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THE ROLE OF VASCULAR HOMEOSTASIS IN WOMEN WITH INFERTILITY TREATED WITH ASSISTED REPRODUCTIVE TECHNOLOGIES AND WITH CONCOMITANT INTRAHEPATIC CHOLESTASIS

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ABSTRACT

Intrahepatic cholestasis of pregnancy (idiopathic jaundice of pregnancy, recurrent familial jaundice of pregnancy) is a benign familial disease manifesting itself in the gestational period in itching and (or) jaundice. As a rule, the disease is observed in the 3rd trimester of pregnancy, that is in the 28th-30th week, and may disappear by itself a few days after labor and recur during future pregnancies.

The aim of this research is to study the role and factors of vascular homeostasis regulation in women with infertility treated with assisted reproductive technologies (ART) and with concomitant intrahepatic cholestasis.

Intrahepatic cholestasis is characterized by the damage of endothelial cells of hepatic sinusoids, changes in the intrahepatic hemodynamics, increased production of cytokines and free radicals. The processes of angiogenesis in the liver are also closely connected with the functional capacity of endothelium. The L-arginine level in blood was measured using the photometric method based. The homocysteine level was measured by the enzymatic cycling method. To measure the level of placental growth factor (PGF) in blood plasma samples, we used the immunochemical method.

The research has found especially severe complications in the course of pregnancy after ART in those women with intrahepatic cholestasis whose placentas developed under the conditions of endothelial dysfunction from the very beginning of pregnancy.

So, these factors may be early prognostic markers of severe obstetrical and perinatal disorders in women with induced pregnancy and hepatobiliary disorders.

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Introduction. Cholestatic hepatitis of pregnancy (or intrahepatic cholestasis of pregnancy - IHCP) is a cholestatic disease that manifests itself in the 2nd or the 3rd trimester of pregnancy in pruritus, increased levels of aminotransferases and bile acids (BA), changes in the activity of hepatic

enzymes and is characterized by spontaneous disappearance of signs and symptoms within two or three weeks after labor [1, 2]. In some studies, IHCP is also called idiopathic intrahepatic cholestasis or hepatosis of pregnancy. Indeed, it is a transitory hepatic dysfunction that disappears within 1 to 3 weeks after labor. IHCP was first mentioned by F. Ahlfeld in 1883 in the Reports and Records of the Clinic of Obstetrics and Gynecology in Hessen [3].

One should also bear in mind that, due to the exhaustion of its reserve potential in the course of pregnancy, the liver becomes more vulnerable (Sidorova I.S., 2003; Friedman S.A., 1998, Girling J., 1997). One should pay special attention to its functioning in case of development of gestational toxicosis; in the pathogenesis of its severe forms, changes in the hepatobiliary system are the key factor (Christina Song, 1998).

Intrahepatic cholestasis of pregnancy is observed in 1-2 out of 1000 pregnancies in the United States [4]. As a rule, it manifests itself in the 3rd trimester, with the onset around the 30th week of pregnancy (on average) and the disappearance of symptoms after labor. The differential diagnosis includes viral hepatitis, autoimmune hepatitis, primary biliary hepatic cirrhosis and cholelithiasis.

Additional risk factors include the pregnant woman's age, multifetal pregnancy, diseases in anamnesis or in relatives, liver diseases before the pregnancy, cholestasis, intake of oral contraceptives [5, 6].

There are several theories of the disease pathogenesis, including the hormonal, the genetic and other theories. According to the hormonal theory, increased secretion of progesterone and other placental hormones in the pregnant woman's body impedes the release of gonadotropic hormones from the hypophysis and leads to increased cholesterol synthesis in the liver and decreased capacity of the membranes of hepatocytes and bile canaliculi to release bile acids. What lies at the basis of pathogenesis is a disorder of cholepoiesis and choleresis as 17 β -estradiol gets to bile canaliculi and inhibits the bile salt export pump, hampering the transport of bile acids into bigger ducts [7,8].

Endothelial dysfunction is known to be a universal pathogenic mechanism of the majority of diseases as endothelium does not only regulate vascular tone but is also involved in the processes of atherogenesis, thrombosis and protection of the integrity of vessel walls [Golovchenko Y.I., Treshchinskaya M.A., 2008]. Besides, endothelium produces a big number of biologically active substances that influence vascular tone and angiogenesis, regulate hemostasis, adhesion and aggregation of platelets, immune and anti-inflammatory responses [Gomazkov O.A., 2000; Aird W.C., 205].

Liver diseases are characterized by the damage of endothelial cells of hepatic sinusoids, changes in the intrahepatic hemodynamics, increased production of cytokines and free radicals [Yermolov S.Y., 2000]. The processes of angiogenesis in the liver are also closely connected with the functional capacity of endothelium. Nitric oxide (NO) is synthesized by hepatocytes and cells of the reticulo-endothelial system of the liver [Ratnikova L.I., Melnikov I.V., 2002]. Affected by chronic processes, the liver suffers from the inhibition of the NO biosynthesis processes, which leads to an imbalance between vasoconstrictors and vasodilators [Hon W.M., 2002].

Aim of research – identifying the role of endothelial dysfunction and factors of vascular homeostasis regulation in the development of obstetrical and perinatal complications in women with infertility treated with ART and with concomitant intrahepatic cholestasis.

Materials and methods of research. We have analyzed and divided into groups 90 women with infertility treated with ART. The basic group consisted of 50 women diagnosed with intrahepatic cholestasis in the 2nd and the 3rd trimester of pregnancy, while the other 40 women did not present the above mentioned disorder (comparison group). In addition and contrast to these 90 women with infertility treated with assisted reproductive technologies, we have also considered 20 women who got pregnant without ART (reference group).

The L-arginine level in blood was measured using the photometric method based on the reaction of α -naphthol with a hypobromite reagent. The homocysteine level was measured by the enzymatic cycling method with the use of the DiaSys kit (Germany) and the Respons 920 analyzer (Germany), following the producer's instructions. To measure the level of placental growth factor (PGF) in blood plasma samples, we used the immunochemical method with electrochemiluminescent immunoassay (ECLIA).

Research results and their discussion. Endothelial dysfunction also plays an important role in reproductive disorders, in particular infertility and recurrent miscarriage [9, 10]. Some authors even consider it to be the basis of pathogenesis of such conditions.

Among the numerous factors of endothelial origin, nitric oxide (NO) is a generally recognized marker of endothelial dysfunction. Nitric oxide is produced from L-arginine under the influence of the nitric oxide synthase enzyme (NOS). It is to this L-arginine / nitric oxide system that the contemporary obstetrics attributes the major vasoregulatory role in the gestational period [11, 12].

Special importance for the development of the vascular network of placenta and for its normal functioning is attributed to the vascular factors that stimulate the proliferation of endothelial cells and increase their viability, which include the vascular endothelial growth factor (VEGF), the placental growth factor (PGF) and the basic fibroblast growth factor (bFGF) [13, 14].

An important factor of reproductive and obstetrical disorders is hyperhomocysteinemia, which leads to the damage and activation of endothelial cells and significantly increases the risk of thrombosis. Homocysteine induces apoptosis of trophoblastic cells and significantly decreases the production of chorionic gonadotropin, which may cause obstetric complications related to implantation disorders [15].

Taking into account the contemporary view of endothelial dysfunction as a universal trigger mechanism of reproductive disorders, the role of vascular changes in the placentation process disorder as well as the genetic susceptibility of women with infertility diagnosed with intrahepatic cholestasis to endothelial dysfunction (unfavorable variants of the eNOS gene polymorphism) that we found in our research, we considered it necessary to study in such women the levels of L-arginine as the NO donor, of homocysteine as an endothelium-damaging factor and of PGF as a factor of angiogenesis [16, 17, 18].

We have researched the indicators that characterize endothelial function and may damage endothelium in 50 women with infertility treated with ART and with concomitant intrahepatic cholestasis (basic group), in 40 women without such hepatobiliary disorders (comparison group) and in 20 women who got pregnant without ART (reference group). The research results (Table 1) have shown in the women of the basic group a decreased level of L-arginine, that is the only source of nitric oxide – the main vasorelaxant factor (44.9 ± 1.1 vs. 49.6 ± 1.3 , $p < 0.05$), which may be indicative of endothelial dysfunction. We have also noticed a trend towards hyperhomocysteinemia, which implies a high risk of endothelium damage, negative influence on the coagulation system, microcirculation disorders and negative influence on the reproductive function. [19, 20, 21].

Table 1. Indicators of vascular hemostasis regulation in women with infertility and functional hepatic disorders

Indicator	Group of women		
	Basic group	Comparison group	Reference group
L-arginine, mmol/l	44.9 ± 1.1	49.6 ± 1.3	53.6 ± 1.4
Homocysteine, micromole/l	6.5 ± 0.36	5.7 ± 0.32	6.2 ± 0.32

Note: * - the difference is significant with regard to the indicator in women from the comparison group ($p < 0.05$).

To assess the condition of vascular regulation in pregnant women with functional hepatic disorders after treatment with ART, depending on the development of obstetric and perinatal complications, we have studied the respective indicators in 50 women with intrahepatic cholestasis (basic group), 40 pregnant women treated with ART but presenting no hepatobiliary disorder (comparison group) and 20 healthy women who got pregnant naturally (reference group) in the 14-16th week of pregnancy. As shown in Table 2, the women of the basic group present significant changes in the analyzed indicators. For instance, one can see a significant decrease in comparison with the reference group of the L-arginine level (44.2 ± 1.1 vs. 52.6 ± 1.4 mmol/l ($p < 0.05$)) and an increase of the homocysteine level. One should also note a significantly decreased level of PGF in blood (91.4 ± 8.6 vs. 132.4 ± 11.5 pg/ml in women from the reference group, $p < 0.05$), which is indicative of disorders of the angiogenesis processes, which, in their turn, have a negative impact on the development and functioning of placenta and lead to complications of pregnancy and disorders of the condition of the fetus, which were often observed in women with hepatobiliary changes.

Table 2. Indicators of vascular homeostasis regulation in pregnant women after ART

Indicator	Group of pregnant women		
	Basic group	Comparison group	Reference group
L-arginine, mmol/l	$(44.2 \pm 1.1)^*$	47.3 ± 1.3	52.6 ± 1.4
Homocysteine, micromole/l	$(6.9 \pm 0.30)^*$	6.2 ± 0.36	5.8 ± 0.33
PGF in blood, pg/ml	$(91.4 \pm 8.6)^*$	118.8 ± 9.5	132.4 ± 11.5

Note: * - the difference is significant with regard to the indicator in women from the reference group ($p < 0.05$).

To identify the role of the disorders of vascular homeostasis regulation in the development of complications of pregnancy in case of hepatic disorders, we have singled out 2 subgroups in the basic group: Subgroup 1 – 19 women with significant obstetrical and perinatal disorders (miscarriages and non-developing pregnancies, severe preeclampsia, decompensated fetal distress), Subgroup 2 – 31 women without such disorders. The research results show (Table 4) especially severe complications in the course of pregnancy after ART in those women with intrahepatic cholestasis whose placentas developed from the very

beginning of pregnancy under the conditions of endothelial dysfunction (decrease of the L-arginine level to 39.8 ± 2.0 mmol/l vs. 45.8 ± 1.6 in women without significant disorders of the gestational process, $p < 0.05$), negative influence of homocysteine (increase to 7.4 ± 0.44 vs. 6.4 ± 0.36 micromole/l, $p < 0.05$), disorders of angiogenesis processes (decrease of the PGF level in blood to 70.5 ± 11.9 vs. 103.7 ± 9.8 pg/ml, $p < 0.05$).

Table 3. Indicators of vascular homeostasis regulation in pregnant women after ART

Indicator	Subgroup of women	
	1	2
L-arginine, mmol/l	$(39.8 \pm 2.0)^*$	45.8 ± 1.6
Homocysteine, micromole/l	$(7.4 \pm 0.44)^*$	6.4 ± 0.36
PGF in blood, pg/ml	$(70.5 \pm 11.9)^*$	103.7 ± 9.8

Note: * - the difference is significant with regard to the indicator in women of Subgroup 2 ($p < 0.05$).

So, these factors may be early prognostic markers of such obstetrical and perinatal disorders in case of induced pregnancy.

Conclusions. Especially severe complications in the course of pregnancy after ART were observed in those women with intrahepatic cholestasis whose placentas developed from the very beginning of pregnancy under the conditions of endothelial dysfunction (decreased L-arginine level), negative influence of homocysteine and disorders of angiogenesis processes (decreased PGF level in blood). Thus, these factors may be early prognostic markers of severe obstetrical and perinatal disorders in case of induced pregnancy.

Conflict of Interest. The authors confirm that there are no conflicts of interest.

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