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ORIGINAL ARTICLE

Gestational progesterone suppresses embryotoxic action of the complement system to chick embryo

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Abstract

OBJECTIVE: In this paper the relation between progesterone levels and embryotoxic effect of serum complement was studied.
DESIGN: The aim of this study was to validate hypothesis that progesterone is strong inhibitor of complement embryotoxic action.
SETTING: We used chick embryo like an experimental model for evidence of our hypothesis. We treated chick embryos by sera acquired from healthy pregnant woman with physiologically elevated levels of progesterone and normal complement activity. We investigated embryotoxicity of these sera.
RESULT: We noticed a significant decrease of sera embryotoxicity inversely related to serum levels of progesterone.
THE MAIN FINDING: The embryotoxicity of sera is reversally dependent on progesterone level.
CONCLUSIONS: These findings bring a new knowledge to the role of progesterone and complement system mainly in initial stages of pregnancy and in some cases of spontaneous abortions.

Introduction

Jelínek and Konícková disclosed a profound embryotoxic effect of the normal human serum on the chick embryo during their pioneering work [1]. They described, after administrating normal human serum intraamniotically, the development of a specific malformation complex that has been referred to as the "strait-jacket" syndrome. The normal human serum induced a specific combination of malformations – spine hyperlordosis, defects of the anterior body wall, eventration of abdominal organs, heart defects, and malformations of the brain – in a dose-dependent manner [2, 3]. The authors claimed that a primary target of the embryotoxic action resided in the amniotic membrane.

Further work in this field has brought experimental evidence that the complement system was responsible for the embryotoxic action [4]. We sustained this fact by experiments using sera deficient in some components of the complement system [5].

The role of the complement system in reproduction has attracted scientific interest since the eighties. Production of complement components in reproductive tissues was reported by a number of authors. Isaacson et al. [6] and Hasty et al. [7] reported the hormonally regulated synthesis of the components in endometrial tissue of normally cycling women. Moreover, complement activity has also been described in liquids present in the female reproductive tracts. The complement activity levels, with the exception of follicular fluid, appeared lower than in the blood serum [8].

Development of immuno-histochemical methods obtained along closer acknowledgment of complement production and regulation at the local levels. The complement-regulatory proteins (CRP) are expressed in endothelial and various epithelial cells, even in sperms, oocytes, cervical fluid and trophoblast as a site of maternal-fetal immune interaction. All these regulators – DAF (decay-accelerating factor), MCP (membrane cofactor protein) and CD59 (leukocyte cluster of differentiation antigen 59) exert an inhibitory effect on complement activation. Studies on CRP expression in female reproductive tracts indicate that the complement system and its regulators appear essential for successful fertilization and implantation. While Jensen et al. [9] reported MCP, DAF and CD59 production in endometrium and fallopian tubes throughout the whole menstrual cycle without hormonal regulation, Hasty et al. [7] described DAF expression in luteal endometrium only. CRP secretion by trophoblast surface cells was noticed at least from the 6th week of gestation [10]. CRP present at the maternal-fetal interface are able to control complement mediated attack to allogenic tissues of the embryo [11].

A study by Tichenor et al. [12] revealed that in about 30% of women with RSA (recurrent spontaneous abortions) an activation of alternative complement pathway appears with consequent hypocomplementemia in maternal serum during abortion.

Our previous study documented that the complement serum activity during menstrual cycle remained almost stable, while embryotoxicity of the same sera samples fluctuated significantly depending upon the phase of ovarian cycle [13]. The lowest embryotoxic activity coincided with the peak of plasma progesterone.

Hypothesis

Provided that the embryotoxicity potential of the serum is inversely dependent on the level of progesterone, it must decrease considerably during pregnancy when massive production of progesterone takes place both in the corpus luteum and in the placenta.

The aim of this study was to verify our previous results acquired with fertile healthy non-pregnant women in the course of the menstrual cycle. We intended to demonstrate the inhibitory effect of progesterone on complement embryotoxicity in the group of pregnant women whose progesterone levels are physiologically high.

Material and methods

Venous blood samples were taken on agreement from a group of healthy pregnant Caucasian women aged 16–41. The group consisted of 30 women in the first trimester of pregnancy (from 6th to 11th week) and of further 30 ones in the course of labor. As a comparative group 30 non-pregnant women volunteered in any phase of the ovarian cycle with physiologically low levels of progesterone. From whole venous blood after spontaneous coagulation we derived serum by centrifugation and followed evacuation.

Complement activity (CH100) was detected by hemolysis of antibody sensitized erythrocytes using a hemolytic complement kit from Binding Site, Birmingham, England, in each sample. By measuring the zones of lysis produced by a number of sera of known complement activity a calibration curve was constructed by plotting the diameter against complement activity on semi-log graph paper. The CH100 in unknown samples was determined by measuring the zone of lysis and reading of the calibration curve. The procedures were routinely performed by the laboratory Imumed, Prague, Czech Republic.

Together with complement activity the levels of progesterone were measured by radioimmunoassay technique [14]. Spectria Progesterone (^{125}I) kit from

Orion Diagnostica, Espoo, Finland, was employed for this purpose in the Biochemical Laboratory of Institute of Mother and Child Care, Prague.

Each serum sample was tested for embryotoxicity on experimental models by the standard method. We used chick embryo on incubation day 4, staged 20–24 following Hamburger and Hamilton [15]. Fertilized eggs of White Leghorn randombred stock were purchased from the farm Dominant, Dobrenice, and incubated for 4 days at 37.5° C and 40–50% relative humidity in a thermostatic oven. After candling and opening eggs with the common window technique, 3μl of normal human serum were injected intraamniotically under the binocular preparation microscope using a glass microcanule with an obliquely ground tip. Each experimental group consisted of approximately 20 externally normal specimens. The windowed eggs were closed with paraffin-sealed glass slides allowing easy control. During 4-days-lasting reincubation the embryos were checked everyday at the same time. On incubation day 8 embryos were harvested and inspected and dissected under the preparation microscope. The embryotoxicity index (Es) comprising survival time and severity of malformations detected, was calculated for each group. [1] All the data were evaluated using basic statistics, regression, factor and cluster analysis (software Statistica – Statsoft).

Results

Association between Es, CH100 and the level of progesterone is best depicted on the 3D graph. The value Es is reversally dependent on the level of progesterone. Hand in hand with an increasing progesterone level the embryotoxicity is less manifested. This interaction is further modified by the hemolytic complement activity (CH100) that does not seem to be influenced by progesterone (Fig. 1).

These associations were confirmed by factor analysis for Es as a dependent variable and CH100 and progesterone as independent variables. It demonstrates the statistically significant relation between Es and progesterone ($p < 0.05$). The dependence of Es on CH100 approaches the level of statistical significance (Table 1).

The result of a single linkage cluster analysis presented on a horizontal hierarchical tree plot offers a synoptic view of the relations mentioned above (Fig.2).

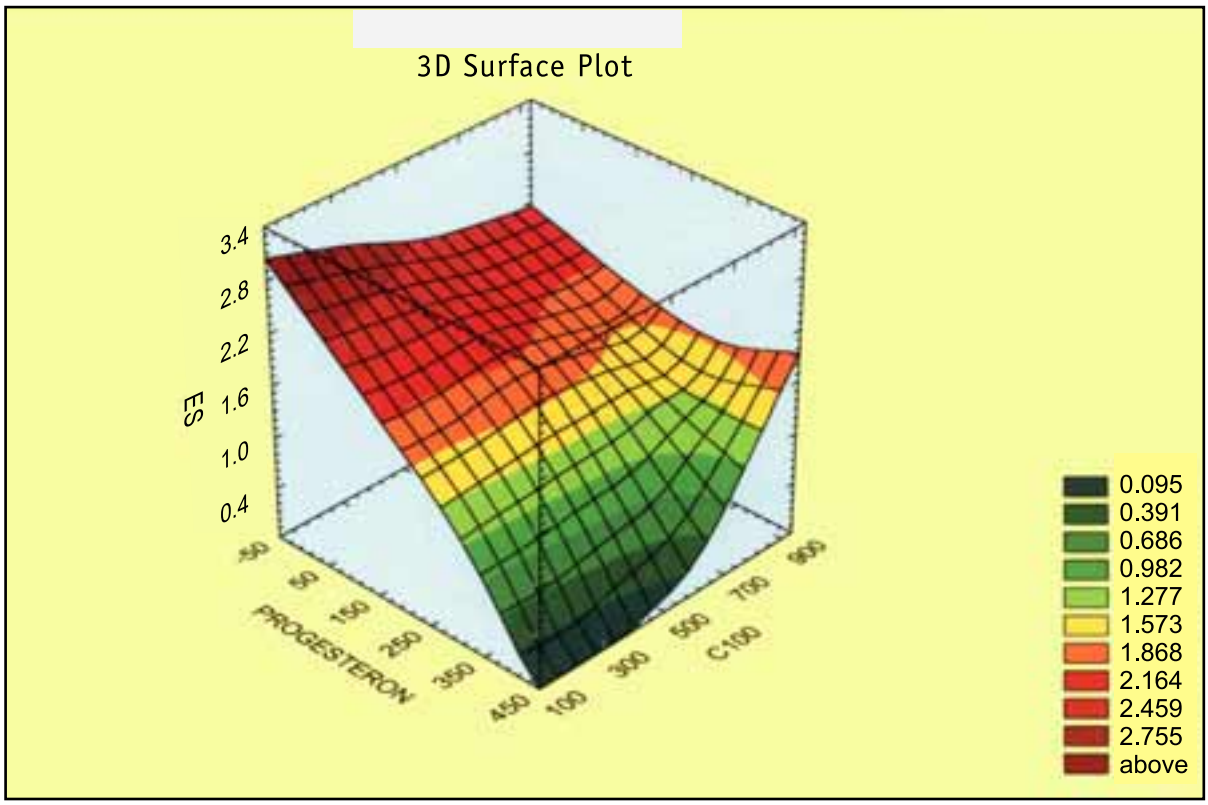

Fig.1 . 3D graph depicting the association between **Es, CH100** and **progesterone levels.** Hand in hand with increasing progesterone level serum embryotoxicity index slopes down. Moreover, this reverse dependence is influenced by complement activity.

Table 1. Table of factor analysis for variables Es, CH100 and progesterone. For insight see the text.

STAT. Regression Summary for Dependent Variable: ES (komplet.sta)
R=.446391 R2^ 199265 Adjusted R—.180858
F(2.87)=10.825 p<.00006 Std.Error of estimate: .73022

N=90	Beta	St. Err. of Beta	B	St. Err. of B	t(87)	p-level
Intercpt			2.577181*	.243563*	10.58116*	.000000*
C100	-.184175	.095941	-.000640	.000333	-1.91966	.058178
PROGEST	-.408467*	.095941*	-.003041*	.000714*	-4.25746*	.000052*

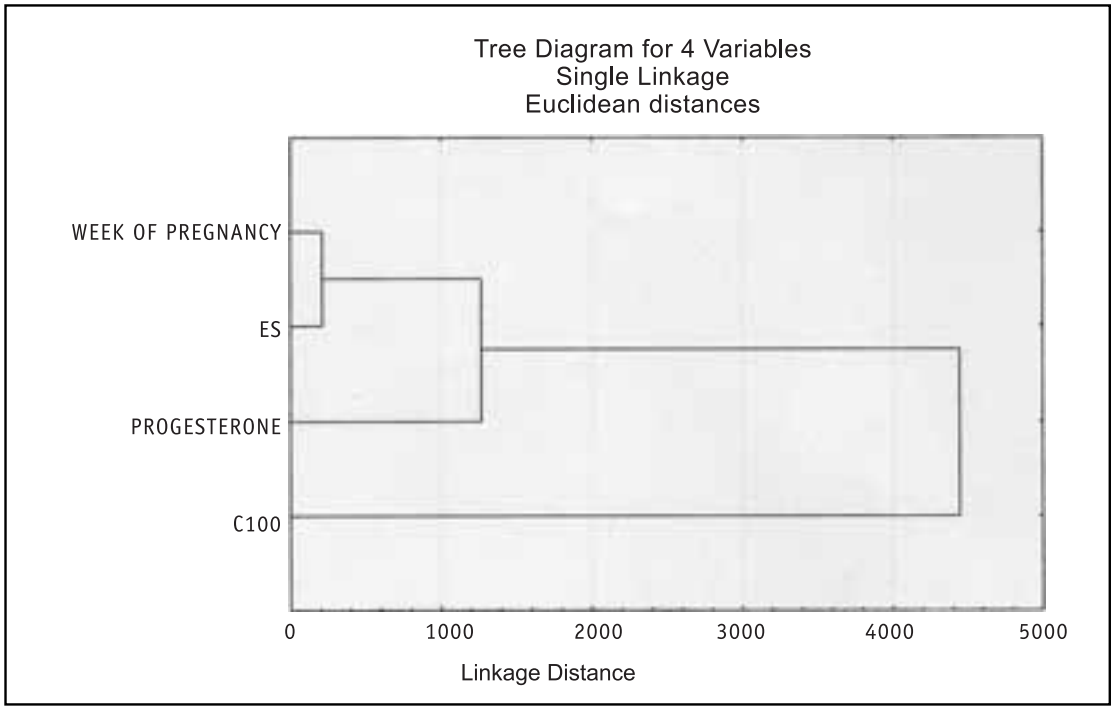

Fig.2. Single linkage cluster analysis of Es, CH100, progesterone and week of gestation. Relationship between progesterone level and serum embryotoxicity is very strong as the well known relation between progesterone level and gestation week. In opposite the dependence of these variables on complement activity is feeble.

Discussion

We intended to characterize the association between embryotoxicity to the chick embryo, on the one hand, and progesterone and complement blood sera activity in human females, on the other hand. Our previous conclusions derived from experiments with blood sera of non-pregnant fertile women were verified and confirmed by the present observation on the group of pregnant women. The complement-mediated embryotoxicity is clearly inhibited by increasing levels of progesterone in both pregnant and non-pregnant women.

Recent studies by many authors demonstrated the complement system as an important regulator of normal reproductive mechanisms (for review see Rooney et al.) [16]. Synthesis of complement components and its regulatory proteins affords evidence of the crucial role of the complement system inhibition in successful implantation and fortuitous development of embryo [6, 7, 10, 11, 17]. Our study points further to progesterone as another great suppressor of complement mediated attack to the allogenic embryo.

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REFERENCES

1 Jelínek R, Konícková Z. Human blood serum – a severe teratogen in the chick. Fol Morphol (Prague) 1975; **23**:40–43.
2 Jelínek R, Doskocil M, Loštický C. The " strait-jacket "syndrome in the chick. I. A morphological analysis. Fol Morphol (Prague) 1976; **24**:98–106.
3 Jelínek R, Doskocil M, Loštický C. The " strait-jacket "syndrome in the chick. II. Mechanism of development. Teratology 1976; **14**:327–334.
4 Loštický C, Jelínek R. The interaction of histone and human serum – a hypothesis of the mechanism of their effect on development of the chick embryo. Fol Morphol (Prague) 1978; **3**:232–234.
5 Zeman M, Nováková P. Immunoteratogenesis – a new field of investigation. Int J Prenatal Perinatal Psychol Med 1996; **8** (Suppl):63–66
6 Isaacson KB, Coutifaris C, Garcia CR, Lyttle CR. Production and secretion of complement component 3 by endometric tissue. J Clin Endocrinol Metab 1989; **69**:1003–1009.
7 Hasty LA, Lambris JD, Lessey BA, Pruksananonda K, Lyttle CR. Hormonal regulation of complement components and receptors throughout menstrual cycle. Am J Obstet Gynecol 1994; **170**:168–175.
8 Vanderpuye OA, Labarrere CA, McIntyre JA. The complement system in human reproduction. Am J Reprod Immunol 1992; **27**:145–155.
9 Jensen TS, Bjorge L, Wollen AL, Polstein M. Identification of the complement regulatory proteins CD46, CD55 and CD59 in human fallopian tube, endometrium, and cervical mucosa and secretion. Am J Reprod Immunol 1995; **34**:1–9.
10 Holmes CH, Simpson KL, Okada H, Okada N, Wainwright SD, Purcell DFJ, et al. Complement regulatory proteins at the fetomaternal interface during human placental development: distribution of CD59 by comparison with membrane cofactor protein (CD46) and decay accelerating factor (CD55). Eur J Immunol 1992; **22**:1579–1585.
11 Cunningham DS, Tichenor JR Jr. Decay accelerating factor protects human trophoblast from complement-mediated attack. Clin Immunol Immunopathol 1995; **74**:156–161
12 Tichenor JR, Bledsoe LB, Opsahl MS, Cunningham DS. Activation of complement in humans with a first trimester pregnancy loss. Gynecol Obstet Invest 1995; **39**:79–82.
13 Nováková P, Zeman M. Progesterone inhibits embryotoxic effect of the complement system. Neuroendocrinol Lett 1999; **20**:105–108.
14 Carlstrom K, Bolton A, Kallner A, Vihko R. Assay of reproductive hormones, when, why and how. In IFCC and Farmos Diagnostica, 2nd edition, Turku,1988:42–46.
15 Hamburger V, Hamilton HL. A series of normal stages in the development of chick embryos. J Morphol 1951; **88**: 49–92.
16 Rooney IA, Oglesby TJ, Atkinson JP. Complement in human reproduction: activation and control. Immunol Res 1993; **12**:276–294.
17 Oglesby TJ, Longwith JE, Huettner PC. Human complement regulator expression by the normal female reproductive tract. Anat Rec 1996; **246**:78–86.