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.

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 outcomes 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 (sVEGFR1) 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/m²) and regular men-
strual cycle with basal follicle stimulating hormone (FSH) <10 IU/

1. 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 (Suprrecin,
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) 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, Austria). Validation test were per-
formed for serum and standard curve was obtained every time of
detection. The concentrations of VEGF, sVEGFR-1 and endothe-
lin 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 spectropho-
metrically using R&D Systems (USA) kit.

Statistical analysis

All data were analysed by STATISTICA 10.0 (StatSoft). 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 metab-
olites 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 vasodilating agents
synthesis (NO and its metabolites) in ART group was demon-
strated in this study. The decrease in synthesis of angiotensin-
oids (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 cardio-
vascular 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 addi-
tional 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 dysfunc-
tion, because endothelium-dependent vasodilatation 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 mecha-
ism may be involved in the fetal programming of the vascular
The synthesis in ART compared with control mice [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**

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**References**