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GENETICS

The haplotype M2 of the *ANXA5* gene is not associated with antitrophoblast antibodies

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Abstract
Purpose The M2 haplotype in ANXA5 as well as antitrophoblast antibodies predispose to recurrent pregnancy loss (RPL). Since M2/ANXA5 can be a factor for development of antiphospholipid antibodies (aPL), this study aimed to trace a possible association of M2 with antitrophoblast antibodies.
Methods One hundred patients with two or more consecutive, idiopathic RPLs were divided in two subgroups, JEG-3^+ (n=42) and JEG-3^- (n=58), according to the anti-JEG-3 reactivity measured in subjects' sera. Both subgroups were genotyped for ANXA5 promoter haplotypes and genetic frequencies were compared to available fertile and control populations, as well as within the subgroups.
Results M2/ANXA5 was generally enriched in the JEG-3 screened cohort of RPL patients in comparison to fertile and population controls. Despite the relatively higher abundance of the haplotype in the JEG-3^- sample as compared to JEG-3^+ patients and in the JEG-3^- primary RPL subset in particular, compared to the rest of patients, there was no statistically significant difference between both, JEG-3^- and JEG-3^+ subgroups.
Conclusion It appears that the haplotype M2/ANXA5 is not associated with the presence of anti-trophoblast antibodies. Our finding indicates that anti-trophoblast antibodies are a class of molecules that differ from aPL and from anti-b2-GPI antibodies, apparently not directed to same or similar epitopes that aPL and anti-b2-GPI would recognize.

Keywords Anti-trophoblast antibodies · Annexin A5 (ANXA5) · JEG-3 · M2/ANXA5 · Recurrent pregnancy loss · Recurrent miscarriages

Capsule M2/ANXA5 carriage appears enriched in a subgroup of RPL patients with primary recurrent miscarriage and negative for antitrophoblast antibodies that is indicative of the haplotype's role as independent risk factor although the observed difference is not statistically significant.

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Introduction

Annexin A5 (ANXA5) is a protein abundantly expressed in placentae with a well-studied anticoagulant function in vitro and in vivo [29, 34]. Recent evidence shows that ANXA5, heavily enriched on the surface of syncytiotrophoblasts (SCT), forming lateral aggregates on the external leaflet of cell membranes, is essential for the membrane repair in living cells [4, 15]. Reduction of ANXA5 expression in placenta should be crucial for its required functions, possibly promoting less efficient repair and mediating procoagulation. Indeed, such reduction has been observed in thrombophila related complications of pregnancy, such as preeclampsia (PE) [5, 32], fetal growth restriction (FGR) [5, 33] and in non-pregnant women with previous recurrent pregnancy loss (RPL) [24]. A promoter haplotype of *ANXA5* identified in 2007 was demonstrated to downregulate ANXA5 mRNA levels [3, 17]. This haplotype named M2 has been shown to be predisposing to RPL in populations of Central Europe [3, 35] and lately also in the Japanese population [21].

Quite recently the haplotype M2 has been found enriched in patients with obstetric antiphospholipid syndrome (APS) [2] that agrees with previously documented decreased

abundance of the protein in APS patients [26]. Significantly lowered ANXA5 levels on the SCT surface may then free shielded phospholipid [25] or protein [6] antigenic determinants that would predispose the development of antiphospholipid antibodies (aPL) [26].

There is a variety of opportunities to induce immune reactions during trophoblast development and invasion in pregnancy [10]. Through the direct contact of fetal and maternal cells and tissues at the feto-maternal interface, as well as through the invasion of fetal cells and their migration in the extracellular matrix of maternal tissues, numbers of morphological sites are engaged in the immunological interaction of mother and fetus that may become involved in pathological situations. Immune reactions to the maternal trophoblast, associated with recurrent miscarriages, include the development of aPL [1, 8, 9, 23, 36] and of antibodies that cross-react with HLA-negative SCT surfaces [11, 12, 18, 19]. In addition it has been demonstrated that activation of SCT toll-like receptors recognizing pathogen associated molecular patterns of infects may induce preterm delivery [13].

The repertoire of individual SCT surface molecules involved in adverse immune reactions of pregnancy is largely unknown, but there is a growing tendency to characterize possible antigenic epitopes. A recent study involving the choriocarcinoma cell line JEG-3 as a test reagent expressing a wide range of trophoblast-specific antigens that are candidate targets of maternal antibodies, identified association of recurrent miscarriage with strong anti-JEG-3 activity in the sera of subjects with unexplained RPL [28].

Since carriage of the M2/ANXA5 haplotype has recently been linked to the development of aPL antibodies on the SCT surface [2], this study aimed to trace the possible role of M2 as a predisposing factor to antitrophoblast antibody generation.

Materials and methods

Study populations

The present study complied with the ethical guidelines of the institutions involved and was approved by the Review Board of the Ludwig-Maximilians University of Munich. Informed consent was obtained from all subjects examined. Included patient and control groups were of German extraction. As far as applicable, the criteria of strengthening the reporting of genetic association studies were observed.

One hundred subjects of German ancestry with available extracted genomic DNA were selected from 194 previously studied [28] patients with recurrent miscarriage before 20 week of gestation. They were recruited between 2004 and 2010 at the Recurrent Pregnancy Loss Clinic of the Division of Gynecological Endocrinology and Reproductive Medicine. All RPL patients were prescreened for any potential cause of their recurrent miscarriages as previously described [16, 28]: Uterine anomalies, endocrinologic dysfunctions (polycystic ovary syndrome according to the Rotterdam criteria [30], thyroidal dysfunctions, thyroid autoantibodies) were excluded, as well as parental chromosomal disorders (numerical and/or structural aberrations). Inherited thrombophilias (factor V-Leiden mutation (FVL), prothrombin (PTm) 20210G>A substitution) and deficiencies in anti-thrombotic factors (protein C, protein S, factor XII, anti-Thrombin) were ruled out. An APS was obviated according to the international consensus statement on an update of the classification criteria for defining antiphospholipid syndrome [20].

According to a previous publication by Rogenhofer et al., positive reactivity with JEG-3 cells was defined to be above the 95 % confidence interval of controls. In this connection a threshold of 39 % anti-JEG-3 reactivity in sera was estimated [28]. The flow-cytometrical measurements were taken between 6 and 8 weeks after the last miscarriage. RPL subjects were divided in high (JEG-3^{+}, median: 47.25 %, min-max: 39.4 %–99.9 %; n=42) and low (JEG-3^{-}, median: 8.7 %, min-max: 0 %–27.6 %; n=58) responder subgroups. Subgroups of RPL patients did not differ for biographic or obstetrical history (see Table 1). Besides, there was no difference regarding the time span between the latest miscarriage and serum collection between JEG-3 positive and JEG-3 negative patients.

DNA was extracted from white blood cells, using the QIAmp DNA blood mini kit (Qiagen, Hilden, Germany) and stored in 100 µl aliquots at −20 °C for further analyses.

Previously recruited control groups consisted of a fertile women cohort (n=500) from the registry of the Institute of Human Genetics, UKM Muenster, and a population control sample drafted from the PopGen biobank at UKSH Kiel (n=533) [3]. Muenster fertile controls had a history of normal pregnancies, > 1 normal term deliveries of healthy, normal weight singletons and without gestational pathology. PopGen population controls were from Northwest Germany, federal state of Schleswig-Holstein. This control group consisted of about equal numbers of healthy male and female subjects, identified through official population registers without specific data on fertility [14].

Determination of anti-trophoblast antibodies reactivity

Antitrophoblast antibodies activities in patients' sera were measured as previously described [28]. Briefly, serum samples (20 ml) were incubated with standardized suspensions of JEG-3 cells (10^5 cells) for 60 min at 4 °C. After washing two times in RPMI, cell suspensions were treated with 1:10 diluted FITC-conjugated goat anti-human secondary antibodies (Dako) for 60 min at 4 °C in the dark. After washing off the unbound signal, cell suspensions were analyzed on a

Table 1 Biographic and historic data of RPL patients with anti-trophoblast antibodies

	Subgroup 1[a] (n=42)	Subgroup 2 (n=58)	p-value
Age years mean (min-max)	32 (24–45)	35 (23–46)	n.s.
Pregancies n mean (min-max)	3 (2–7)	3 (2–9)	n.s.
Deliveries n mean (min-max)	0 (0–2)	0 (0–2)	n.s.
Miscarriages n mean (min-max)	3 (2–6)	3 (2–9)	n.s.
Early RPL≤12 weeks of gestation	36 (85.5 %)	51 (88 %)	
Late RPL 12–20 weeks of gestation	2 (5 %)	0 (0 %)	
Early and late RPL	4 (9.5 %)	7 (12 %)	
Primary RPL[b]	27 (64 %)	35 (60 %)	
Secondary RPL[c]	15 (36 %)	23 (40 %)	
Number of miscarriages			
2	18 (43 %)	21 (36 %)	
3	12 (28,5 %)	21 (36 %)	
4	7 (16.5 %)	7 (12 %)	
≥5	5 (12 %)	9 (16 %)	

n number, *%* percentage, *RPL* recurrent pregnancy loss, *n.s.* non significant)

[a] Subgroup 1, JEG-3$^+$ patients; subgroup 2, JEG-3$^-$ patients; [b] Primary RPL: no infant born either dead or alive after the 20th completed week of gestation and/or weighing more than 500 g before the series of miscarriages [WHO]; [31] [c] Secondary RPL: at least one infant born either dead or alive after the 20th completed week of gestation or weighing more than 500 g before the series of abortions [WHO] [31]

Beckton-Dickinson flow cytometer (FACScan) and reactivities were measured in mean channel shifts. Anti-JEG-3 reactivity was calculated as a percentage from the ratio of differences between the reactivities of the test sample and a low reacting standard over the difference of high and low reacting standards reactivity.

Genotyping and statistical analysis

Genotyping of extracted DNA was performed through direct amplicon Sanger sequencing as previously described [3]. Genotypes were scored in electronic table format and four digit coded for further processing. Additionally, the JEG-3 cell line was genotyped for the ANXA5 promoter haplotypes from extracted DNA as indicated for patients' DNA above. Departures from the Hardy–Weinberg equilibrium were assessed, using a MCMC implementation of an exact test, integral part of the Genepop package [27]. Odds ratios with 95 % confidence intervals were calculated using the FREQ procedure intrinsic to the SAS statistical software package V8 (SAS Institute, Cary, NC, USA). Differences of M2 carriage in JEG-3$^+$ and JEG-3$^-$ patient subgroups were assessed using the two-tailed Fisher's exact test. Statistical significance was interpreted at odds ratios of 2 (±0,25) and above, characteristic for the RPL populations genotyped so far [3, 17, 35]. The power of the analysis was set at 60 %, with significance level p<0,05.

Genetic backgrounds of population and fertile controls were previously examined using the STRUCTURE program [7, 22], essentially as described [3].

Results

The JEG-3 cell line, used as test target for the flow cytometric analysis of antitrophoblast antibodies reactivties was found to be of normal (N/N) genotype for the ANXA5 promoter alleles, confirming that annexin A5 expression in these cells would not be decreased due to carriage of M2. The association between M2 carriage and idiopathic RPL was first verified by comparing the 100 patients (see Table 2) with population and Muenster fertile controls. The RPL patient group was in the Hardy-Weinberg equilibrium for the ANXA5 haplotypes (MCMC P=0.874). The allele frequency (AF) of M2 (see Table 2) was found to be somewhat higher in patients (0.105) than among fertile (0.051) and population controls (0.082). Consequently carriers appeared to face a 2.2 times higher risk of RPL than non-carriers (odds ratio 2.8, 95 % confidence interval 1.15–5.2) compared to the fertile controls and a moderate risk of 1.3 (odds ratio 1.4, 95 % confidence interval 0.8–2.4) in comparison with the population controls.

In addition, possible differences in the distribution of the M2 haplotype were evaluated in the JEG-3$^-$ and JEG-3$^+$ patients, by comparing genetic frequencies with the fertile and population controls, as well as between the subgroups. JEG-3$^-$ and JEG-3$^+$ subgroups were both in the Hardy-Weinberg equilibrium for the ANXA5 haplotypes (MCMC P=0.368 and P=0.36, accordingly).

In JEG-3$^-$ patients the AF of M2 was 0.112 that consequently resulted in a relatively elevated risk trend of 2.7 (odds ratio 3.2, 95 % confidence interval 1.5–6.8) as compared to fertile controls and in a relative risk trend of 1.5

Table 2 Genotype frequencies of *ANX45* gene promoter haplotypes in German RPL patients with anti-trophoblast antibodies and two different control groups

Genotype	RPL patients all		RPL patients subgroup 1[a]		RPL patients subgroup 2		Muenster fertile controls		PopGen controls	
	Observed	Expected[b]	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
N/N	66 (66 %)	66.3	28 (66.7 %)	27.4	38 (65.6 %)	38.8	356 (71.2 %)	343.6	415 (77.9 %)	413.3
N/M1	13 (13 %)	13.1	7 (16.7 %)	6.6	6 (10.3 %)	6.6	87 (17.4 %)	99.5	35 (6.6 %)	47.8
M1/M1	1 (1 %)	0.6	0 (0 %)	0.3	1 (1.7 %)	0.2	16 (3.2 %)	7.2	1 (0.2 %)	1.5
N/M2, M1/M2[c]	19 (19 %)	18.9	6 (14.3 %)	7.4	13 (22.4 %)	11.6	31 (6.2 %)	48.4	77 (14.4 %)	69.0
M2/M2	1 (1 %)	1.1	1 (2.3 %)	0.3	0 (0 %)	0.8	10 (2 %)	1.4	5 (0.9 %)	1.4
Total	100	100	42	42	58	58	500	500	533	533

% percentage, *RPL* recurrent pregnancy loss)

[a] Subgroup 1, JEG-3+ patients; subgroup 2, JEG-3- patients; [b] Expected: genotype frequency expected at Hardy-Weinberg equilibrium, calculated with the Genepop package; [c]: genotype *M1/M2* was only observed in one patient, one Muenster and five PopGen controls

(odds ratio 1.6, 95 % confidence interval 0.8–3.2) in comparison to population controls. JEG-3$^+$ patients exhibited somewhat lower M2 AF of 0.095, relatively close to the AF noted in the general population (0.082), resulting in a relative risk trend of 2 (odds ratio 2.2, 95 % confidence interval 0.8–5.6) as compared to fertile controls. No statistically discernible relative risk trend (relative risk value of 1.01; odds ratio 1.1, 95 % confidence interval 0.4–2.7) was estimated when comparing the JEG-3$^+$ subgroup to the PopGen controls. The intergroup comparison JEG-3$^-$ vs. JEG-3$^+$ (two-tailed Fischer's exact test) indicated that despite the relatively lower M2 abundance in JEG-3$^+$ patients, there is no statistically significant difference in the haplotype distributions of both subgroups (p=0,455). Furthermore, the JEG-3$^-$ and the JEG-3$^+$ -subgroup were divided into patients with primary and secondary RPL to evaluate possible differences in the distribution of M2 carriage in these clinically relevant subsets. Among JEG-3$^+$ patients, there was no trend of observable M2 prevalence in any RPL category. In contrast, in JEG-3$^-$ patients with primary recurrent miscarriages an enrichment of M2 carriers could be assessed. Their relative risk was estimated with 1.3 and odds ratio with 1.9 (95 % confidence interval 0.5–8.9) versus the whole patient group.In comparison to fertile controls, relative risk for M2 carrying JEG-3$^-$ women with primary RPL was 3.4 (odds ratio 3.9, 95 % confidence interval 1.6–9.4) and 1.8 compared to population controls (odds ratio 1.9, 95 % confidence interval 0.8–4.4).

Discussion

As expected, the M2 haplotype was consistently rarer in both control groups, as compared to the 100 idiopathic RPL patients selected for this study. The resulting relative risk is moderate, when examined with population controls. However, considering the JEG-3$^-$ subgroup of patients only, the estimated risk value close to 2 is in agreement with previously assessed RPL risks of M2 carriers in populations of Central Europe [3, 35] and in the Japanese population [21]. In contrast, the JEG-3$^+$ sample exhibits considerably lower abundance of the haplotype, almost as low as in the general population, so that the noted slight enrichment is at the level of statistically tolerable difference. The last might be due at least in part to a positive ascertainment bias in the population controls that would potentially include aPL positive individuals, who were definitely excluded from the JEG-3 tested RPL cohort. On the other hand a negative ascertainment bias in the JEG-3$^+$ subgroup through an overwhelming effect of the antitrophoblast antibodies risk factor, if not associated with M2 carriage, could also be a plausible explanation.

The intergroup comparison of JEG-3$^-$ with JEG-3$^+$ patients indicates that although the noted higher relative abundance of the M2 haplotype in the JEG-3$^-$ is not statistically

significant, it is still remarkable. Such enrichment would be expected, when other potential causes of idiopathic RPL, including the presence of antitrophoblast antibodies, are excluded. The assumption of M2/ANXA5 being an independent risk factor for recurrent miscarriage from this study, was affirmed by the additional enrichment of M2 carriers in the subgroup of JEG-3^{-} patients with primary RPL, having in mind that these patients would certainly exhibit less frequently harmful antibodies generated within a successful pregnancy [11, 18, 28].

Another conclusion that can possibly be drawn from this M2 distribution among 'high' vs. 'low' activity JEG-3 responders is the non-importance of the haplotype for the generation of antitrophoblast antibodies, previously identified as RPL risk [28].

Since reduced abundance of ANXA5 on the SCT surface would be expected to 'unmask' phospholipid antigenic determinants [2], or to contribute to the enrichment on beta-2-glycoprotein (b2-GPI) epitopes [6], but probably not to block or interfere with the antigenicity of other surface molecules, this finding is in line with the proposed biological functions of this protein. It appears relevant that this is the first description of the JEG-3 cell line to have a normal (N/N) genotype for the ANXA5 promoter haplotypes. This result suggests expression levels of the protein not to be reduced due to genetically altered ANXA5 promoter alleles. Further, such normal ANXA5 levels would not predispose to exposure of phospholipid [2] and/or protein [6] antigenic determinants. Altogether, our finding indicates that antitrophoblast antibodies are a class of molecules that differ from aPL and from anti-b2-GPI antibodies, apparently not directed to same or similar epitopes that aPL and anti-b2-GPI would recognize.

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Conflict of interest The Authors wish to declare no conflict of interest.

References

1. Bennett SA, Bagot CN, Arya R. Pregnancy loss and thrombophilia: the elusive link. Br J Haematol. 2012;157:529–42.
2. Bogdanova N, Baleva M, Kremensky I, Markoff A. The annexin A5 protective shield model revisited: inherited carriage of the M2/ANXA5 haplotype in placenta as a predisposing factor for the development of obstetric antiphospholipid antibodies. Lupus. 2012;21:796–8.
3. Bogdanova N, Horst J, Chlystun M, Croucher PJ, Nebel A, Bohring A, et al. A common haplotype of the annexin A5 (ANXA5) gene promoter is associated with recurrent pregnancy loss. Hum Mol Genet. 2007;16:573–8.
4. Bouter A, Gounou C, Bérat R, Tan S, Gallois B, Granier T, et al. Annexin-A5 assembled into two-dimensional arrays promotes cell membrane repair. Nat Commun. 2011;2:270.
5. Chinni E, Tiscia GL, Colaizzo D, Vergura P, Margaglione M, Grandone E. Annexin V expression in human placenta is influenced by the carriage of the common haplotype M2. Fertil Steril. 2010;91:940–2.
6. de Laat B, Wu XX, van Lummel M, Derksen RH, de Groot PG, Rand JH. Correlation between antiphospholipid antibodies that recognize domain I of beta2-glycoprotein I and a reduction in the anticoagulant activity of annexin A5. Blood. 2007;109:1490–4.
7. Falush D, Stephens M, Pritchard JK. Inference of population structure: extensions to linked loci and correlated allele frequencies. Genetics. 2003;164:1657–87.
8. Galarza-Maldonado C, Kourilovitch MR, Pérez-Fernández OM, Gaybor M, Cordero C, Cabrera S, et al. Obstetric antiphospholipid syndrome. Autoimmun Rev. 2012;11:288–95.
9. Hughes G. Hughes Syndrome: the antiphospholipid syndrome–a clinical overview. Clin Rev Allergy Immunol. 2007;32:3–12.
10. Huppertz B, Berghold VM, Kawaguchi R, Gauster M. A variety of opportunities for immune interactions during trophoblast development and invasion. Am J Reprod Immunol. 2012;67:349–57.
11. Johnson PM, Barnes RM, Hart CA, Francis WJ. Determinants of immunological responsiveness in recurrent spontaneous abortion. Transplantation. 1984;38:280–4.
12. Kishore R, Agarwal S, Halder A, Das V, Shukla BR, Agarwal SS. HLA sharing, anti-paternal cytotoxic antibodies and MLR blocking factors in women with recurrent spontaneous abortion. J Obstet Gynaecol Res. 1996;22:177–83.
13. Koga K, Cardenas I, Aldo P, Abrahams VM, Peng B, Fill S, et al. Activation of TLR3 in the trophoblast is associated with preterm delivery. Am J Reprod Immunol. 2009;61:196–212.
14. Krawczak M, Nikolaus S, von Eberstein H, Croucher PJP, El Mokhtari NE, Schreiber S. PopGen: population-based recruitment of patients and controls for the analysis of complex genotype-phenotype relationships. J Commun Genet. 2006;9:55–61.
15. Krikun G, Lockwood CJ, Wu XX, Zhou XD, Guller S, Calandri C, et al. The expression of the placental anticoagulant protein, annexin V, by villous trophoblasts: immunolocalization and in vitro regulation. Placenta. 1994;15:601–12.
16. Li TC, Makris M, Tomsu M, Tuckerman E, Laird S. Recurrent miscarriage: aetiology, management and prognosis. Human Reprod Update. 2002;8:463–81.
17. Markoff A, Gerdes S, Feldner S, Bogdanova N, Gerke V, Grandone E. Reduced allele specific annexin A5 mRNA levels in placentas carrying the M2/ANXA5 allele. Placenta. 2010;31:937–40.
18. McIntyre JA, Faulk WP. Recurrent spontaneous abortion in human pregnancy: results of immunogenetical, cellular and humoral studies. Am J Reprod Immunol. 1983;4:165–70.
19. McIntyre JA, Faulk WP, Nichols-Johnson VR, Taylor CG. Immunological testing and immunotherapy in recurrent spontaneous abortion. Obstet Gynecol. 1986;67:169–75.
20. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost. 2006;4:295–06.
21. Miyamura H, Nishizawa H, Ota S, Suzuki M, Inagaki A, Egusa H, et al. Polymorphisms in the annexin A5 gene promoter in Japanese women with recurrent pregnancy loss. Mol Hum Reprod. 2011;17:447–52.
22. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000;155:945–59.
23. Quenby S, Farquharson RG, Dawood F, Hughes AM, Topping J. Recurrent miscarriage and long-term thrombosis risk: a case–control study. Hum Reprod. 2005;20:1729–32.
24. Rand JH, Arslan AA, Wu XX, Wein R, Mulholland J, Shah M, et al. Reduction of circulating annexin A5 levels and resistance to annexin A5 anticoagulant activity in women with recurrent spontaneous pregnancy losses. Am J Obstet Gynecol. 2006;194:182–8.

25. Rand JH, Wu XX. Antibody-mediated disruption of the annexin-V antithrombotic shield: a new mechanism for thrombosis in the antiphospholipid syndrome. Thromb Haemost. 1999;82:649–55.
26. Rand JH, Wu XX, Guller S, Scher J, Andree HA, Lockwood CJ. Antiphospholipid immunoglobulin G antibodies reduce annexin-V levels on syncytiotrophoblast apical membranes and in culture media of placental villi. Am J Obstet Gynecol. 1997;177:918–23.
27. Raymond M, Rousset F. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Heredity. 1995;86:248–9.
28. Rogenhofer N, Ochsenkühn R, Von Schönfeldt V, Assef RB, Thaler CJ. Anti-trophoblast antibodies associated with recurrent miscarriages. Fertil Steril. 2012;97:361–6.
29. Romisch J, Seiffge D, Reiner G, Paques EP, Heimburger N. In-vivo antithrombotic potency of placenta protein 4 (annexin V). Thromb Res. 1991;61:93–104.
30. Rotterdam ESHRE / ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril. 2004;81:19–25.
31. Rowe P, Comhaire F, Hargreave T, Mellows H. WHO manual for the standardized investigation and diagnosis of the infertile couple. World Health Organization, Cambridge University Press. 1993.
32. Shu F, Sugimura M, Kanayama N, Kobayashi H, Kobayashi T, Terao T. Immunohistochemical study of annexin V expression in placentae of preeclampsia. Gynecol Obstet Invest. 2000;49:17–23.
33. Sifakis S, Soufla G, Koukoura O, Soulitzis N, Koutroulakis D, Maiz N, et al. Decreased annexin A5 mRNA placental expression in pregnancies complicated by fetal growth restriction. Thromb Res. 2010;125:326–31.
34. Thiagarajan P, Tait J. Binding of annexin V/placental anticoagulant protein I to platelets. Evidence for phosphatidylserine exposure in the procoagulant response of activated platelets. J Biol Chem. 1990;265:17420–3.
35. Tiscia G, Colaizzo D, Chinni E, Pisanelli D, Sciannamè N, Favuzzi G, et al. Haplotype M2 in the annexin A5 (ANXA5) gene and the occurrence of obstetric complications. Thromb Haemost. 2009;102:309–13.
36. Vinatier D, Dufour P, Cosson M, Houpeau JL. Antiphospholipid syndrome and recurrent miscarriages. Eur J Obstet Gynecol Reprod Biol. 2001;96:37–50.