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% to 3% of all births in many developed nations involve ARTs. 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; these factors may in turn be linked to long-term cardiovascular risk. Data from meta-analyses have also suggested an approximate 20% to 30% increase in the risk of major malformations in ART babies. 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 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. 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 cardiovascular 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 cardiovascular risk profiles as control subjects, which suggests that the mechanism for the observed vascular abnormalities was unrelated to hormonal, parental, or postnatal environmental factors. However, others have documented higher blood pressure and fasting blood sugar levels in ART children aged 8 to 18 years compared with naturally conceived children, which suggests these as potential mechanisms of late cardiovascular risk.

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 age. We found similar early vascular functional abnormalities in the pulmonary circulation in high-risk children with congenital heart disease. In 1997, Napoli et al found aortic lipid deposition in fetuses of hypercholesterolemic mothers, and in 2005, we found increased aortic wall thickness in growth-restricted newborns in the first days of life, which implicates fetal events in the modification of potential vascular risk. The present data from Scherrer et al suggest that even the environment of the embryo might alter cardiovascular risk later in life. 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 pickup from the mature ovarian follicles. Gametes (oocytes and sperm) are then coincubated 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 to 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% to 30% of such treatment cycles result in a live delivery, consistent with a degree of cellular stress to the gamete(s) or embryo (for example, from physical 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 offspring. Certainly in several animal species studied, in vitro culture and manipulation alter gamete and embryo physiology by initiating stress-induced cellular responses, which may then modify gene expression patterns. A particular concern has been “epigenetic” modification during ARTs.

Epigenetics refers to heritable alterations in gene expression, which involve 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,” whereby imprinted genes may be...
expressed in a parent-specific manner (that is, the allele from one or the other parent is expressed and the other is silent, the latter because of epigenetic modifications). The more extreme examples of epigenetic abnormalities include monoallelic 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) 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). Collectively, these studies suggest an association between ART and loss of maternal DNA methylation, perhaps in turn related to greater vulnerability of the oocyte (compared with 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 postnatal vascular structure and function. This does not appear to depend on the type of ART, because 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 present report by Scherrer et al) have documented that ART culture medium alters methylation patterns of genes, which is 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 postnatal 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 caused by epigenetic changes, which then could beheritable by the offspring, with adverse but different phenotypic consequences. Alternatively or additionally, the physical manipulation of pickup, injection, and extracorporeal exposures could elicit epigenetic changes. Furthermore, exposure of the gametes or embryos to chemical-rich culture media could alter DNA methylation. Khosla et al, for example, have shown that the addition of serum to mouse embryos cultured in M16 medium results in DNA methylation and changes in expression of several genes, in association 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, and thus, it is quite conceivable that physical manipulations or chemically artificial environments might alter these processes in a potentially maladaptive way. Because 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 present findings is the implication that cellular or epigenetic changes are occurring in a cluster of pluripotent progenitor cells (in the embryo or blastocyst) that are sufficient to alter cell physiology in the postnatal 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 cardiovascular viewpoint? The authors note a similar magnitude of systemic arterial dysfunction in the ART children studied compared with children with type 1 diabetes mellitus studied by others; however, one cannot necessarily infer a similar degree of cardiovascular risk (because 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 by 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 2 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 that might ameliorate or reverse any potentially harmful health consequences.

Disclosures

None.

References


**Key Words:** Editorials ■ arterial stiffness ■ arteries ■ endothelium ■ pathophysiology ■ pulmonary circulation
Manipulating Nature: Might There Be a Cardiovascular Price to Pay for the Miracle of Assisted Conception?

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