miscarriage [16,17]. Fibrillin-1 organization and distribution in the various regions of the endometrial stroma were found to be pregnancy stage dependent – it was found among endometrial fibroblasts and decidual cells, respectively, in the pre- and post-implantation periods [10]. King and Blankenship [18] examined the developmental appearance of fibrillin in macaque and human placentas and fetal membranes. At early gestational ages (26–30 days), fibrillin was found in cell columns and cytotrophoblastic shell, with weak staining in the villous stroma. Staining was abundant in the shell and columns at 53 days as well, and stronger staining was seen in the stroma of the chorionic plate and stem villi. Staining in the shell and remnants of the cell columns in later gestation continued to be positive, though variable. Fibrillin was abundant in the stromal cores of human term placental villi. The authors suggested that fibrillin provides attachment points for cells while at the same time

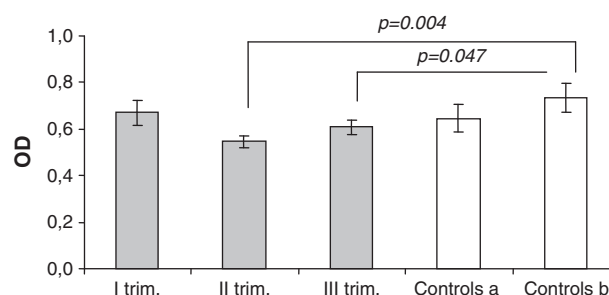


Fig. 3. Anti-fibrillin-1 IgM autoantibodies in the sera of the three trimesters pregnant women and non-pregnant control groups: (a) women who had already had at least one previous successful pregnancy, (b) women who had never been pregnant. The results are presented as mean values of the optical density (OD) \pm standard error (SE). The significant p values vs. non-pregnant controls ($p<0.05$) are shown.

providing a strong, yet flexible, matrix to accommodate growth particularly in areas subject to shear stress.

Fibrillin and elastin are colocalized in the elastic fibers. In our previous study [15] we investigated elastin turnover during the three trimesters of normal pregnancy. We used the serum levels of anti-tropoelastin and anti- α -elastin antibodies and their ratio as markers of elastin synthesis and degradation. In all trimester groups we established significantly decreased levels of anti- α -elastin IgM antibodies compared to controls and significantly reduced anti- α -elastin IgG during the third trimester. Synthesis/degradation ratio was significantly increased during the last trimester. These findings all together suggested decreased elastin degradation during pregnancy in terms of constant synthesis since the levels of anti-tropoelastin antibodies did not show significant differences compared to non-pregnant controls. We suggested that decreased elastin degradation had a protective role in pregnancy.

This study continues our previous investigation and focuses on fibrillin turnover during pregnancy. To our knowledge, this is the first study on the serum levels of anti-fibrillin-1 IgG and IgM autoantibodies during the three trimesters of normal pregnancy. We compared them with two non-pregnant controls: (a) women who had already had at least one previous successful pregnancy and (b) women who had never been pregnant. Significant differences of anti-fibrillin-1 IgM antibodies were established between second and third trimester pregnant women and control group (b) – IgM was significantly decreased in both trimesters compared to the controls. These results are similar to those established for anti- α -elastin IgM antibodies during normal pregnancy, excluding the first trimester, where anti-elastin IgM antibodies were significantly decreased compared to the controls. We define the levels of anti-fibrillin-1 IgM antibodies in non-pregnant nulligravida controls as “normal” levels of natural IgM antibody resembling the physiological fibrillin remodeling, including degradation of fibrillin substrates by matrix metalloproteinases (MMPs) followed by autoantibody production to cryptic or misfolded antigenic epitopes, which become available after proteolytic fragmentation of fibrillin. IgM is the first antibody isotype

Table 1
Serum levels (average optical density values) of anti-fibrillin-1 IgG and IgM antibodies in the study groups. P value vs. the two groups of non-pregnant controls is shown for each study group. (a) Women who had already had at least one previous successful pregnancy, (b) women who had never been pregnant, * $p<0.05$ was considered significant.

Study group (N)	Anti-fibrillin-1 IgG antibody			Anti-fibrillin-1 IgM antibody		
	O.D.	p vs. control (a)	p vs. control (b)	O.D.	p vs. control (a)	p vs. control (b)
1st trimester (n=15)	0.248 \pm 0.037	p=0.549	p=0.936	0.67 \pm 0.54	p=0.731	p=0.321
2nd trimester (n=16)	0.208 \pm 0.022	p=0.746	p=0.348	0.546 \pm 0.26	p=0.157	p* = 0.004
3rd trimester (n=17)	0.251 \pm 0.038	p=0.492	p=0.899	0.608 \pm 0.031	p=0.584	p* = 0.047
RPL patients (n=18)	0.233 \pm 0.024	p=0.791	p=0.752	0.857 \pm 0.044	p* = 0.004	p=0.064
Control (a) (n=11)	0.221 \pm 0.01	p=0.578	p=0.578	0.646 \pm 0.06	p=0.210	p=0.210
Control (b) (n=15)	0.246 \pm 0.019			0.735 \pm 0.01		

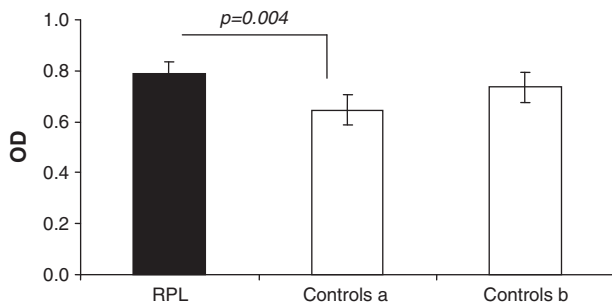


Fig. 4. Anti-fibrillin-1 IgM autoantibodies in the sera of RPL patients and non-pregnant control groups: (a) women who had already had at least one previous successful pregnancy, (b) women who had never been pregnant. The results are presented as mean values of the optical density (OD) \pm standard error (SE). The significant *p* value vs. non-pregnant control group (a) ($p < 0.05$) is shown.

to appear in response to initial exposure to antigen and usually reappear, to a less extent, after further exposure.

Ashworth et al [19], investigated the potential catabolic effects of six matrix metalloproteinases (MMP-2, MMP-3, MMP-9, MMP-12, MMP-13 and MMP-14) on fibrillin molecules and on intact fibrillin-rich microfibrils. Using newly synthesized recombinant fibrillin molecules, major cleavage sites within fibrillin-1 were identified – the six different MMPs generated a major degradation product of approximately 45 kDa from the N-terminal region of the molecule. According to the authors [19], degradation of fibrillin substrates by MMP-2, MMP-3 and MMP-13, which are mainly expressed by stromal cells constitutively or after growth factor induction, suggests that these enzymes may contribute to physiological fibrillin remodeling. The expression of MMP-2 and MMP-3 was also detected in human endometrial stromal cells cultured separately, as revealed by immunoblot, immunoprecipitation and northern analysis [20,21].

Autoantibodies against recombinant fibrillin-1 polypeptide were studied in humans affected by SSc or primary pulmonary hypertension syndrome [8,22]. Reactivity against recombinant polypeptides covering the N-terminal end (residues 15 to 193), the proline-rich region (residues 367 to 425), and a stretch of calcium-binding epidermal growth factor-like domains (residues 1326 to 1549) was investigated [9]. Sera from Caucasian SSc patients showed the presence of anti-fibrillin-1 antibodies in 42% of patients [9]. However, in a later study of Brinckmann et al. in 41 Caucasian patients with SSc, none of the sera showed positive reactivity against the recombinant polypeptide spanning either the N-terminal half or the C-terminal half of fibrillin-1 [23]. The data of the authors clearly showed that SSc in Caucasians was not characterized by the presence of autoantibodies against properly folded fibrillin-1. It is possible that the anti-fibrillin-1 autoantibodies recognize cryptic or misfolded antigenic epitopes, which may become available after proteolytic fragmentation of fibrillin-1. In this light, one could speculate that these autoantibodies are a secondary phenomenon following fibrillin fragmentation. This assumption is further substantiated by the data of another *in vitro* study indicating that the amount of fibrillin-1 in the extracellular matrix produced by SSc cells diminished faster than in the matrix of control cells, arguing for a higher susceptibility to proteolytic degradation [24].

If anti-fibrillin-1 antibodies are produced to degradation products, not to properly folded fibrillin-1, according to the above mentioned authors, it seems that according to our results, the highest level of fibrillin-1 degradation is detected in the control group of non-pregnant, nulligravida, cycling women – they had the highest levels of anti-fibrillin-1 IgM antibody among all study healthy groups. Actually, the endometrium undergoes dramatic tissue sloughing and remodeling during the menstrual cycle. Studies of primate endometrium show that several MMPs are highly expressed during menstruation, where the vast majority of tissue breakdown occurs

[25]. Generally, MMP-2 is expressed at relatively constant values throughout the entire menstrual cycle. During the proliferative phase, MMP-11 and MMP-7 are expressed in moderate abundance, while MMP-1, MMP-3, and MMP-9 are expressed at low levels focally [25].

According to our study, first trimester pregnant women had the same high levels of anti-fibrillin-1 IgM antibody as the mentioned control group which is an evidence for a similar level of fibrillin-1 degradation. This finding is in accordance with the number of studies of degradative properties of placental cytotrophoblast cells (CTB) during the implantation and pregnancy trimesters – the invasive activity peaks during the twelfth week of pregnancy and declines rapidly thereafter. Investigators have determined that human trophoblasts produce both MMP-2 and MMP-9 [26–28] with the highly invasive first trimester trophoblasts secreting greater amounts of both MMPs when compared with third trimester cells. During the first trimester, MMP-2 is expressed in extravillous trophoblast, whereas MMP-9 is mainly expressed in villous CTB [29], and *in vitro*, human CTB cells secrete MMP-2 and MMP-9 [30,31]. Experiments using a culture system of cytotrophoblasts isolated from first, second and third trimester human placentas plated on the basement membrane-like extracellular matrix produced by the PF HR9 teratocarcinoma cell line showed that degradative activity appeared to be unique to first trimester cytotrophoblast cells which express a number of MMPs that were not found in the cytotrophoblasts of the later gestational age [31].

We established significantly decreased serum levels of anti-fibrillin-1 IgM antibodies during the second and third trimester of pregnancy compared to the non-pregnant controls – a finding in accordance with the reported protective decrease of cytotrophoblast degradative activity after the placentation. Of course these first results and suggestions need further confirmation. For example, other explanations of decreased second and third trimester autoantibodies could come from the characteristics of the maternal immune response during pregnancy, i.e. the achieved immunological tolerance, the immunological masking, preventing the rejection of placenta and fetus, etc. However, serum anti-fibrillin-1 IgG antibodies did not differ significantly between pregnant and non-pregnant controls.

In the group of RPL patients we established significantly increased anti-fibrillin-1 autoantibodies compared to control group (a) – women who had already had at least one previous successful pregnancy. It is unclear whether this enhanced immune response plays a primary role in pregnancy loss pathogenesis or is a secondary phenomenon resulting from increased presentation of proteolytic fibrillin fragments in the decidual extracellular matrix. Skrzypczak et al. [32] established higher MMP-2 expression in 43% of the endometria from women with recurrent miscarriages compared to normal endometria from the control group. The investigators suggest that dysregulated TGF-beta, MMP-2 and MMP-9 expression are associated with infertility and early pregnancy loss. The question is: are the increased anti-fibrillin-1 IgM antibodies in RPL only a “mirror” of increased fibrillin degradation or they are already pathological autoantibodies which could further contribute to autoimmune reactions causing disturbances of the immunoregulation of pregnancy? One possible autoantibody induced mechanism added to the pathogenesis of pregnancy loss could be inappropriate complement activation since activated complement components are present in normal placentas in successful pregnancy [31]. The uncontrolled complement activation is prevented by regulatory proteins present on the trophoblast membrane but antigen–antibody reaction could cause complement activation and complement-mediated immune attack at the fetomaternal interface [33].

In conclusion, we could assume that even being in general colocalization in the structure of elastic fibers, elastin and fibrillin-1 slightly differ in their endometrial/decidual organization and expression, in their metabolism during normal pregnancy as well as in the autoantibody levels in normal pregnancy and RPL. These findings

need further confirmation by other methods and in larger study groups. Investigation of elastin and fibrillin turnover and autoimmunity during normal pregnancy and RPL is provocative and could be important for the clinical diagnosis and prognosis of patients with RPL as well as for new therapeutic approaches in these patients.

Take-home messages

- The established significantly decreased serum levels of anti-fibrillin-1 IgM antibodies during the second and third trimester of pregnancy compared to the non-pregnant controls may reflect the protective decrease of cytotrophoblast degradative activity after the placentation.
- Anti-fibrillin IgM autoantibodies were increased in patients with RPL and may contribute to the pathogenesis of pregnancy losses.
- Investigation of elastin and fibrillin-1 turnover and autoimmunity during normal pregnancy and RPL is provocative and could be important for the clinical diagnosis and prognosis of patients with RPL as well as for new therapeutic approaches in these patients.

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Genetic in RA at a glance: the shared epitope world

It is widely known that genetic factors contribute for an approximately 60% of the total risk for developing Rheumatoid Arthritis (RA). The strongest association has been found between RA and the HLA-DRB1 alleles that share the RAA (Arg, Ala, Ala) aminoacid motif that acts as a functional unit, the so called shared epitope (SE). These alleles have also been linked with the production of anti-citrullinated protein antibodies (ACPAs). Far to be fully explained, several authors reported that different SE alleles confer different risk of predisposition. The aminoacids that confer the highest risk are located at position 70 and 71 and are basic for the new classification system of the SE in RA that divides the alleles into S1, S2, S3P, S3D and denotes all non-RA motifs as X. This new classification by du Montcel has been validated for the susceptibility in large cohorts; on the contrary data are contrasting concerning the capability of distinguishing the predisposing and protective alleles for RA-specific antibody production. Recently, Gyetvai and coworkers (*Rheumatology (Oxford)*. 2010;49:25-33) examined the impact of S1, S2, S3P, S3D alleles on antibody production individually, using the X/X genotype as a reference. The authors found that not only S2 and S3P but also S1 and S3D allele predispose to the production of ACPAs, with the hierarchy S2+S3P>S1+S3D>X/X. These evidences stress the hypothesis that ACPA positive RA represents a distinct subgroup of disease, with a more severe behavior. The approach that was used, which is completely different from the previously adopted, allowed a better identification of the risk alleles associated with RA phenotype, and could be useful in future works in the identification of specific RA risk patterns and subgroups.