Gynecological Endocrinology

ISSN: 0951-3590 (Print) 1473-0766 (Online) Journal homepage: http://www.tandfonline.com/loi/igye20

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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.

http://informahealthcare.com/gye
ISSN: 0951-3590 (print), 1473-0766 (electronic)

GYNECOLOGICAL ENDOCRINOLOGY

Gynecol Endocrinol, 2015; 31(S1): 31–33
 DOI: 10.3109/09513590.2015.1085199

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ORIGINAL ARTICLE

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

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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- anti-angiogenic factors balance and increase in vascular impedance in ART-conceived embryos.

Keywords

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

History

Received 17 August 2015
Accepted 2 September 2015
Published online 5 October 2015

Introduction

Assisted reproductive technologies (ART) have aided millions of couples worldwide to have children. *In vitro* fertilization has been performed for more than three decades, and children born after ART now estimated up 1–4% of the births in developed countries [1]. With continued ART success and utilization, any long-term health risks due to ART treatment have the potential to affect a substantial proportion of the population and increase the future health care burden.

Epidemiological work in humans has put forward the hypothesis that intrauterine environmental influences may predispose the children to chronic cardiovascular and metabolic disease in adulthood [2]. Therefore, the safety of ART for long-term health has a growing importance, but there is little information on this issue. This could be related, at least in part, to the young age of these persons because clinically manifest disease may not yet have had time to develop.

Although ART are generally considered safe, the potential association of these technologies with poorer pregnancy outcomes has long been investigated. There is evidence that ART is associated with increased risk for adverse perinatal outcome and congenital malformations [3]. Preliminary evidence has recently suggested that ART could be associated with long-term cardiovascular changes. It was demonstrated the increased blood pressure in late childhood after ART conception [4]. Another study demonstrated the presence of signs of systemic and pulmonary vascular dysfunction in children conceived by ART [5]. Systemic endothelial dysfunction represents the first step in the development of atherosclerosis and is already detectable in apparently healthy children at increased cardiovascular risk [6,7].

One of the consequences of the endothelial dysfunction is the increase in vascular resistance and impedance. The isovolumic relaxation time (IRT) has been reported to be a useful, non-invasive, Doppler-derived left ventricular (LV) relaxation index that could serve as an index of ventricular afterload and peripheral vascular resistance [8,9]. By incorporating only time interval, the index is less dependent on anatomy or precise imaging. Furthermore, IRT is independent of ventricular geometry [10]. We hypothesized that IRT would be technically feasible to obtain in the embryo using the pulsed Doppler technique. By simultaneously obtaining the mitral valve inflow waveforms and the aortic outflow waveforms from the LV outflow tract, the IRT interval could be measured.

Accumulating evidence suggests that imbalance between circulating angiogenic factors such as vascular endothelial growth factor (VEGF) and anti-angiogenic factors such as soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) and the soluble form of endoglin (sEng) is the central key in endothelial dysfunction and pathophysiology of preeclampsia [11].

We therefore assessed stable metabolites of nitric oxide (NO), endothelin concentration, vascular pro- and anti-angiogenic growth factors and early embryo hemodynamics (IRT) in women who became pregnant as the result of ART and in women who conceived naturally.

The hypothesis was that ART induced endothelial dysfunction and early embryonic hemodynamic changes, which are related to epigenetic mechanisms.

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

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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/m^2) 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 18 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 – sVEGF-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 vasodilation agents synthesis (NO and its metabolites) in ART group was demonstrated in this study. The decrease in synthesis of angiosuppressors (endoglin and sVEGF-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

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

Analyte	ART group ($n = 39$)	Control group ($n = 68$)	p Value
Endothelin 1 (fmol/l)	1.82 (0.63–3.11)	0.04 (0.004–0.45)	0.0001
VEGF, pg/ml	0.14 (0.0–5.16)	0.09 (0.0–0.66)	n.s.
sVEGFR-1, pg/ml	0.54 (0.03–1.08)	1.06 (0.46–1.96)	0.01
NO_2 gen., mkM/l	16.38 (14.23–21.83)	19.35 (15.24–26.26)	n.s.
NO_2 end., mkM/l	1.66 (1.09–2.34)	3.18 (1.26–5.16)	0.004
NO_3, mkM/l	14.56 (12.42–19.65)	16.07 (11.92–20.7)	n.s.
sEndoglin, ng/ml	5.31 (4.61–6.19)	8.56 (6.99–10.41)	0.0001

The results are expressed as median (range).

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

Gestational age (wk)	ART group	Control group	p Value
11	35 ± 3	27 ± 2	0.01
12	37 ± 3	28 ± 3	0.01
13	39 ± 3	30 ± 2	0.01

system [15]. 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 evidence 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.

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