Manipulating Nature: Might There be a Cardiovascular Price to Pay for the Miracle of Assisted Conception?

Running title: Celemajer; Manipulating nature and CV risk

David S. Celemajer, MB, BS, PhD, DSc, FAHA
Sydney Medical School, University of Sydney, Sydney, Australia

Address for Correspondence:
David S. Celemajer, MB, BS, PhD, DSc, FAHA
C39 - Royal Prince Alfred Hospital
The University of Sydney
NSW 2006 Australia
Tel: +61 2 9515 6519
Fax: +61 2 9550 6262
Email: david.celemajer@email.cs.nsw.gov.au


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Assisted Reproductive Technologies (ARTs) have brought the miracle of childbirth to literally hundreds of thousands of adults who would otherwise not have conceived children; indeed it is now estimated that 1-3% of all births in many developed nations involve ARTs\(^1\). The first ART birth, however, was not until 1978 and so even the oldest such offspring are only now entering young adult life. Will they have the same health outcomes as those babies conceived “naturally”? 

There have been some health problems documented after ART. In studies to date, ART has been consistently associated with multiple births and low birth weight in offspring\(^2\); these factors may in turn be linked to long term cardiovascular risk\(^3\). Data from meta-analyses have also suggested an approximately 20-30% increase in the risk of major malformations in ART babies\(^4\). The absolute risks of such outcomes is low, however; of greater concern would be any significant increase in the risk of more common adverse health outcomes, such as premature cardiovascular (CV) disease. 

In this issue of *Circulation*, Scherrer *et al* document significant vascular dysfunction in 65 children conceived by ART, examined at an average age of 12 years\(^5\). They find adverse changes in both the systemic and pulmonary circulations, including structural and functional alterations, some of which have been prospectively linked to higher risk of increased CV events in later life (for example, increased carotid intima-media thickness and systemic arterial stiffness). Strengths of this provocative paper include the careful examination of relevant control groups, such as offspring of mothers who had ovarian hyperstimulation without ART, and siblings of ART children conceived naturally. The ART children were of similar birth size and had similar traditional CV risk profiles compared with controls, suggesting that the mechanism for the observed vascular abnormalities was unrelated to hormonal, parental or post-natal environmental factors. It is worth noting, however, that others have documented higher blood
pressure and fasting blood sugar levels in ART children aged 8-18 years compared with naturally conceived children, suggesting these as potential mechanisms of late CV risk.6

Can environmental exposures so early in life actually alter vascular phenotype and risk? In 1992, we first demonstrated arterial abnormalities (systemic endothelial dysfunction) in high risk children as young as 8 years of age7. We found similar early vascular functional abnormalities in the pulmonary circulation, in high risk children with congenital heart disease8. In 1997, Napoli et al found aortic lipid deposition in fetuses of hypercholesterolemic mothers9 and in 2005, we found increased aortic wall thickness in growth-restricted newborns, in the first days of life10, implicating fetal events in the modification of potential vascular risk. The current data of Scherrer et al suggest that even the environment of the embryo might alter CV risk, later in life5. To understand the possible mechanisms whereby this might occur, the events involved in ART processes need to be examined.

A typical ART cycle starts with hormonal ovarian hyperstimulation, followed by oocyte pick-up from the mature ovarian follicles. Gametes (oocytes and sperm) are then co-incubated in a culture-medium for a few hours, or a sperm may be injected directly into the oocyte in culture, to aid fertilization. The resulting embryo is cultured for 2-3 days to form a cleavage embryo (6-8 cells) or for several more days to form a blastocyst (70-100 cells), before reimplantation into the mother’s womb (either at that time or after a period of freezing then thawing). The “In Vitro” parts of these events occur, therefore, during a critical developmental period of the nascent embryo. This may be one reason why only approximately 15-30% of such treatment cycles result in a live delivery11, consistent with a degree of cellular stress to the gamete(s) and/or embryo (for example, from physical and/or chemical stress). Concern was raised a decade ago that such manipulations of gametes and embryos may have adverse long-term health consequences for the
Certainly in several animal species studied, in vitro culture and manipulation alter
gamete and embryo physiology by initiating stress-induced cellular responses\textsuperscript{13}, which may then modify gene expression patterns. A particular concern has been “epigenetic” modification during ARTs\textsuperscript{14}.

Epigenetics refers to heritable alterations in gene expression, involving changes other than those in the actual sequence of nucleotides in the DNA. Examples include methylation status and modifications of histones. These biochemical modifications of DNA can suppress gene expression, without altering gene sequence. This can particularly affect gene “imprinting”, where imprinted genes may be expressed in a parent-specific manner (that is, the allele from one or other parent is expressed and the other is silent, the latter due to epigenetic modifications). The more extreme examples of epigenetic abnormalities include mono-allelic gene expression, with potentially devastating phenotypic consequences.

Early studies of ART children suggested a significantly higher than expected incidence of genetic imprinting syndromes, such as Angelman syndrome (which includes severe developmental delay, absent speech, seizures and ataxia, with gene inactivation of the maternal copy of chromosome 15 region p11-13\textsuperscript{15}) or Beckwith-Wiedemann syndrome (which includes prenatal overgrowth, macroglossia, abdominal wall defects and gene inactivation of the maternal copy of chromosome 11 region p15.5\textsuperscript{16}). Collectively, these studies suggest an association between ART and loss of maternal DNA methylation\textsuperscript{15}, perhaps in turn related to greater vulnerability of the oocyte (compared to the sperm) to epigenetic changes in response to the in vitro environment.

More recently, it has been appreciated that more subtle epigenetic changes associated with ART can cause less dramatic but still potentially relevant changes in the phenotype of ART
offspring. Scherrer et al propose that the ART procedures themselves might lead to epigenetic changes that alter post-natal vascular structure and function. This does not appear to depend on the type of ART, as abnormalities were seen in ART offspring with or without sperm injection into oocytes (as also found by Ceelen et al in their study of blood pressures and blood sugars in ART children). In a series of intriguing studies in mice, Rexhaj et al (from the same group as the current Scherrer paper) have documented that ART culture media alters methylation patterns of genes, associated with vascular dysfunction and hypertension in offspring. They have also demonstrated that maternal undernutrition (which is known to increase oxidative stress in the placenta) is associated with altered pulmonary DNA methylation and vascular dysfunction, which can be reversed by post-natal administration of inhibitors of histone deacetylation.

What might cause these putative epigenetic changes in ART embryos? One potential confounder is that parental subfertility (which resulted in the need for ART in the first place) might itself be due to epigenetic changes, which then could be heritable by the offspring, with adverse but different phenotypic consequences. Alternatively or additionally, the physical manipulation of pick up, injection and extracorporeal exposures could elicit epigenetic changes. Furthermore, exposure of the gametes and/or embryos to chemical-rich culture media could alter DNA methylation. Khosla et al, for example, have shown that adding serum to mouse embryos cultured in M16 medium results in DNA methylation and changes in expression of several genes, associated with reductions in fetal weight. Niemitz and Feinberg have hypothesized that methionine content of certain commercial ART media might be critically involved in inducing epigenetic changes and expressed concern that the chemical content of such media are not always clearly disclosed by the manufacturers.
The epigenome changes considerably during gametogenesis and embryogenesis in “normal” circumstances\textsuperscript{14} and thus it is quite conceivable that physical manipulations and/or chemically artificial environments might alter these processes in a potentially maladaptive way. As methylation and histone modifications in DNA sequences can be easily measured by current techniques, serially in parents, cord blood and/or offspring, these are potentially fruitful areas for future research into epigenetic changes before, during and after ART processes and their potential consequences.

One particularly worrisome aspect of the current findings\textsuperscript{5} is the implication that cellular and/or epigenetic changes are occurring in a cluster of pluripotent progenitor cells (in the embryo and/or blastocyst), sufficient to alter cell physiology in the post-natal systemic and pulmonary circulations; might this mean that other organ systems might also express significant phenotypic abnormalities? Clearly such questions need to be examined, in ART offspring.

Need children born after ART be treated differently, from a CV viewpoint? The authors note a similar magnitude of systemic arterial dysfunction in the ART children studied compared to children with Type 1 diabetes mellitus, studied by others; however one cannot necessarily infer a similar degree of CV risk (as the “injury” in diabetes may be ongoing, from persistent hyperglycemia and its consequences). We do not yet know the prognostic significance of the arterial abnormalities documented in these ART children, although such abnormalities may indeed prove to be clinically relevant. Furthermore, the study of Scherrer et al is a small study of only 65 ART children. Thus there is insufficient evidence, at this time, to screen or treat such children and young adults differently. These data do serve as a clarion call, however, for two types of studies; those examining the effects of the relevant physical and chemical manipulations
of gametes and embryos on cellular physiology, epigenetic changes and their possible consequences; and those addressing disease surveillance in all health domains of ART children.

ART has brought untold joy to many. With social changes mandating greater choices for family planning at older parental ages, the demand for ART will likely continue to rise. In addition, the advent and more ready availability of Preimplantation Genetic Diagnosis suggests that there may be even further increases in the number of ART offspring in the near future. In this context, work such as that by Scherrer et al now underscores the urgent need for us to understand the possible adverse late outcomes of ART and to focus on finding possible technical changes to the In Vitro procedures, which might ameliorate or reverse any potentially harmful health consequences.

Conflict of Interest Disclosures: None

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David S. Celermajer

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