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## THE EFFECT OF HUMORAL FACTORS RELEASED BY HYPOPERFUSED PLACENTA ON FETAL DEVELOPMENT

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**Abstract.** Simulation of hypoperfusion of the right uterine horn in rats on the 14<sup>th</sup> day of pregnancy resulted in disruption of microcirculation in placentas of the left uterine horn, decrease of fetal weight and length as well as in fetal death after implantation.

The obtained data complement the overall understanding of the pathogenesis of preeclampsia and placental insufficiency and create preconditions for development of new areas of creation of effective drugs for treatment and prevention of these pathologies.

**Keywords:** rats, preeclampsia, placental insufficiency, placental ischemia, fetal hypoplasia.

### Introduction

Preeclampsia is the most frequent disease of pregnant women and holds the first place among the reasons of maternal and perinatal mortality. The pathogenesis of this severe disease is still far from being understood completely. However in recent years many authors report a specific histological pattern at time of morphological examination of the placenta, i.e. disproportional development of the marginal area between the maternal and the fetal portions [1, 21, 24, 28]. There has been observed incomplete invasion of cytotrophoblast into the coiled artery of the uterus. In case of gestosis the level of alteration of the coiled arteries themselves does not reach the level specific to normal pregnancy [18, 26, 27]. Increased distance between the coiled arteries and the chorionic villae as well as immaturity of the coiled arteries themselves induces trophoblast ischemia and the fetoplacental barrier permeability increase.

The produced humoral factors in response to ischemia as well as possibly to fetal antigens which passed through the fetoplacental barrier with the increased permeability after penetration into a maternal body encourage accumulation of asymmetric dimethylarginine (ADMA) in plasma, development of generalized endothelial dysfunction,

secondary ischemic events and oxidative stress [5, 6, 7, 14, 15, 16, 16]. Due to the above search of new medicinal preparations for treatment and prevention of preeclampsia which would have endothelioprotective, anti-ischemic and antioxidant activity becomes of great importance [1, 5, 11, 12, 23, 25]. However the information on the influence of the humoral factors released by the hypoperfused placenta on fetal development and healthy portions of the placenta in the available literature is rather scarce. In this connection the presented work was dedicated to research of fetal development and the state of placenta under the conditions of placental hypoperfusion modeling.

**Research objective:** To study the peculiarities of fetal development and the state of placenta under the conditions of hypoperfusion modeling in rats.

### Research methodology

The experiment involved 20 white female Wistar rats with the weight of 250–300 g. In order to form groups of pregnant animals with the specific gestational age male rats (2 animals) were introduced into a cage with female rats (3 animals) for the period of 24 hours, at that the animals before the experiment were maintained in separate cages. After that the animals were caged in separate cages and after the

period of 14 days pregnancy was established by palpation under the ether narcosis conditions. Our experiments showed 30-40% of pregnancy event frequency. The pregnant rats were divided into the groups (n=10): I – intact; II – with modeling of hypoperfusion in the right uterine horn on the 14<sup>th</sup> day of gestation. A model of the uterine hypoperfusion as described by many authors (Ошибка! Источник ссылки не найден., Ошибка! Источник ссылки не найден., Ошибка! Источник ссылки не найден.) and modified by us was taken as a prototype. On the 14<sup>th</sup> day of gestation 10 animals underwent surgery involving application of silver clamps on the right iliac artery (0.2 mm) and the right ovarian artery (0.1 mm). Thus we've ensured hypoperfusion only in the right uterine horn in contrast to the described prototype.

On the 21<sup>st</sup> day of gestation in order to register the indices of systolic and diastolic blood pressure (SBP, DBP) catheterization of the right carotid artery of the anesthetized (chloral hydrate in the dose of 300 mg/kg) animals was performed, bolus dosing of pharmacological substances was carried out into the right femoral vein. There were performed vascular tests in order to determine endothelium-dependent and endothelium-independent vasodilatation along with calculation of an endothelial dysfunction coefficient (EDC) [9].

The study of the placental microcirculation was made with the aid of the equipment produced by «Biopac systems» company: a polygraph MP100 with a laser Doppler flowmetry module (LDF) LDF100C and a sensor TSD144. The LDF results recording was performed by means of the program Acqknowledge of 3.8.1 version, the values of microcirculation were expressed in perfusion units (PU) [2, 3, 4]. NO-production function of endothelium was assessed on the basis of the data on the content of stable NO metabolites, i.e. nitrite ions (NOx) in blood plasma. For the purpose of determining fluid content in the greater omentum the same was weighted, dried at the temperature of 37<sup>o</sup>C within the period of 24 hours and weighed once again [8, 13].

### Results and discussion

Application of the clamps on the right iliac artery and the right ovarian artery did not resulted in the statistically significant change of the blood pressure and the endothelial dysfunction coefficient (Table 1). The animals in this group demonstrated decrease of the placental microcirculation from 452.4±27.16 to 204.4±14.30 PU in the right uterine horn and to 309.0±15.80 PU in the left uterine horn

(p<0.05). No statistically significant change of diuresis, proteinuria and NO-synthesizing function of endothelium was observed in these animals.

Modeling of reduced blood flow in the placentas of the right uterine horn produced changes of ischemic genesis, i.e. there were observed extensive necroses and hemorrhages in the labyrinth zone, tissues dissection on the border between the placenta and the uterine wall (partial separation).

The comparative evaluation of the state of fetuses revealed reduction of the fetal weight and size as compared to the group of intact pregnant animals and post-implantation fetal death.

Table 1

The values of morphological and functional parameters in pregnant rats at time of modeling the reduced blood flow in the right uterine horn (M±m; n=10)

Parameter \ Group	Pregnant (intact)	Pregnant (hypoperfusion of the right uterine horn)
SBP, mm hg	134.5±2.3	138.8±4.4
DBP, mm hg	92.0±2.1	89.8±5.7
EDC, c.u.	1.10±0.11	1.29±0.08
Microcirculation, PU	446.3±27.46	204.4±14.30 <sup>*R</sup> 309.0±15.80 <sup>*L</sup>
NOx, micromole/dL	2.28±0.11	2.15±0.09 <sup>*</sup>
Fluid content in the greater omentum tissues, %	49.89±0.82	48.78±2.00
Weigh of fetuses, g	1.73±0.06	1.13±0.06 <sup>*R</sup> 1.31±0.08 <sup>*L</sup>
Length of fetuses, mm	24.59±0.42	20.89±0.45 <sup>*R</sup> 22.90±0.45 <sup>*L</sup>
Lwngth/weight, mm/g	14.91±0.28	19.36±0.76 <sup>*R</sup> 18.63±0.68 <sup>*L</sup>
Post-implantation death, %	0	34.16±3.37 <sup>*R</sup> 18.66±5.66 <sup>*L</sup>

Note: SBP, DBP – systolic and diastolic blood pressure (mm hg); EDC – endothelial dysfunction coefficient (c.u.); microcirculation in the placenta (PU); nitrite ions concentration (NOx); \* - p < 0.05 as compared to the intact animals group; R – the right uterine horn; L – the left uterine horn.

Therefore the results of the carried out experiment give evidence of the absence of marked effect of the right uterine horn hypoperfusion on systemic hemodynamic values. The local changes in the right uterine horn are expected and in line with the literature data. The changes observed in the left uterine horn blood supplying vessels of which remain intact have the biggest importance. To all seeming the humoral factors released by the ischemic placenta of the right uterine horn [23] have local marked effect

on the intact placenta vessels since they are the most sensitive.

On the basis of the obtained data in relation to the pathogenesis of development of preeclampsia and placental insufficiency it is possible to state with some degree of certainty that the areas of ischemic placenta may induce deterioration of morphological and functional state of its healthy areas due to the released humoral factors. The obtained data give opportunities for creation of medicines for treatment and prevention of the discussed pathology which would have new mechanisms of action.

### References

1. Gureev V.V., Pokrovsky M.V., Korokin M.V. ADMA – eNOS – deterministic ways of pharmacological correction of preeclampsia. Belgorod: Publishing House of Belgorod State University. 2014. 265 p. ; Ill. [\[Full text\]](#)
2. Gureev V.V. Investigation of the role of distant ischemic preconditioning in correction of morphological and functional disturbances by short episodes of ischemia-reperfusion in the condition of ADMA-like preeclampsia. *Kursk Scientific-Practical Bulletin "Man and his health"*. № 3 (2012): P. 5-9. [\[eLIBRARY\]](#) [\[Full text\]](#)
3. Gureev V.V. The role of iNOS in experimental correction of endothelial dysfunction in case of ADMA-like preeclampsia by short ischemia-reperfusion episodes. *Basic Research*. № 8, Part 2 (2012): P. 298-301. [\[eLIBRARY\]](#)
4. Gureev V.V. The role of ATP-sensitive K<sup>+</sup> channels in experimental correction of endothelial dysfunction in case of ADMA-like preeclampsia by short ischemia-reperfusion episodes [electronic resource]. *Modern Problems of Science and Education* № 5 (2012). <http://www.science-education.ru/105-7053>.
5. Gureev V.V., Zhilinkova L.A., Stupakova Ye.G. Correction of endothelial dysfunction by nicorandilum, tetrahydrobiopterine and resveratrol in the course of experimental gestosis modeling // *Basic research*. – 2015. – № 1-1. – S. 58-62. [\[eLIBRARY\]](#) [\[Full text\]](#)
6. Gureev, V.V. Endothelial dysfunction – a central element in the pathogenesis of preeclampsia / V.V. Gureev // *Scientific Bulletin of Belgorod State University. Ser. Medicine. Pharmacy*. – 2012. – V. 17. № 4-1 (123), issue 17/1. – P. 5-12. [\[eLIBRARY\]](#) [\[Full text\]](#)
7. Zaynulina M.S., Mozgovaya Ye.V., Nianuri D.A. Diagnostic significance of graphical chart with characteristics of endothelium in pregnant women with late preeclampsia and diabetes // *Journal of Obstetrics and Gynecological Diseases*. – 1999. – № 2. – P.22-24.
8. Zainulina M.S., Petrishchev N.N. Endothelial dysfunction and its markers in gestosis // *Journal of Obstetrics and Gynecological Diseases* – 1997. – № 3. – P.18-22.
9. Ivanova, L.B. The effect of GABA derivatives on endothelial function in rats with experimental preeclampsia /L.B. Ivanova, V.I. Karamysheva, V.N. Perfilova, // *Problems of Reproduction*. – 2012. – № 1. – P. 28-30. [\[eLIBRARY\]](#)
10. Correction of ADMA-like preeclampsia in experiment / V.V. Gureev, S.A. Aliokhin, A.A. Dolzhikov, A.S. Mostovoy // *Kursk Scientific-Practical Bulletin "Man and his health"*. – 2012. – № 1. – P. 14-19. [\[eLIBRARY\]](#)
11. Pokrovsky M.V., Kochkarov V.I., Pokrovskaya T.G. et al. Methodological approaches to quantify the development of endothelial dysfunction in L-NAME-induced model of nitric oxide deficiency in experiment // *Cuban Scientific Medical Bulletin*. – 2006. – № 10 (91). – P. 72 – 77. [\[eLIBRARY\]](#)
12. Pokrovsky M.V., Pokrovskaya T.G., Gureev V.V. et al. Use of L-arginine in correction of endothelial dysfunction in experimental preeclampsia // *Experimental and Clinical Pharmacology*. – 2012. – V. 75. № 2. – P. 14-16. [\[eLIBRARY\]](#)
13. Realities and perspectives of pharmacological correction of ADMA-eNOS-associated pathways in preeclampsia / M.V. Pokrovsky, N.G. Filippenko, V.V. Gureev [et al.] // *Rational Pharmacotherapy in Cardiology*. – 2010. – № 6 (6). – P. 882-887. [\[eLIBRARY\]](#)
14. Reznikova, L.B. Endothelioprotective activity of GABA derivatives in experimental gestosis. Thesis of. ... PhD in Medical Science / L.B. Reznikova. / Volgograd, 2013. – P. 165. [\[eLIBRARY\]](#)
15. The role of oxidative metabolism disorders in development of endothelial dysfunction of pre-eclampsia. *Georgian Medical News. Tbilisi-New York*. №7-11 (115) October 2004, P. 21-24.
16. The role of oxidative stress in placenta in the pathogenesis of preeclampsia. *Georgian Medical News. Tbilisi-New York*. №10 (115). October 2004. P. 21-24.
17. The factors involved in the nitric oxide metabolism disorder and in development of endothelial dysfunction in case of preeclampsia. *Allergology and Immunology*. Moscow, Russia, vol. 5, №3. 2004, p. 393-395.
18. Pharmacological correction of ADMA-eNOS-associated targets in case of preeclampsia / M.V. Pokrovsky, T.G. Pokrovskaya, V.V., Gureev [et al.] // *Obstetrics and Gynecology*. – 2011. – № 2. – P. 16-20. [\[eLIBRARY\]](#)
19. Animal Models of Preeclampsia / E. Podjarny, G. Losonczy and C. Baylis / *Semin Nephrol*. – 2004. – Vol. 24(6). – P. 596–606. [\[Free PMC\]](#)
20. Circulating and utero-placental adaptations to chronic ischemia in the rat / J.S. Gilbert, A.J. Bauer, A. Gingery [et al.] // *Placenta*. – 2012. – Vol. 33. P. 100-105. [\[PubMed\]](#)
21. Crocker I Gabor Than Award Lecture 2006: preeclampsia and villous trophoblast turnover: perspectives and possibilities // *Placenta*. – 2007 – Apr; 28 Suppl A: S4 – 13. [\[PubMed\]](#)
22. Ducray J.F., Naicker T., Moodley J. Pilot study of comparative placental morphometry in pre-eclamptic and normotensive pregnancies suggests possible maladaptations of the fetal component of the placenta. // *European Journal of Obstetrics Gynecology and*

Reproductive Biology. 2011 May;156(1):29-34. [\[PubMed\]](#)

23. Endothelin type A receptor antagonist attenuates placental ischemia-induced hypertension and uterine vascular resistance / K.B. Tam Tam, E. George, K. Cockrell [et al.] // *Am J Obstet Gynecol.* – 2011. – Vol. 204: 330. – P. 331–e334. [\[PubMed\]](#)

24. Effect of l-arginine, vitamin b6 and folic acid on parameters of endothelial dysfunction and microcirculation in the placenta in modeling of l-name-induced no deficiency / Korokin M.V., Pokrovsky M.V., Novikov O.O. [et al.] // *Bulletin of Experimental Biology and Medicine.* – 2011. – № 1. P. 70-72. [\[PubMed\]](#)

25. Pathophysiology of hypertension during preeclampsia: linking placental ischemia with endothelial dysfunction / Jeffrey S. Gilbert, Michael J. Ryan, Babbette B. LaMarca, Mona Sedeek, Sydney R. Murphy and Joey P. Granger / *Am J Physiol Heart Circ Physiol* 294: H541–H550, 2008. First published November 30, 2007; doi:10.1152/ajpheart.01113.2007. [\[PubMed\]](#)

26. Purkerson M.L. Vekerdy L. A history of eclampsia, toxemia and kidney in pregnancy // *Am. J. Nephrol.* – 1999. – 19:2. – P. 313-319. [\[PubMed\]](#)

27. Remote Ischemic Preconditioning Correction in Adma-Like Gestosis Model / Gureev V.V., Alehin S.A., Pokrovskiy M.V. [et al.] // *Research Journal of Pharmaceutical, Biological and Chemical Sciences.* – 2014. – №5. – P. 1095-1098.

28. Robertson W.B., Khong T.Y. Hypertension in pregnancy//Eds F. Sharp, E.M Symonds. – New York, 1987. – P.101-193.

29. Yamamoto T., Takahashi Y., Kuno S. et al. International Society for the Study of Hypertension in Pregnancy: Book of Abstract of the 10-th World Congress. – Washington, 1996. – P.238.

30. Van Oppenraaij R.H., Bergen N.E., Duvekot J.J., de Krijger R.R., Hop Ir W.C., Steegers E.A., Exalto N. Placental vascularization in early onset small for gestational age and preeclampsia. // *Reproductive Science* 2011 Jun;18(6):586-93. [\[PubMed\]](#)