Endothelial function, regulation of angiogenesis and embryonic central hemodynamics in ART-conceived pregnancies

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To cite this article: N. V. Bashmakova, P. B. Tsyvian, G. N. Chistiakova, I. A. Gazieva, Y. M. Trapeznikova & D. O. Mazurov (2015) Endothelial function, regulation of angiogenesis and embryonic central hemodynamics in ART-conceived pregnancies, Gynecological Endocrinology, 31:sup1, 31-33, DOI: 10.3109/09513590.2015.1085199

To link to this article: http://dx.doi.org/10.3109/09513590.2015.1085199

Published online: 08 Oct 2015.

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Endothelial function, regulation of angiogenesis and embryonic central hemodynamics in ART-conceived pregnancies

N. V. Bashmakova, P. B. Tsyvian, G. N. Chistiakova, I. A. Gazieva, Y. M. Trapeznikova, and D. O. Mazurov

Abstract
This study was undertaken to compare the concentrations of pro- and anti-angiogenic growth factors, nitric oxide (NO) stable metabolites in maternal serum and embryonic left ventricular (LV) isovolumic relaxation time (IRT, ms) during the first trimester in two groups of women: with pregnancy conceived by assisted reproductive technologies (ART, n = 39) and normally conceived (control group, n = 68) pregnancy. The concentration of vasoconstrictor endothelin 1 was 45.5 times more in ART than in control group. On the contrary, the concentrations of NO stable metabolites in ART were 1.9 times less than in control women. The assessment of angiogenic suppressors in ART women demonstrates the decrease in s-endoglin concentration was 1.6 times and in soluble receptor to vascular endothelial growth factor concentration was 2.0 times in comparison with control group. There was a significant increase in LV IRT in ART embryos in comparison to control ones. These data suggest significant changes in pro-angiogenic factors balance and increase in vascular impedance in ART-conceived embryos.

Keywords
Assisted reproductive technologies, embryonic vascular impedance, pro-anti-angiogenic factors balance

Materials and methods
We conducted a prospective study utilizing a clinical pregnancy as endpoint. A total of 39 consecutive unfertile women received first or repeated in vitro fertilization – embryo transfer treatment for
tubal, endometriosis and unexplained factors (ART group) in the department of reproductive medicine at Mother and Child Research Institute (Yekaterinburg, Russia). The control group consisted of 68 women with naturally conceived pregnancy. All women had a normal body mass index (BMI, 19–23 kg/m²) and regular menstrual cycle with basal follicle stimulating hormone (FSH) <10 IU/l. There was no history of ovarian operation in women of both groups. Patients undergoing controlled ovarian hyperstimulation with low ovarian response (<5 follicles with diameter >16 mm and E2 <1000 pg/ml on the day of hCG injection) were excluded. Also, exclusion criteria were: history of recurrent miscarriage (three consecutive miscarriages), distortion of the uterine cavity shown on ultrasound scan and ectopic pregnancy following IVF treatment. The study was approved by institutional ethics committee and all subjects provided written informed consent.

All ART group women were pre-treated with buserelin (Suprecur, Hoechst, Frankfurt, Germany) nasal spray 150 mg four times a day from the mid-luteal phase of the cycle preceding the treatment cycle and received human menopausal gonadotrophin (hMG), (Pergonal, Serono, Geneva, Switzerland) for ovarian stimulation. Human chorionic gonadotrophin (hCG) (Profasi, Serono, Geneva, Switzerland) was given intramuscularly when the leading follicle reached 16 mm in diameter and there were at least three follicles of 16 mm in diameter. Serum estradiol (E2) concentration was measured on the day of hCG administration. Transvaginal ultrasound-guided oocyte retrieval was scheduled 36 h after the hCG injection.

All ultrasound examinations were performed using a Voluson 730 Expert (GE Medical Systems, Milwaukee, WI) ultrasound system equipped with RIC 5–9H vaginal and RAB 4–8L abdominal transducers. Ultrasonography was performed strictly adhering to the ALARA (as low as reasonably achievable) principle, and the total time of ultrasound exposure was restricted to a maximum of 20 min. After confirming fetal viability and excluding the presence of any obvious fetal anomaly, the crown-ramp length was measured. Echocardiography was performed transabdominally in all cases, and additional transvaginal examination was performed when the transabdominal image was sub-optimal. A systematic assessment of fetal heart structure was performed, obtaining standard two dimensional views [12]. Valve clicks were used to identify the closure and opening of the atrioventricular and semilunar valves while measuring the time intervals [10]. The LV inflow and outflow blood velocity waveforms were obtained simultaneously and the IRT (ms; time interval between the closure of the aortic valve and the opening of the mitral valve) was measured. All the Doppler recordings were performed during fetal quiescence over four to six cardiac cycles. For all the parameters assessed, an average of three separate measurements was used for statistical analysis.

Maternal serum concentrations of VEGF, sVEGFR-1 and endothelin 1 were evaluated using commercially available ELISA kits (Bender Medsystems, Vienna, Austria). Validation test were performed for serum and standard curve was obtained every time of detection. The concentrations of VEGF, sVEGFR-1 and endothelin 1 were determined by interpolation from the standard curve. All samples were examined in duplicate. The sensitivity of the ELISA kits to VEGF and sVEGFR-1 was 25 and 15 pg/ml, respectively. The intra- and inter-assay coefficients of variation (CVs) for VEGF, sVEGFR-1 and endothelin 1 were both lower than 10%. Serum concentration of soluble Endoglin (sEng) was assessed by R&D Systems (USA) kit. Nitrite and nitrate, the stable metabolic products of NO, were measured spectrophotometrically using R&D Systems (Minneapolis, MN) kit.

Statistical analysis

All data were analysed by STATISTICA 10.0 (StatSoft, Tulsa, OK). The values of IRT measurement data are expressed as mean±SD. The results for pro- and anti-angiogenic factors concentrations are expressed as median (range). Differences between the groups were tested for significance using independent-samples t-test. Bonferroni correction was adopted for multiple comparisons. Statistical significance was defined as p<0.05.

Results

Table 1 demonstrates the concentrations of the main pro- and anti-angiogenic growth factors and stable metabolites of NO. The concentration of pro-angiogenic agent endothelin 1 was 45.5 times more in the serum of the women of ART group than in control. On the contrary, the concentration of the stable metabolites of NO in ART group was 1.9 times less than in the control group. There was no significant difference in the concentration of VEGF and control groups. The assessment of angiogenesis suppressors content (endoglin and soluble receptor to VEGF – sVEGFR-R1) demonstrates the decrease in concentrations of these agents in ART group correspondently 1.6 and 2.0 times in comparison to the control group.

Echocardiographic assessments of embryonic LV IRT (IRT) in ART and control groups are presented in Table 2. The mean values of ART (ms) at 11, 12 and 13 weeks of gestation were significantly less in ART group in comparison with the control group.

Discussion

The significant increase in one of the strongest vasoconstrictors – endothelin 1 and concomitant decrease in vasodilators synthesis (NO and its metabolites) in ART group was demonstrated in this study. The decrease in synthesis of angiosuppressors (endoglin and sVEGFR-R1) could reflect the down-regulation of these agents as a result of general vasoconstrictive reaction in ART women during early pregnancy. We also demonstrated a significant increase in embryonic LV IRT in ART group. This finding can reflect the increase in vascular impedance at this early stage of ART embryos development.

Due to young age of the ART population in humans, it is not known yet whether ART is associated with increased risk for clinical cardiovascular endpoints. However, there is abundant evidence that in population at risk, atherosclerosis and cardiovascular diseases already start in childhood many years before the first clinical events occur [6,7]. We propose the endothelial dysfunction as a main mechanism of changes that we observed in maternal serum angiogenic – anti-angiogenic agents balance and IRT changes in embryos of ART group.

There are several facts obtained from studies in ART mice which could support this idea. It was shown that in ART mice, endothelium-dependent mesenteric artery dilation was defective and carotid artery stiffness was increased [13]. In ART, this defective vascular function in vitro was translated into significant arterial hypertension in vivo [13].

In humans, it is difficult to completely exclude that parental factors contribute to vascular dysfunction in ART children. The findings that in normal mice ART induces premature vascular aging and arterial hypertension, however, provide strong additional evidence for the concept that ART per se is the main cause of the observed changes. The findings in mice also strengthen the concept that hormonal stimulation of the ovulation in the mother is not important determinant of ART-induced vascular dysfunction, because endothelium-dependent vasodilation of mesenteric artery was normal in offspring of super-ovulated mice [14].

In offspring of mice with protein-restricted diet during pregnancy, pulmonary vascular dysfunction is associated with altered lung DNA methylation suggesting that epigenetic mechanism may be involved in the fetal programming of the vascular
Moreover, it was demonstrated that epigenetic alteration may participate in ART mice vascular changes. It was found that the methylation of the promoter of the gene coding for endothelial nitric oxide synthase (eNOS) was altered in the aorta of ART mice [15]. This demethylation had important consequences, as evidenced by decreased eNOS and eNOS RNA expression in the vascular bed and impaired vascular NO synthesis in ART compared with control mice [15].

Declaration of interest

This study was supported by a grant from the Russian Foundation for Basic Research-Ural (No: 13-04-96080). The authors report no declarations of interest.

References


Table 1. Serum concentrations of pro- and anti-angiogenic agents in maternal groups.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>ART group (n = 39)</th>
<th>Control group (n = 68)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelin 1 (fmol/l)</td>
<td>1.82 (0.63–3.11)</td>
<td>0.04 (0.004–0.45)</td>
<td>0.0001</td>
</tr>
<tr>
<td>VEGF, pg/ml</td>
<td>0.14 (0.0–5.16)</td>
<td>0.09 (0.0–0.66)</td>
<td>n.s.</td>
</tr>
<tr>
<td>sVEGFR-I, pg/ml</td>
<td>0.54 (0.03–1.08)</td>
<td>1.06 (0.46–1.96)</td>
<td>0.01</td>
</tr>
<tr>
<td>NO₂ end., mkM/l</td>
<td>1.66 (1.09–2.34)</td>
<td>3.18 (1.26–5.16)</td>
<td>0.004</td>
</tr>
<tr>
<td>sEndoglin, ng/ml</td>
<td>5.31 (4.61–6.19)</td>
<td>8.56 (6.99–10.41)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

The results are expressed as median (range).

Table 2. Left ventricular isovolumic relaxation time (ms) values in maternal groups.

<table>
<thead>
<tr>
<th>Gestational age (wk)</th>
<th>ART group</th>
<th>Control group</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>35 ± 3</td>
<td>27 ± 2</td>
<td>0.01</td>
</tr>
<tr>
<td>12</td>
<td>37 ± 3</td>
<td>28 ± 3</td>
<td>0.01</td>
</tr>
<tr>
<td>13</td>
<td>39 ± 3</td>
<td>30 ± 2</td>
<td>0.01</td>
</tr>
</tbody>
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