

The antiphospholipid syndrome in pregnant rabbits and their offspring. Neuropathological aspects

Przemysław Nowacki¹, Jadwiga Ossowicka-Stępińska², Elżbieta Ronin-Walknowska², Justyna Tabaka¹

¹Department of Neurology, ²Department of Feto-Maternal Medicine, Pomeranian Medical University, Szczecin, Poland

Folia Neuropathol. 2005; 43 (1): 15-22

Abstract

Objective: Although the obstetric consequences of antiphospholipid syndrome (APS) in pregnant rabbits have been described, there are no data on the serological and neuropathological aspects of the syndrome in their offspring. It would also be interesting to recognize whether the CNS abnormalities in rabbit fetuses relate to placental damage or depend on the antiphospholipid antibodies, transmitted from the pregnant animal through the placenta to the fetal serum.

Material and methods: A post-mortem neuropathological examination was done on 36 adult female New Zealand rabbits, and their offspring (100 fetuses). The material was divided into 4 groups: Group I – 26 pregnant rabbits with experimental APS, Group IC 10 – pregnant rabbits without APS (control group I), Group II – 64 fetuses derived from animals included in Group I, and Group IIC – 36 fetuses derived from individuals included in Group IC (control group II). The platelet count, activated partial thromboplastin time (APTT), antiplatelet antibodies in serum and coated on the platelets were evaluated to identify the APS in adult rabbits and their offspring.

Results: A significantly higher number of fetuses demonstrating weaker vitality and shorter survival time was observed in Group II. The percentage of dead and reabsorbed fetuses was also considerably higher in Group II. The serum markers of APS occurred both in Group I and II while the neuropathological evidences of APS: the thrombo-necrotic and inflammatory changes were found exclusively in APS pregnant animals. Moreover, cytoarchitecture of the fetal brains was intact. There were no disturbances in neuronal migration and abnormalities of cytodifferentiation.

Conclusions: 1. The antiphospholipid syndrome in pregnant rabbits results in serological markers of the syndrome in their offspring. 2. The central nervous system of fetuses delivered from pregnant rabbits with the antiphospholipid syndrome remains intact despite the serological markers of the syndrome in fetus circulation. 3. The miscarriages in pregnant rabbits with the antiphospholipid syndrome depend rather on placental pathology related to the syndrome than on the syndrome per se transmitted from adult females to fetal circulation.

Key words: antiphospholipid syndrome, rabbit fetuses, neuropathology

Communicating author:

Professor Przemysław Nowacki, Department of Neurology, Pomeranian Medical University, Unii Lubelskiej 1, 71-252 Szczecin, Poland, tel. +48 91 425 32 51, fax +48 91 425 32 60, e-mail: nowacprz@sci.pam.szczecin.pl

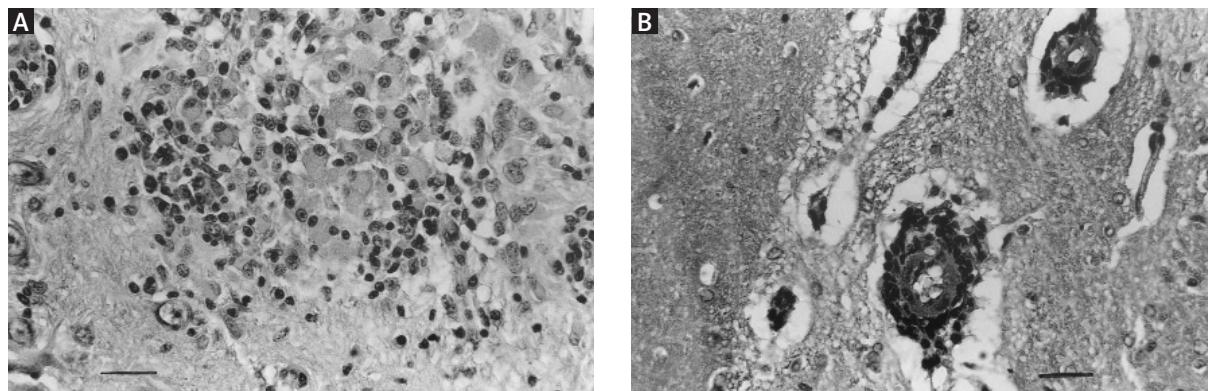


Fig. 1. The necrotic focus (A) and perivascular infiltrates (B) within the white matter of the APS pregnant rabbit. Hematoxylin and eosin. The bars indicate 15 μ m

Introduction

In our series of reports on experimental model of antiphospholipid syndrome (APS) in pregnant rabbits, we described an influence of the APS on the central nervous system (CNS) in adult animals with special reference to the time after immunization. We have previously suggested that two main types of morphological changes may be evoked by the APS: thrombonecrotic and inflammatory. The former probably results from extent vasculopathy related to APS [14,15]. The active inflammatory reaction and

thrombonecrotic changes gradually diminished after immunization had been finished [15]. Vasculopathy, both in the CNS and other organs is generally accepted to be a main factor leading to APS clinical manifestations [3,17,22]. The APS may cause thrombotic macro- and microangiopathy within many organs resulting in renal and liver failure, pulmonary hypertension, valve abnormalities, thrombocytopenia, and various neurological complications – migraine episodes, transient ischemic attacks, ischemic stroke, choreatic movements [1,2,7,8,11]. Since its first reports the APS has been known predominantly among obstetricians [5,6,10,12]. The association of the APS with recurrent miscarriages is one of the most constant. The spontaneous abortions among young women and low fetal mass probably depend on thrombotic, embolic and necrotic changes within the placenta, related to APS [13,19]. High level circulating IgG and IgM antiphospholipids (aPL) may cause pregnancy loss in 80% of cases [9].

Although the obstetric consequences of the APS in pregnant rabbits have been described, there are no data on the serological and neuropathological aspects of the syndrome in their offspring. It would also be interesting to recognize whether the CNS abnormalities in rabbit fetuses relate to placental damage or depend on the aPL transmitted from the pregnant animal through the placenta to the fetal serum.

Material and methods

A post-mortem neuropathological examination was done on 36 adult female New Zealand rabbits, aged 5-6 months, 3900-4600 g of body weight and

Table I. General data concerning the APS in the rabbits' offspring

General data	Group II	Group IIC
living	68.5%	93.1%
dead	12.3%	3.45%
reabsorbed	19.2%	3.45%
vitality scale		
I°	49.1%	16.7%
II°	20.7%	42.6%
III°	30.2%	40.7%
survival time (minutes)		
15	17%	1.9%
30	17%	5.5%
45	66%	92.6%

Group II/IIC $p < 0.001$ in all parameters

their offspring (100 fetuses). The material was divided into 4 groups: Group I – 26 pregnant rabbits with experimental APS, Group IC – 10 pregnant rabbits without APS (control group I), Group II – 64 fetuses derived from animals included in Group I, and Group IIC – 36 fetuses derived from individuals included in Group IC (control group II). The APS was evoked by subcutaneous injection of cardiolipin in an increasing dose from 0.3 to 0.4 ml (2250-3000 µg) with adjuvant (2 % solution of aluminum hydroxide) mixed 1:1. Pregnancies were terminated on the 30th day by means of the caesarean section under general anesthesia with Vetbutal. The platelet count (PLT) (Coulter STKS adapted for rabbit blood analysis), the activated partial thromboplastin time (APTT) (Hemolab Silimat), antiplatelet antibodies in serum and coated on the platelets (IF – immunofluorescence test) were evaluated to identify the APS in adult rabbits and their offspring. The intensity of IF was described as distinctively positive (++), positive (+) and negative (-). Only living and vital fetuses were taken into consideration to avoid the influence of other factors than the APS on fetal brain. The procedure of experimental APS in our animals has been previously described [16,20]. The formalin-fixed, paraffin-embedded brain specimens were examined per case. All specimens were stained with hematoxylin and eosin (HE), van Gieson, and Heidenhain's methods. Immunohistochemical evaluations were carried out with GFAP and factor VIII-related antigen.

An image-computerized analysis was made by means of morphological material scanned in light microscope and a two-tube color television camera. The illumination source was a 100 W halogen bulb. The Achroplan 20 x and Plan-Neofluar 40 x objectives were used for scanning and measurements. Each scanned image comprised 0.0096 mm² and 0.0048 mm² for magnification at 20 and 40x, respectively. Five to eight images were scanned from consecutive areas: 1/ frontal and occipital cortex, 2/ hippocampal formation comprising CA1 and CA3 fields, 3/ midbrain structures, including: globus pallidus and thalamus, 4/ cerebellar cortex. For analysis the images were defined on gray (brightness) scale. A gray value of 0 represented black (maximal density), a gray value of 255 represented white (minimal density). The distribution of values in scanned areas (including background and neurons) along the x coordinate was examined by means of the function 'PROFILE'. The PROFILE curve consists of

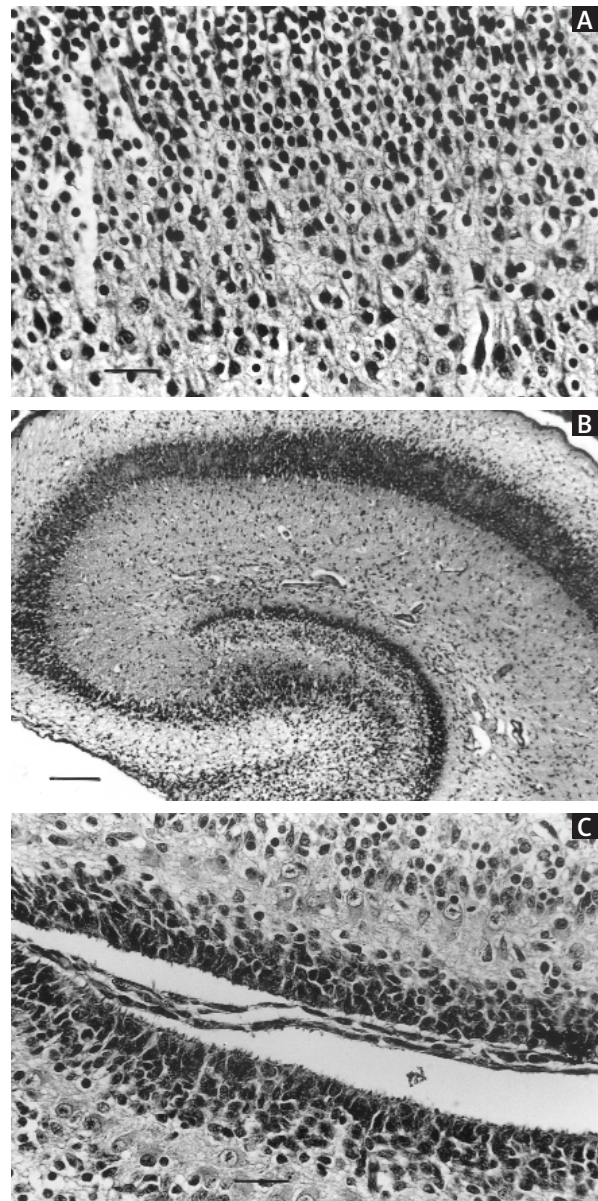


Fig. 2. The normal appearance of frontal cortex (A), hippocampal structures (B) and cerebellar cortex in the fetus delivered from the APS female. Hematoxylin and eosin. The bars indicate 30 µm

three true color components: – red, green, and blue (RGB). Each color component is also described by means of corresponding numerical value (0-255) measured for every pixel between the first and the end point of the PROFILE. The PROFILE procedure allows us to estimate the concentration of neurons with relation to the background of the measured area

Table II. The serum markers of the APS in pregnant rabbits and their offspring

	Group I	Group IC	Group II	Group IIC
PLT (10 ³) mean ± SD	162.5±50.3	464.3±32.3	296.4±43.6	362.5±68.5
APTT (s) mean ± SD	130.6±43.4	42.3±3.1	68.4±24.5	44.7±10.4
IF	++	0	2	0
	+	4 } 10	1 } 8	0 } 8
	-	0	5	8

PLT Group I/IC $p < 0.001$ Group II/IIC $p < 0.05$
 APTT Group I/IC $p < 0.001$ Group II/IIC $p < 0.01$

Table III. The neuropathological markers of the APS in pregnant rabbits and their offspring

	Group I	Group IC	Group II	Group IIC
thrombo-necrotic changes	14/23* 61%	0/10*	0/62*	0/24*
perivascular infiltrates	18/29 78.3%	0/10	0/62	0/24
meningeal infiltrates	6/23 26.1%	0/10	0/62	0/24

* The number of investigated animals in the group.

(amplitude of waves). The morphometric analysis was made by means of KONTRON imaging system KS-100, v. 2.0 (license number 0100176).

Statistical differences between PROFILE numerical values were determined by means of the Student's t-test and Fisher's F test (one way analysis of variance). Statistical differences between groups were analyzed by chi-squared test with a value of $p < 0.05$ and with 95% confidence intervals.

Consent to the experiments on rabbits was obtained from the Szczecin Ethical Board for Animal Experiments.

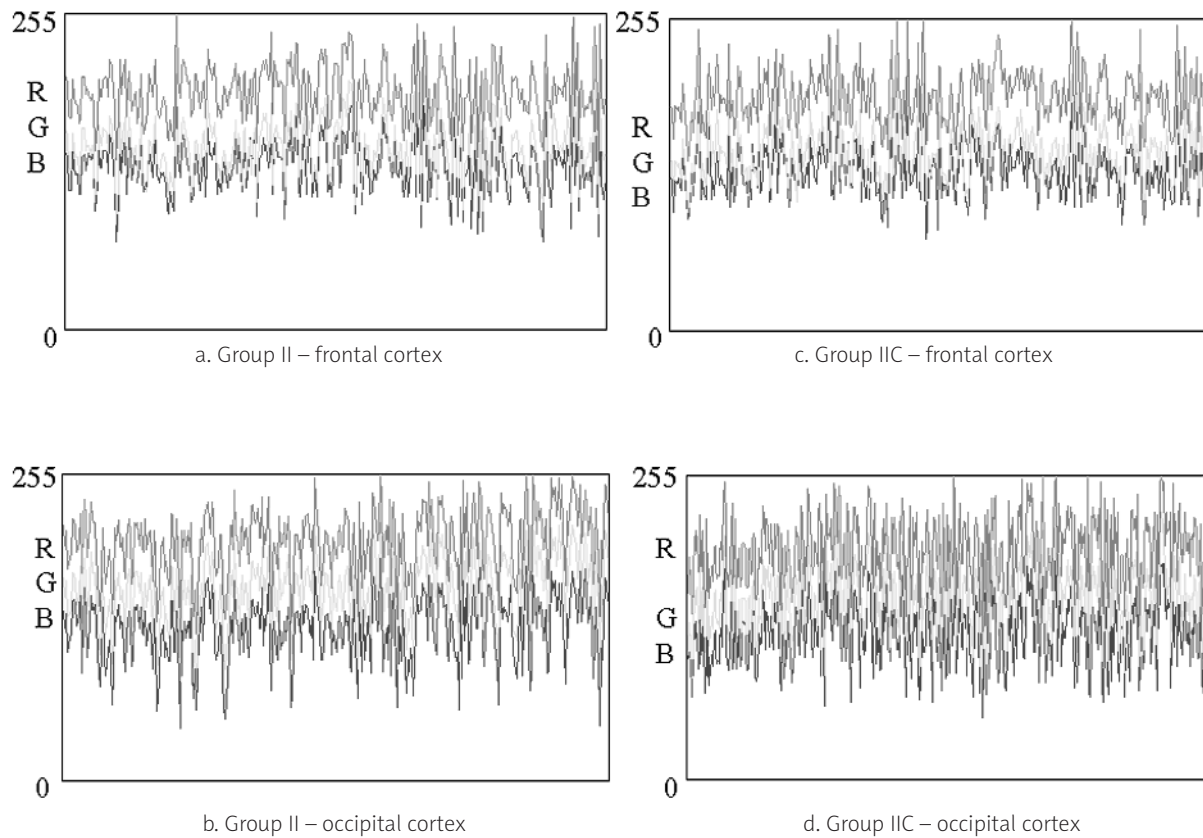
Results

The general data on offspring delivered from pregnant rabbits with experimental APS is presented in Table I. A significantly higher number of fetuses demonstrating weaker vitality and shorter survival time was observed in animals of Group II. The percentage of dead and reabsorbed fetuses was also considerably higher in this group.

The serum markers of the APS were found both in pregnant animals (Group I) and in their fetuses (Group

II). Immunized pregnant rabbits and their offspring demonstrated a significant decrease of mean PLT count and elongation of APTT. The antiplatelet antibodies IgM and IgG class, detected by immunofluorescence test (IF) in the blood serum and coated on platelets were also exclusively observed in APS animals, both adult and fetuses (Table II).

A macroscopic appearance of the brains of pregnant rabbits and their living fetuses appeared to be normal. There were two forms of microscopic changes within the brains, reflecting the APS: thrombo-necrotic and inflammatory ones. The former consisted of necrotic foci and macrophage clusters surrounded by mononuclear cells (Fig. 1a). The inflammatory component was characterized by small perivascular infiltrates consisting of lymphocytes, predominantly located within the white matter of brain hemispheres, brain stem and cerebellum (Fig. 1b). The infiltrates were also observed in meninges. The neuropathological changes within the CNS, related to the APS were described in our previous paper [14]. These abnormalities were exclusively found within the brains of pregnant rabbits, whereas they were totally absent in fetal brains (Table III). Also cytoarchitecture of fetal



* Differences between PROFILE values shown in Fig. 3a and 3c, and between PROFILE values shown in Fig. 3b and 3d statistically non-significant ($p>0.05$)

	R	G	B		R	G	B
Fig. 3a	mean=204.3 SD ± 25.34	mean=176.3 SD ± 37.35	mean=148.2 SD ± 33.11	Fig. 3c	mean=196.6 SD ± 32.12	mean=163.5 SD ± 34.25	mean=145.3 SD ± 32.87
Fig. 3b	mean=219.3 SD ± 32.26	mean=183.3 SD ± 34.54	mean=159.6 SD ± 32.44	Fig. 3d	mean=226.3 SD ± 33.27	mean=176.3 SD ± 33.22	mean=152.3 SD ± 32.86

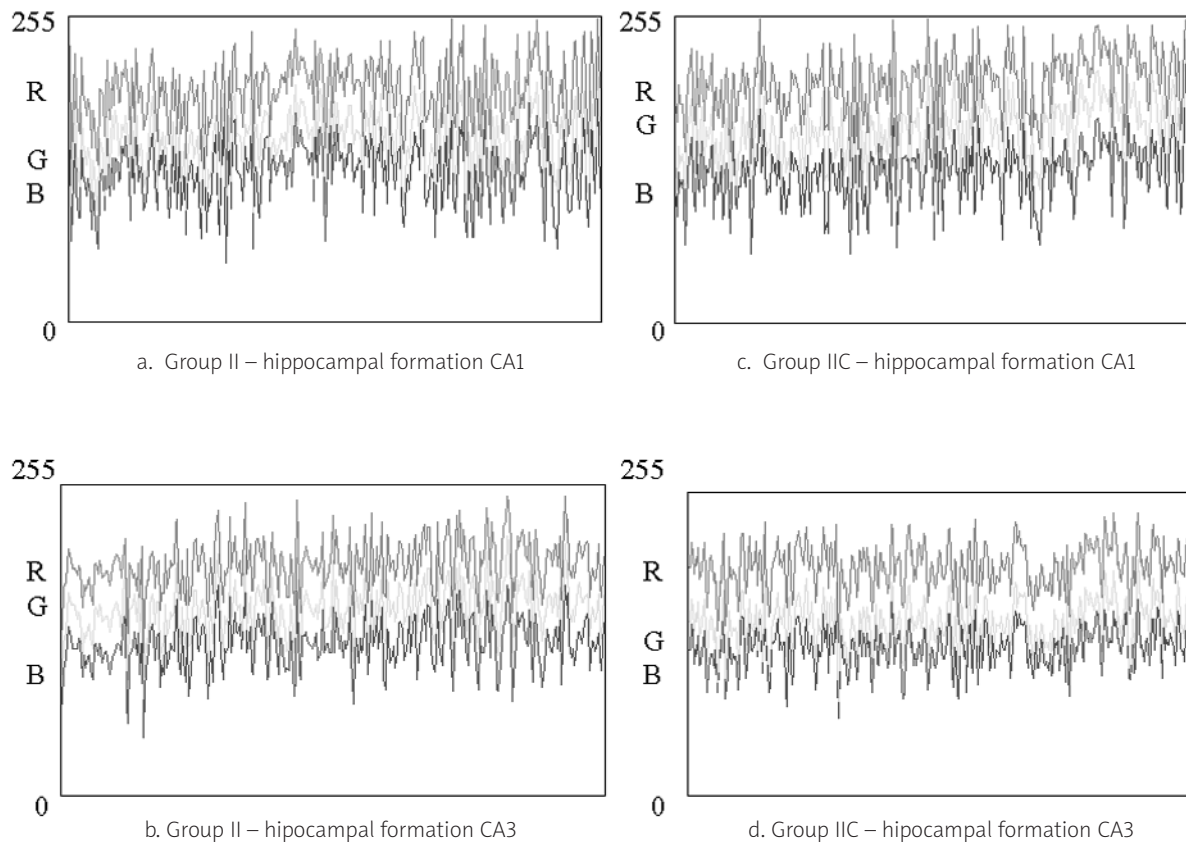
Fig. 3. Distribution of PROFILE values along x coordinate of frontal and occipital cortex in fetuses derived from the APS pregnant rabbits (Group II) and non-APS pregnant rabbits (Group IIC)

brains was not involved – its appearance was found to be normal, relatively to physiological maturation (Fig. 2a, b, c). There were no disturbances in neuronal migration and abnormalities of cytodifferentiation within the examined areas. The PROFILE curves demonstrated no significant differences in distribution and concentration of neurons. The selected examples of PROFILE curves and values within cortical and hippocampal structures were shown in Fig. 3 and 4. Contrary to APS pregnant rabbits there was no vasculopathy within the brains of their offspring.

Discussion

The APS is characterized by coexistence of aPL and/or lupus anticoagulant with thrombo-embolic episodes resulting in many organs involvement, including recurrent pregnancy loss [4].

The APS in our adult animals appeared to influence their offspring. The APS pregnant rabbits demonstrated a considerably higher percentage of dead and reabsorbed fetuses comparing with non-APS animals. Considering the APS pathogenesis in pregnant rabbits



* Differences between PROFILE values shown in Fig. 4a and 4c, and between PROFILE values shown in Fig. 4b and 4d statistically non-significant ($p>0.05$)

	R	G	B		R	G	B
Fig. 4a	mean=227.3 SD ± 52.51	mean=184.3 SD ± 38.3	mean=157.3 SD ± 32.34	Fig. 4c	mean=232.6 SD ± 17.9	mean=193.2 SD ± 22.6	mean=147.3 SD ± 24.7
Fig. 4b	mean=223.4 SD ± 3.8	mean=177.3 SD ± 10.2	mean=159.3 SD ± 9.22	Fig. 4d	mean=230.7 SD ± 6.36	mean=185.2 SD ± 10.56	mean=163.4 SD ± 10.23

Fig. 4. Distribution of PROFILE values along x coordinate of hippocampal formations CA1 and CA3 in fetuses derived from the APS pregnant rabbits (Group II) and non-APS pregnant rabbits (Group IIC)

and their offspring, the main attention should be focused on placental pathology. Many abnormalities were described in the placenta in the APS individuals. Decidua presents necrosis, acute and chronic inflammation, and vascular thrombo-embolic changes. Intravascular fibrin depositions, syncytial knot formation, and fibrosis are also observed [22,25]. Histopathological abnormalities within the placenta of the APS animals were found in our rabbits as well. There were thrombo-necrotic changes, congestions, perivascular bleedings, calcifications, and diminished

trophoblast proliferation activity [18]. Also other authors emphasize the role of placental trophoblast in the pathophysiology of the APS [21].

Based on the mentioned placental changes we could expect the APS pregnant animal fetuses to demonstrate the brain damage. There could be two possible ways to develop probable damage to the CNS: directly related to the APS transmitted to fetuses or indirect CNS involvement resulting from thrombo-necrotic destruction of the placenta. All

examined fetuses delivered from the APS rabbits demonstrated the APS serological evidence: elongation of APTT, decrease of PLT count and appearance of aPL in IgG and IgM type. It gives evidence that antibodies may penetrate through the placenta to the fetuses. It is difficult to answer whether they are able to penetrate through normal or primarily damaged placenta, because all examined placentas taken from the APS female appeared to be abnormal [18]. In pregnant mice human IgG and anti-La autoantibodies were transported efficiently into the fetal circulation. Organ-specific IgG binding was found in the fetal heart, skin, liver, and bone [24]. The thrombo-embolic changes and mild inflammatory infiltrates are common phenomena for the APS, resulting from antiphospholipid autoantibodies presence within the organs [14,15,23]. The thrombo-necrotic and inflammatory changes resulting from the APS have been exclusively observed in APS pregnant animals. Although we detected serum markers of the APS in fetuses, we did not find any neuropathological evidence of the APS within the fetal brains. Moreover, the cytoarchitecture of the fetal brains was intact. Neither the retardation in the brain development nor heterotopies were found. Based on neuropathological investigations it seems that the aPL antibodies and other serological markers of the APS in fetal serum are not able to cause damage to the brain. In experimental model of the APS, transplacental anti-La/SSB antibodies did not bind to apoptotic cells in the fetal thymus, lung, brain, or gut [24]. Maybe the lack of the APS neuropathological markers results from immature immunocompetence of fetal rabbit CNS. There was significantly lower percentage of living fetuses with normal vitality among offspring of the APS pregnant rabbits. In the light of the above-described results, the pathophysiological mechanisms that lead to high rates of intrauterine fetal death and reabsorption seem to be rather associated with placental pathology than fetal APS occurrence.

Conclusions

1. The antiphospholipid syndrome in pregnant rabbits results in serological markers of the syndrome in their offspring.
2. The central nervous system of fetuses delivered from pregnant rabbits with the antiphospholipid syndrome remains intact despite the serological markers of the syndrome in fetus circulation.

3. The miscarriages in pregnant rabbits with the antiphospholipid syndrome depend rather on placental pathology related to the syndrome than on the syndrome *per se* transmitted from adult females to fetal circulation.

References

1. Amigo MC, Garcia-Torres R, Robles M. Renal involvement in primary antiphospholipid syndrome. *J Rheumatol* 1992; 19: 1181-1185.
2. Asherson RA, Mackworth-Young CG, Boey ML, Harris EN, Hughes GR. Pulmonary hypertension in SLE: a report of 3 cases. *J Rheumatol* 1986; 12: 416-418.
3. Cherg SC, Wang YF, Fa YM, Yan SP, Huang WS. Iliofemoral vein thrombosis and pulmonary embolism associated with a transient ischemic attack in a patient with antiphospholipid syndrome. *Clin Nucl Med* 2001; 26: 84-85.
4. Cuadrado MJ, Lopez-Pedraza C. Antiphospholipid syndrome. *Clin Exp Med* 2003; 3: 129-139.
5. Derksen M, De Groot G, Niewenhuis HK, Christiaes GC. How to treat women with antiphospholipid antibodies in pregnancy. *Ann Rheum Dis* 2001; 60: 1-3.
6. Derue GJ, Englert JH, Harris EN. Fetal loss in systemic lupus: association with anticardiolipin antibodies. *J Obstet Gynecol* 1985; 5: 207-209.
7. Harris EN, Gharavi AE, Hegde U. Anticardiolipin antibodies in autoimmune thrombocytopenic purpura. *Brit J Haematol* 1985; 59: 231-234.
8. Hess DC. Models for central nervous system complications of antiphospholipid syndrome. *Lupus* 1994; 3: 253-257.
9. Hughes GR. The antiphospholipid syndrome: ten years on. *The Lancet* 1993; 342: 341-344.
10. Kim J, Smith KJ, Toner C, Skelton H. Delayed cutaneous reactions to heparin in antiphospholipid syndrome during pregnancy. *J Int J Dermatol* 2004; 43: 252-260.
11. Kinkaid-Smith P, Fairley KE, Kloss M. Lupus anticoagulant associated with renal thrombotic microangiopathy and pregnancy-related renal failure. *Q J Med* 1988; 69: 795-815.
12. Kondo A, Shida M, Suzuki T, Izumi SI, McIntyre JA, Makino T. Anti-annexin A5 antibodies in reproductive failures in relation to antiphospholipid antibodies and phosphatidylserine. *Am J Reprod Immunol* 2003; 50: 202-208.
13. Lakasing L, Campa JS, Parmar K, Poston R, Hunt BJ, Poston L. Normal expression of cell adhesion molecules in placenta from women with systemic lupus erythematosus and the antiphospholipid syndrome. *Placenta* 2000; 21: 142-149.
14. Nowacki P, Ronin-Walknowska E, Ossowicka-Stępińska J. Central nervous system involvement in pregnant rabbits with experimental model of antiphospholipid syndrome. *Folia Neuropathol* 1998; 36: 38-44.
15. Nowacki P, Ronin-Walknowska E, Ossowicka-Stępińska J. Neuropathological changes within the brain of rabbits with experimental model of antiphospholipid syndrome in different time after immunization. *Folia Neuropathol* 1999; 37: 269-272.
16. Ossowicka-Stępińska J, Engel-Pietrzak K, Ronin-Walknowska E. Therapeutic application of TFX and ubiquitin complex in

- pregnant rabbits with antiphospholipid syndrome. Intern J Thymol 1999; 7: 596-600.
17. Pamuk ON, Cakir N, Soy M, Aktoz M, Celik Y, Akdemir O. Mitral valve vegetation and cerebral emboli in a primary antiphospholipid syndrome patient who had hepatitis C virus infection: Report of a case and review of the literature. Clin Rheumatol 2003; 22: 136-139.
 18. Parafiniuk M, Ossowicka-Stepińska J, Ronin-Walknowska E. Influence of TUC (thymus ubiquitin complex) – Jelfa on the rabbit placentas morphology in experimentally induced antiphospholipid syndrome. A Perin Medicine 2002; 8: 10-15.
 19. Pierangeli S, Barker J, Sticovac D, Ackerman D, Anderson G, Barquinero J, Acland R, Harris EN. Effect of human IgG antiphospholipid antibodies on an *in vivo* thrombosis model in mice. Haemost 1994; 71: 670-674.
 20. Ronin-Walknowska E, Ossowicka-Stepińska J, Kucharska E, Skoczowska M, Czajkowska M. The experimental antiphospholipid syndrome in female rabbits. Int J Thymology 1998; 6: 545-551.
 21. Rote, NS, Vogt E, De Vere G, Obringer AR, Ng AK. The role of placental trophoblast in the pathophysiology of the antiphospholipid antibody syndrome. Am J Reprod Immunol 1998; 39: 125-136.
 22. Sebire NJ, Backos M, El Gaddal S, Goldin RD, Regan L. Placental pathology, antiphospholipid antibodies, and pregnancy outcome in recurrent miscarriage patients. Obstet Gynecol 2003; 101: 258-263.
 23. Shoenfeld Y, Ziporen L. Lessons from experimental APS models. Lupus 1998; 7 Suppl 2S: 158-161.
 24. Tran HB; Macardle PJ; Hiscock J; Cavill- D; Bradley J; Buyon JP; Gordon TP. Anti-La/SSB antibodies transported across the placenta bind apoptotic cells in fetal organs targeted in neonatal lupus. Arthritis Rheum 2002; 46: 1572-1579.
 25. Van Horn JT, Craven C, Ward K, Branch DW, Silver RM. Histologic features of placentas and abortion specimens from women with antiphospholipid and antiphospholipid-like syndromes. Placenta 2004; 25: 642-648.