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Influence of ubiquitin on fetal survival and full term rabbits derived from mothers with induced antiphospholipid syndrome

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Summary

An assessment of the effect of bovine testis ubiquitin complex (TeUC) administered to pregnant rabbits with experimentally induced antiphospholipid syndrome (APS) on the rabbit-fetuses intrauterine growth and survival rate was conducted. Group TeUC – 44 offspring from mothers treated by an i.m. administration of TeUC on the 20th, 21st, 22nd and 26th days of pregnancy, 10 mg daily. Group IM – 62 offspring from mothers with experimentally induced antiphospholipid syndrome (APS) (immunized by cardiolipin administration). Group IM-TeUC – 48 offspring from rabbits with induced antiphospholipid syndrome (APS) and administered additionally TeUC in the same way as in the TeUC group. Control group consisted of 56 offspring, from rabbits treated by physiological saline. Conclusion: TeUC given during uncomplicated pregnancy has positive influence on neonatal rabbits' birth weight, survival and viability. TeUC given to pregnant rabbits with APS improves the rate of live born neonates as well as viability and postnatal survival in preterm born rabbits.

Keywords: ubiquitin, rabbits, fetus, neonate, antiphospholipid syndrome

The antiphospholipid (aPL) antibody syndrome is an autoimmune disorder in which vascular thrombosis or recurrent pregnancy losses – often attributable to thrombosis within the placental vasculature – occur in patients having laboratory evidence for antibodies against phospholipids or phospholipid-binding protein cofactors in their blood.

There are a variety of manifestations of antiphospholipid syndrome (APS). Most common of these are: deep vein thrombosis (32%), thrombocytopenia (22%), livedo reticularis (20%), stroke (13%), superficial thrombophlebitis (9%), pulmonary embolism (9%), fetal loss (8%), transient ischemic attack (7%) and hemolytic anemia (7%) (2, 9, 17).

In many animal studies with experimental models of antiphospholipid syndrome (APS) the effects of maternal disease on the developing fetus were described. The possible immediate side effects of maternal antibodies are recurrent pregnancy loss, resorption of fetuses and increased number of still births (6, 21). On the other hand some experiments were also described indicating that the therapeutic use of aspirin and steroids during pregnancy can prevent adverse effect of maternal APS on fetal development and health (6). In the last years good results were noticed from the use of Thymus factor X (TFX) in pregnant rabbits with experimentally induced antiphospholipid syndrome. The results indicate a decreasing rate in fetal resorption and increasing number of live births as well as neonatal viability and survival (7, 8).

Thymus tissue is rich in ubiquitin, which was isolated for the first time in 1975 (23, 24). Ubiquitin is a peptide of molecular weight of about 8,450 D, containing 74-76 amino acid residues. The complete amino acid sequence of ubiquitin (UC), an adenylate cyclase stimulating polypeptide, is probably universal in living cells (19). The discovery of the ubiquitin--proteasome system resulted in another important development – the realization that regulated proteolysis is involved in controlling a broad array of cellular processes such as the cell cycle and cell division, apoptosis, transcription, antigen presentation, signal transduction, receptor-mediated endocytosis, protein quality control and the modulation of diverse metabo-

lic pathways (4, 14). Intracellular proteolysis was therefore transformed from a neglected process and research area into an important field in modern biology (3).

The beneficial effect of both the TFX and thymus ubiquitin complex (TUC) treatment were observed in several diseases (5, 13). At present not only thymus UC but also ubiquitin complex derived from bovine lungs, hearts and testis are available. Up to now the influence of testis UC (TeUC) given to healthy pregnant rabbits and pregnant rabbits suffering from antiphospholipid syndrome on the condition of their offspring at birth has not been described.

The aim of the present study was the assessment of the effect of TeUC application to pregnant rabbits with and without experimentally induced antiphospholipid syndrome on fetal body mass, viability and neonatal survival.

Material and methods

The study involved live and dead neonates born to 40 pregnant rabbits (White New Zealand breed) divided into four groups: 3 experimental and one control.

The experimental group TeUC – consisted of 46 offspring (42-live birth, 2-still birth, 2-fetal resorption), from 10 pregnant rabbits, treated by intramuscular Testis Ubiquitin Complex (derived from bovine testis produced by the Pharmaceutics Company Jelfa – Poland) administered on 20th, 21st, 22nd and 26th day of pregnancy at a dose 10 mg daily. The evaluated concentration of testosterone in the TUC did not exceed the level found in female rabbits.

In the two subsequently examined groups, pregnant rabbits with experimentally induced antiphospholipid syndrome (APS) were involved. Due to an easier clinical assessment of the rabbit does and their fetuses and also in consideration of the similarity in the structure of the doe's and human placenta (blood-chorioamiotic) this model has been used for conducting the experiments.

The model of antiphospholipid syndrome (APS) in non-pregnant and pregnant rabbits was designed in the Department of Feto-Maternal Medicine in Szczecin (18). These experimental groups were labeled as follows: IM-TeUC and IM.

Group IM – consisted of 73 offspring (53 – live born; 9 – stillborn, 11 – fetal resorption) from 10 pregnant rabbits, immunized by a subcutaneous administration of cardiolipin at doses of 2250--3000 µg administrated (Biomed--Kraków) with adjuvant (2% solution of aluminium hydroxide) at a proportion 1 : 1, since the 10th day of pregnancy, twice weekly, until the termination of the pregnancy.

Group IM-TeUC – consisted of 49 offspring (46 – live born, 2 – stillborn, 1 – fetal resorption) from 10 pregnant rabbits, immunized by a subcutaneous administration of cardiolipin mixed with adjuvant (2% solution of aluminium hydroxide), as described above and treated additionally with intramuscular Testis Ubiquitin Complex (TeUC) administrated on the 20th, 21st, 22nd and 26th days of pregnancy at a dose of 10 mg daily.

The control group (C) – consisted of 58 offspring (54 – live born, 2 – stillborn, 2 – fetal resorption), descended from 10 pregnant rabbits, treated with physiological saline in volumes and terms analogical with the others pregnant rabbits.

Pregnancies were terminated before term, on the 30th day, by caesarean section under general anaesthesia with vetbutal. After delivery the newborn rabbits were placed in an incubator at a temperature of 37°C.

The rate of live and dead neonates as well as fetal resorption was calculated and compared between the groups. Survival rate was calculated in 15 minute periods up to 45 minutes of life based on the number of still living neonates. Furthermore, a neonatal vitality score was used for differentiation of the preterm rabbits vitality in the analyzed groups. Neonatal vitality was assessed on the basis of the evaluation of 3 features such as: body tone, spontaneous activity and responsiveness to stimuli. The evaluation of each feature was marked on a 3-point scale. Neonates received 1 point if they had weak body tone, low activity and weak responsiveness to stimuli and 3 points with normal body tone, good spontaneous activity and brisk reaction to stimuli. Preterm rabbits with different behavior from that described by 1 or 3 points received 2 point for each feature. The results were coded as follows: 1 point for each feature was noticed as poor vitality, 2 points – as medium and 3 points – as good.

Statistical analysis was conducted by using Fisher exact test, U Mann-Whitney's test, and Pearson chi-square test. Values of $p < 0.05$ were considered significant.

Consent to the experiments was obtained from the Ethical Board for Animal Experimentation PAM-BN-060/11/98.

Results and disscusion

In these examinations the highest average birth mass of the rabbit newborns has been observed in the TeUC group (53.45 g ± 4.7) and it statistically differed ($p < 0.0001$) from the average body mass of the rabbit new-

Tab. 1. Body weight, number of live and stillborns and fetal resorption in control (C) and experimental groups of rabbits

Parameter analyzed	Groups			
	C	TeUC	IM	IM-TeUC
Live born	54 (93.4%)**	42 (91.3%)**	53 (72.6%)	46 (93.9%)**
Still born	2 (3.45%)**	2 (4.35%)**	9 (12.3%)	2 (4.1%)**
Fetal resorption	2 (3.45%)	2 (4.35%)	11 (15.1%)	1 (2.04%)
Total number of subject	58 (56)	44 (46)	62 (73)	48 (49)
Body weight (g)	44.2 ± 4.1	53.45 ± 4.7*	43.1 ± 6.6	50.1 ± 4.1

Explanations: * – $p < 0.05$, ** – $p < 0.01$ in comparison with group IM; wTeUC – bovine testis ubiquitin coplex injected i.m. to pregnant rabbits; IM – immunised subcutanealy with cardiolipid; IM-TeUC – immunised with cardiolipid and injected with bovine testis ubiquitin complex (TeUC)

Tab. 2. Neonatal vitality in control and experimental groups of rabbits: number of rabbits (%)

Vitality	Groups							
	C		TeUC		IM		IM-TeUC	
	n = 54		n = 42		n = 53		n = 46	
Poor	9	(16.7)	0	(0.0)#*	26	(49.1)##	4	(8.7)*
Medium	23	(42.6)	4	(9.5)##	11	(20.8)#	8	(17.4)#
Very good	22	(40.7)	38	(90.5)##*	16	(30.2)	34	(73.9)##*

Explanations: * – $p < 0.001$ in comparison with group IM; # – $p < 0.05$, ## – $p < 0.001$, in comparison with group C

Tab. 3. Time of postnatal survival evaluated in 15 minutes periods up to 45 minutes of neonatal life in rabbits from the control and experimental groups: number of rabbits (%)

Time of survival (min.)	Groups							
	C		TeUC		IM		IM-TeUC	
	n = 54		n = 42		n = 53		n = 46	
15	1	(1.9)*	0	(0.0)*	9	(17.0)	0	(0.0)*
30	3	(5.6)	0	(0.0)*	9	(17.0)	4	(8.7)
45	50	(92.6)**	42	(100.0)**	35	(66.0)	42	(91.3)**

Explanation: * – $p < 0.01$; ** – $p < 0.001$, in comparison with group IM

borns from the control group (C) (tab. 1). A significant statistical difference ($p < 0.006$) has also been found between the average weight of the newly born rabbits, originating from the does from the IM group, the average body mass of the rabbits from the does of the IM-TeUC group and between the average body mass of the rabbit newborns in the IM-TeUC and the control group (C) ($p < 0.004$) (tab. 1). Simultaneously, in the IM group a statistically significant percentage of live born rabbits in comparison with the IM-TeUC group has been observed. The highest vitality characterized 90.5% of the newly born rabbits in the TeUC group, 73.9% born in the IM-TeUC group, 40.7% of the control group and 30.2% of the rabbit newborns from the immunized group (IM).

In the TeUC group no newborns of low vitality have been observed. The diversity newborns of vitality between the groups (tab. 2) has also been statistically shown in percentages.

In the immunized group the quantity of the rabbit newborns, which have lived through 45 min. was statistically significantly lower than in the remaining groups ($p < 0.001$). In the TeUC group all the newborns lived through 45 min. The highest differentiation of the survival time concerned the IM group (tab. 3).

The clinical manifestations of the antiphospholipid syndrome (APS) included venous and arterial thrombosis and embolism, disseminated large and small vessel thrombosis with accompanying multiorgan ischemia and infarction, stroke, premature coronary disease, and spontaneous pregnancy losses (16). Some therapeutic efforts were conducted using animal models of APS to decreased negative influence of maternal APS on fetal development and pregnancy outcome. The dominating experimental model of APS in animals was the one induced in mice (1).

That is probably the reason that up until now most data were published basing on mice experiments. In mice with experimentally induced APS administration of small doses of aspirin, low molecular heparin or immunoglobulin reduced the number of fetuses resorbed and increased the fetal body mass (6, 22). In 1998 Ronin-Walknowska et al. (18) published a model of APS achieving in pregnant and non-pregnant female rabbits. Only limited data concerns rabbit offspring born after APS induced during pregnancy (7, 8, 10). Several lines of evidence suggest that the ubiquitin proteasome pathway may play an important role in regulating intermediary metabolism and cell functioning in catabolic disease states, such as sepsis, burns or ischemia-reperfusion injury (14). Regarding pregnant rabbits with experimentally induced APS and subsequently treated with TFX or thymus ubiquitin complex (TUC) Engel-Pietrzak et al. (8) found an increase in the number of living offspring, increase in their body mass, reduction of resorption rate, improvement of viability and survival rate, compared to untreated rabbit offspring. Similar results have been obtained after the introduction of bovine testis ubiquitin complex (TUC) to pregnant rabbits suffering from APS. Additionally, no side effects of TeUC given during normal, not immunized pregnancy, were discovered on fetal development. The rate of live birth was not significantly different from that of control groups consisting of healthy animals. What is more interesting, newborn rabbits from the TeUC were born with better health than control newborns from healthy untreated mothers. They also had better vitality and all of them survived the period of the analyzed 45 minutes. Moreover, a positive influence of TeUC on fetal body mass in uncomplicated and complicated by experimentally induced APS was noticed.

Further studies are necessary to explain fully the positive ubiquitin action in pregnant rabbits with experimentally induced APS on fetuses and newborns. At present it is suggested that ubiquitin immunomodulating action (19) decreases the area of embolia and thromboses in placenta and increases the proliferative activity of trophoblast (10, 11, 15). The ubiquitin-proteasome pathway might be a therapeutic target to improve the metabolic processes (14).

All these actions can lead to an improvement of fetal and neonatal survival rate in preterm and newborn rabbits developing during prenatal life in a condition of maternal antiphospholipid syndrome.

Conclusions

1. TeUC given during uncomplicated pregnancy has positive influence on the neonatal rabbits' birth weight, survival and vitality.

2. TeUC given to pregnant rabbits with APS improves the rate of live born neonates as well as vitality and postnatal survival in preterm born rabbits.

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