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REVIEW

Clinical update

Cardiovascular dysfunction in children conceived by assisted reproductive technologies

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Epidemiological studies demonstrate a relationship between pathological events during foetal development and future cardiovascular risk and the term 'foetal programming of cardiovascular disease' has been coined to describe this phenomenon. The use of assisted reproductive technologies (ARTs) is growing exponentially and 2–5% of children are now born by this procedure. Emerging evidence indicates that ART represents a novel important example of foetal programming. Assisted reproductive technology may modify the cardiovascular phenotype in two ways: (i) ART involves manipulation of the early embryo which is exquisitely sensitive to environmental insults. In line with this concern, ART alters vascular and cardiac function in children and studies in mice show that ART alters the cardiovascular phenotype by epigenetic alterations related to sub-optimal culture conditions. (ii) Assisted reproductive technology markedly increases the risk of foetal insults that augment cardiovascular risk in naturally conceived individuals and are expected to have similar consequences in the ART population. Given the young age of the ART population, it will take another 20–30 years before data on cardiovascular endpoints will be available. What is clear already, however, is that ART emerges as an important cardiovascular risk factor. This insight requires us to revise notions on ART's long-term safety and to engage on a debate on its future. There is an urgent need to better understand the mechanisms underpinning ART-induced alteration of the cardiovascular phenotype, improve the procedure and its long-term safety, and, while awaiting this aim, not to abandon medicine's fundamental principle of doing no harm (to future children) and use ART parsimoniously.

Keywords *In vitro* fertilization • Endothelium • Epigenetic • eNOS • Arterial hypertension • Pulmonary hypertension

Introduction

Based on epidemiological studies showing an association between adverse events during early life and the prevalence of cardiovascular disease later in life,[1] Barker put forward the hypothesis of 'foetal programming of cardiovascular diseases'. These early epidemiological studies focused on hard clinical cardiovascular endpoints. Progress in the detection of early cardiovascular alterations known to increase cardiovascular risk later in life,[2,3] has opened up the possibility to search for such alterations in young apparently healthy populations at risk and design interventions to prevent premature cardiovascular morbidity and mortality. This shift of focus is illustrated by studies in offspring of mothers suffering from pre-eclampsia who are at increased risk for stroke later in life[4] and in whom alterations of the systemic and pulmonary circulation are already detectable during childhood.[5]

Assisted reproductive technologies (ARTs) which initially were developed to treat women with tubal disease, over the past decade have been applied to an ever expanding list of other situations, resulting in an exponential growth of the number of babies born by this procedure who now account for 2–5% of births in developed countries.[6] Based on emerging data showing that the early embryo is particularly sensitive to environmental insults, we speculated that ART alters cardiovascular function in the offspring. Here, we summarize this evidence in animals and humans and explore how ART-induced epigenetic alterations in the embryo and ART-related foetal insults may shape cardiovascular function and determine future cardiovascular risk.

Assisted reproductive technologies-induced cardiovascular alterations in humans

Premature atherosclerosis

There is abundant evidence that in populations at risk for premature cardiovascular morbidity and mortality, subclinical atherosclerosis is

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already detectable in childhood.[3] Several non-invasive techniques allow in populations at increased cardiovascular risk to detect early, subclinical, vascular alterations, and predict outcome.[2,7] Using such techniques, recent studies demonstrate that young apparently healthy ART children display generalized endothelial dysfunction and exaggerated stiffening and morphological alterations of the vasculature in the systemic circulation (*Figure 1*).[8]

Systemic endothelial dysfunction represents the first step in the development of atherosclerosis and can be assessed by determining the increase of the brachial artery diameter evoked by reactive hyperaemia (flow-mediated vasodilation, FMD).[2] Flow-mediated vasodilation of the brachial artery is roughly 25% smaller in ART than in control children.[8] Endothelial dysfunction in ART children is not limited to the systemic circulation, since high-altitude exposure induces exaggerated hypoxic pulmonary hypertension in this population. Moreover, systemic and pulmonary endothelial dysfunction are correlated, indicating that endothelial dysfunction is a robust finding in the ART population.[8–11] Stiffening of the vasculature also takes place during the early development of atherosclerosis and has been shown to predict future cardiovascular risk in children suffering from diseases known to predispose to premature cardiovascular morbidity.[3,12–14] Carotid-femoral pulse wave velocity, a proxy of arterial stiffness, is significantly faster in ART children than in controls.[8] Finally, premature atherosclerosis in children is associated with structural changes of the vasculature that can be assessed by ultrasound measurement of the carotid intima-media thickness (IMT).[3,15–17] Carotid IMT is significantly increased in ART children.[8] Structural changes of the vasculature in ART children appear to develop very early, given that increased aortic IMT *in utero* has been reported.[18] In summary, these findings demonstrate vascular alterations which suggest premature vascular ageing in young apparently healthy ART children.

Arterial hypertension

Increased arterial blood pressure is expected to be one of the first clinical consequences of ART-induced vascular dysfunction. In line with this expectation, Ceelen and colleagues reported higher systolic and diastolic office blood pressure in ART children than in spontaneously conceived control children.[19] Consistent with these observations, preliminary data using 24-h ambulatory blood pressure

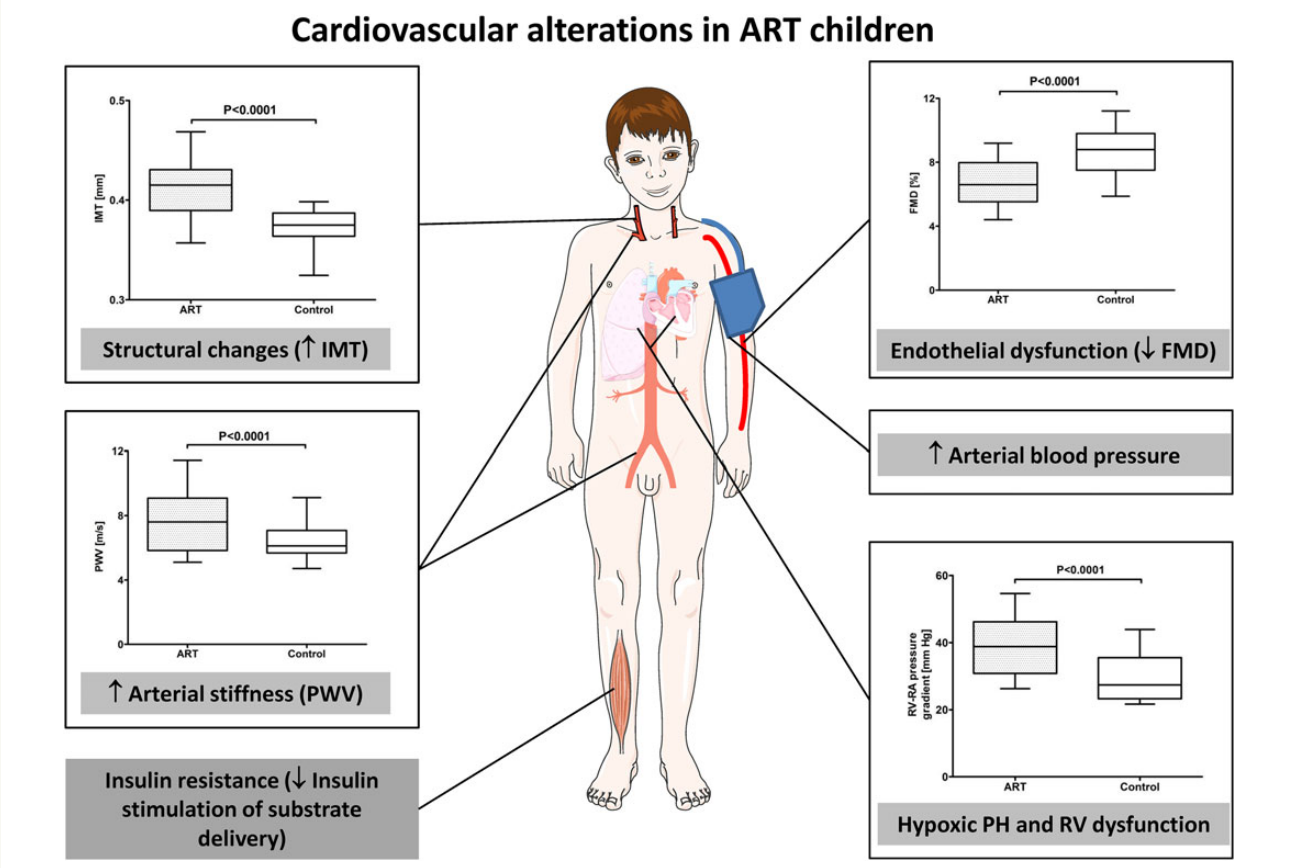

Figure 1 Assisted reproductive technology-induced cardiovascular alterations and consequences thereof in young apparently healthy children. Assisted reproductive technology alters systemic and pulmonary vascular function. There is evidence that systemic vascular dysfunction translates into increased arterial blood pressure and contributes to insulin resistance, whereas pulmonary vascular dysfunction predisposes to exaggerated hypoxic pulmonary hypertension and right ventricular dysfunction. IMT, carotid intima-media thickness; PWV, pulse wave velocity; FMD, flow-mediated dilation; PH, pulmonary hypertension; RV, right ventricle.

measurements show that in ART children, vascular dysfunction is associated with increased systolic and diastolic blood pressure compared with control children.[20] These differences in blood pressure between ART and control children are clinically important, since childhood blood pressure tracks into later life.[21]

Pulmonary hypertension

Pulmonary vascular dysfunction predisposes ART children to an exaggerated increase of pulmonary artery pressure during acute high-altitude exposure.[8,9] This suggests that ART persons permanently living at high altitude or suffering from diseases associated with chronic hypoxaemia may be predisposed to pulmonary hypertension. Moreover, exaggerated hypoxic pulmonary hypertension is a risk factor for high-altitude pulmonary oedema,[22,23] and ART needs to be taken into account when giving advice for the prevention of this problem.

Cardiac dysfunction and remodelling *in utero* that persist into adolescence

There is evidence for foetal programming of cardiac function in humans, as evidenced by remodelled and less efficient hearts in children born after foetal growth restriction.[24] In line with this concept, cardiac remodelling and dysfunction has been reported in ART compared with control fetuses.[18] These problems persisted in 6-month-old infants and predominated in the right heart.[18] The recent observation of right ventricular dysfunction under the stressful conditions of high-altitude exposure in ART adolescents who were born at term with normal birth weight suggests that cardiac alterations persist into adulthood.[25] These findings suggest that in addition to cause premature vascular ageing translating into increased arterial blood pressure, ART also alters cardiac function.

Insulin resistance

Insulin stimulates blood flow and substrate delivery to the skeletal muscles by an endothelium-dependent mechanism.[26–28] Defective nitric oxide-dependent insulin stimulation of blood flow and substrate delivery to skeletal muscle tissue leads to insulin resistance in experimental animal models and humans.[27–29] In line with this concept, a recent study demonstrated insulin resistance in young adults conceived by ART.[30] These findings suggest that ART-induced vascular dysfunction not only increases cardiovascular risk but may also facilitate diabetes later in life.

An often overlooked issue, when discussing consequences of ART on cardiovascular risk later in life, is that ART not only induces cardiovascular dysfunction per se but also significantly increases the prevalence of pathological events during foetal life that markedly increase cardiovascular risk in naturally conceived persons (*Table 1*).[4,6,31–34]

Assisted reproductive technology increases the risk of pathological events during foetal life which are known to increase cardiovascular risk in naturally conceived humans

Two examples serve to illustrate this problem. First, ART increases the risk of the mother to suffer from pre-eclampsia. Pre-eclampsia is known to induce generalized vascular dysfunction and hypoxic pulmonary hypertension in the naturally conceived offspring that is detectable already during childhood[5] and known to more than double the risk for stroke later in life.[4] Secondly, ART markedly increases the risk of premature birth.[6,34] In naturally conceived young adults born pre-term, left ventricular mass, an independent predictor of cardiovascular morbidity and mortality,[35] is increased.[36] Interestingly, it appears possible that perinatal interventions used to increase pre-term perinatal survival may contribute to some of the observed cardiovascular alterations.[37] For example, antenatal glucocorticoid administration to the mother to prevent respiratory distress in the neonate and intravenous lipid infusions used for nutrition of pre-term babies have been suggested to contribute to increased aortic stiffness and cardiac dysfunction observed in young adulthood in this population.[37] Finally, it should be noted that in many places, it is still current practice to implant more than one embryo at the time, in order to increase the success rate of ART. This practice results in a marked increase in the number of multiple pregnancies that are associated with a further increase of the risk of pre-eclampsia, pre-term birth, and low birth weight compared with singleton pregnancies.[6] Intriguingly, single embryo transfer with extended embryo culture, another procedure used to increase the success rate of ART, also increases the risk of pre-term birth.[38]

Collectively, these data indicate that even if ART would not cause any cardiovascular alteration itself, by increasing the prevalence of pathological foetal events known to increase cardiovascular risk later in life, ART is expected to significantly increase premature cardiovascular morbidity and mortality in the offspring.

Table 1 Unfavourable perinatal outcomes in assisted reproductive technology children known to be associated with increased cardiovascular risk in naturally conceived children

Perinatal outcomes in ART children		Long-term outcomes in naturally conceived children	
		Arterial hypertension	Ischaemic heart disease
	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
Pre-term birth (<37 weeks)	1.5 (1.5–1.6)	1.7 (1.3–2.2)	0.96 (0.80–1.16)
Low birth weight (<2500 g)	1.7 (1.6–1.8)	1.2 (1.1–1.3)	1.2 (0.88–1.44)
Small for gestational age	1.4 (1.3–2.1)	—	1.6 (1.2–2.2)

ART, assisted reproductive technologies.

Mechanisms underpinning assisted reproductive technology-induced cardiovascular dysfunction

Information from studies in assisted reproductive technologies mice

Premature vascular aging, arterial hypertension, insulin resistance and shortened life span in assisted reproductive technologies mice

Consistent with findings in humans, endothelium-dependent mesenteric artery dilation is defective and carotid artery stiffness and arterial blood pressure are increased in ART mice.[39] Moreover, ART mice display insulin resistance.[30] These data in normal mice further demonstrate the potential of ART to alter the cardiovascular and metabolic phenotype and strengthen the concept that these problems are related to ART per se. Moreover, and of potential importance for the long-term outcome of the ART population in humans, ART has consequences on the life span; when challenged with a Western style high-fat diet, the life span of ART mice was shortened by roughly 25% compared with control mice. Assisted reproductive technologies mice also provide unique insight into mechanisms underpinning ART-induced cardiovascular dysfunction.

In humans, it is difficult to definitively exclude the possibility that parental factors (i.e. older age of parents necessitating to resort to ART, transmission by ART of sterility-associated vascular dysfunction to the offspring) contribute to alteration of the cardiovascular phenotype in the ART population. The finding that in normal mice, ART induces premature vascular aging and arterial hypertension, strongly supports the contention that ART itself is an important factor in the programming of offspring cardiovascular dysfunction but it does not exclude the possibility that other mechanisms may also play a part, particularly in humans. The findings in mice also suggest that hormonal stimulation of the ovulation in the mother is not an important determinant of ART-induced vascular dysfunction.[39]

Role of epigenetic mechanisms underpinning cardiovascular dysfunction

There is increasing data indicating that epigenetic mechanisms play an important role in the foetal programming of phenotypic alterations that may determine the risk of adult diseases.[40,41] The term 'epigenetic' refers to changes of gene expression that are not related to modifications of the DNA sequence, but to altered methylation of cytosine residues, chromatin structure, or histone acetylation which modify the availability of DNA for transcription. During gametogenesis, fertilization, and early embryo development, the epigenome undergoes a series of changes,[41] suggesting that these stages are particularly vulnerable to epigenetic dysregulation. In line with this speculation, the methylation of the insulin-like growth factor-2 gene is altered in offspring of parents exposed periconceptually to famine,[42] and the frequency of rare imprinting disorders associated with epigenetic dysregulation is higher-than-expected in ART children.[40,43] This suggests that epigenetic mechanisms may underpin ART-induced cardiovascular dysfunction. Studies in ART mice provide direct support for this hypothesis.

Epigenetic alterations are maintained during cell division may persist throughout the life span of the individual[41,44,45] and sometimes be transmitted to the next generation.[46] Consistent with this concept, vascular dysfunction in the progeny of male ART mice mated with normal females is comparable with the one observed in the fathers and is associated with similar alterations of the methylation of genes in the vasculature.[39] More specifically, in ART mice the methylation of the promoter of the gene coding for endothelial nitric oxide synthase (eNOS) is altered in the aorta. This dysmethylation has important consequences, because it is associated with decreased eNOS expression, decreased eNOS mRNA expression, and lower nitric oxide plasma concentration in ART than in control mice.[39] Of note, the methylation of the endothelin-1 and the ACE gene is not altered in ART mice, indicating that ART does not induce global changes of DNA methylation of genes involved in vascular regulation.[39]

Histone deacetylase-inhibitors have been shown to reverse epigenetic and phenotypic changes induced by pathologic events during early life and to prevent transmission of these changes to the progeny.[39,47] Consistent with these observations, butyrate administration to adult male ART mice normalizes the methylation and expression of the eNOS gene in vascular tissue together with vascular responsiveness to acetylcholine and prevents the transmission of these alterations to the progeny.[39,47]

Collectively, these findings indicate that in mice, ART alters the cardiovascular phenotype by an epigenetic mechanism which changes the entire chain of events starting from altered eNOS methylation in the vasculature over endothelial dysfunction and premature vascular senescence to arterial hypertension and possibly premature mortality.[39]

Studies in mice also provide mechanistic information that could be important for ART in humans. Culture time may be a determinant of ART-induced epigenetic and cardiovascular alterations. In mice, endothelial dysfunction and arterial hypertension are comparable in ART mice generated by implantation of two-cell embryos and blastocysts, suggesting that the time needed to obtain two-cell embryos is sufficient to cause these changes.[39] In keeping with this observation, altered methylation patterns and gene expression are already detectable in two-cell embryos.[48] In humans, embryos are generally implanted at the blastocyst stage. The findings in mice suggest that in humans shortening the time lag between fertilization and implantation is unlikely to prevent ART-induced vascular dysfunction.

Current culture media used for ART are suboptimal, as shown by reduced pregnancy rates, viability, and growth of cultured compared with *in vivo* embryos.[39] Culture media may lack, or contain at different concentration, key metabolites and/or growth factors present in oviductal fluid and are not capable of reproducing the dynamic changes of oviductal fluid naturally occurring along the female reproductive tract. In line with this concept, suboptimal culture conditions *in vitro* compromise the ability of the embryo to maintain genetic imprinting.[48] Taken together, these findings suggest that suboptimal culture conditions contribute to ART-induced cardiovascular dysfunction.

Information from studies in humans

While mechanistic studies are difficult to perform in young apparently healthy children, these studies nevertheless provide some information on potential underlying mechanisms. Vascular dysfunction does not appear to depend on the technique used for ART, because it is similar in children born after *in vitro* fertilization and intra-cytoplasmic sperm injection and in children born after the transfer of frozen zygotes or the transfer of fresh embryos.[8] Assisted reproductive technology-induced cardiovascular dysfunction is also

not dependent upon the presence of low birth weight or prematurity, since it occurs in children born with normal birth weight and at term.[8,25] Parent-related factors, such as increased prevalence of cardiometabolic disease in sterile parents, maternal age, or hormonal stimulation of the ovulation, do not appear to play a role.[8] Sterility in the parents is not associated with vascular dysfunction that could be transmitted to the offspring by ART.[8] Finally, some[49] but not other studies[50] have suggested that parental psychological stress during pregnancy alters the cardiovascular phenotype in the offspring. To the best of our knowledge, this potential mechanism has not been investigated in ART. Collectively, these observations suggest that parent-related factors do not play an important role and that vascular dysfunction in offspring of ART is related to the procedure itself. Interestingly, an impairment of endothelial function was found not only in ART children but also in offspring of mothers suffering from pre-eclampsia,[5,7] suggesting that pathological events occurring during embryonic and late foetal life have similar long-term consequences for the circulation.[7]

How to avoid assisted reproductive technologies-induced cardiovascular dysfunction in the future and how to prevent the long-term consequences of this problem in those already born

Figure 2 depicts possibilities for interventions that may allow attaining this aim. First, studies in mice show that modification of culture media may attenuate ART-induced epigenetic and cardiovascular alterations.[39] This observation suggests that some culture conditions could be less detrimental for the offspring than others. In this context, it is very surprising that there exists no information on the exact composition of culture media used for ART and no detailed information on the ART-procedures used in fertility clinics. There is an urgent need to oblige suppliers and clinics to provide this information in order to identify procedures that may have less detrimental effects on cardiovascular function and long-term health in the offspring than others. The identification of best practice of ART will also need

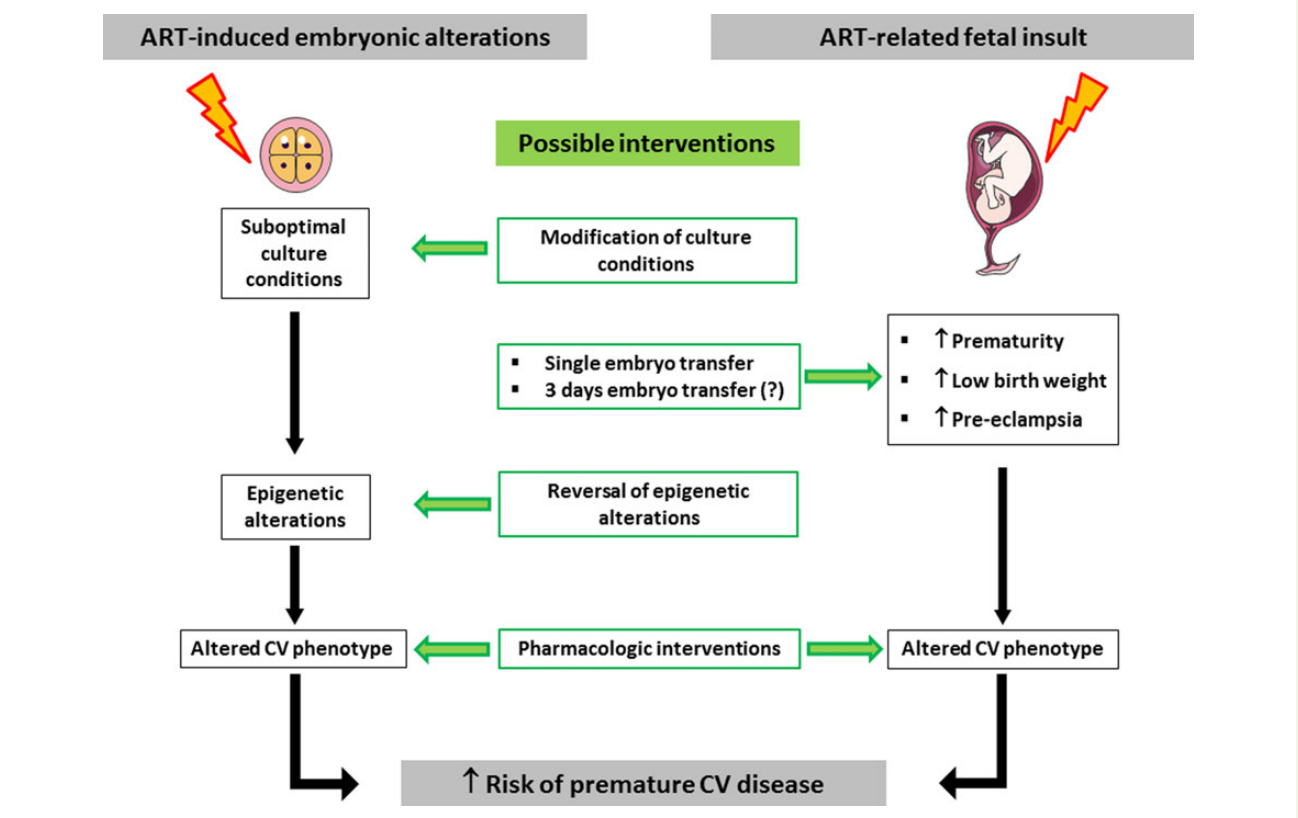

Figure 2 Mechanisms underpinning assisted reproductive technology-induced alteration of the cardiovascular phenotype and possibilities for prevention and/or intervention. Assisted reproductive technology alters the cardiovascular phenotype and increases cardiovascular risk in two ways. (i) Suboptimal culture conditions cause epigenetic changes in the embryo that result in an altered cardiovascular phenotype.[41] Improvement/modification of culture conditions may attenuate assisted reproductive technology-induced epigenetic alterations and administration of drugs that restore epigenetic changes reinstate a normal cardiovascular phenotype.[39] (ii) Assisted reproductive technology increases the prevalence of pathological events during the foetal period which are known to increase cardiovascular morbidity and mortality in naturally conceived persons.[6,34] Single embryo transfer is expected to eliminate the additional risk of foetal insults related to multiparity,[6,34] implantation of 3-day embryos appears to be associated with a lower risk of foetal insults than blastocyst transfer.[6] Finally, pharmacological interventions aimed at restoring a normal cardiovascular phenotype are expected to decrease ART-related cardiovascular risk in the offspring.[9]

long-term studies on outcome in the ART population. Surprisingly, funding bodies appear to have little interest in financing such studies. The reason(s) for this reluctance are not clear, but the enormous financial interests related to ART, the apparently healthy looking aspect of this young ART population, and considerations related to political correctness, to name just a few, may play a role.

Second, studies in ART mice show that administration of drugs that normalize altered methylation of genes implicated in cardiovascular regulation normalizes cardiovascular function, suggesting that interventions aimed at normalizing epigenetic alterations may decrease long-term cardiovascular risk in the ART population and prevent potential transmission of this risk to the progeny.[39]

Thirdly, single embryo transfer is expected to eliminate the additional augmentation of cardiovascular risk related to the increased prevalence of prematurity, low birth weight and pre-eclampsia associated with multiple gestation.[6]

Fourthly, early detection of altered cardiovascular function and cardiovascular risk factors in the ART population is expected to allow for timely intervention to prevent/postpone premature cardiovascular disease. For example, recent observations in children suggest that ART-induced vascular dysfunction is related to altered redox regulation and reversible.[9] In this regard, medical history taking needs to include questioning about mode of conception and foetal/perinatal complications, as more aggressive reduction of other, modifiable cardiovascular risk factors may be needed among some of these populations.

Conclusions and future perspectives

Many questions remain open regarding the exact underlying mechanisms involved in ART-induced alterations of cardiovascular function and its long-term consequences. What is already clear, however, is that ART emerges as a major novel cardiovascular risk factor expected to be present in 2–5% of the population in industrialized countries and with the potential to be transmitted to future generations.

Assisted reproductive technology alters cardiovascular function and increases cardiovascular risk in two ways. First, ART alters the cardiovascular phenotype by epigenetic changes that are induced during a period encompassing harvesting of oocytes and sperm, *in vitro* fertilization and embryo culture. Second, ART markedly increases the prevalence of prematurity, low birth weight and pre-eclampsia, perinatal conditions known to increase cardiovascular morbidity and mortality in naturally conceived humans. A major problem at this time is that given the young age of the ART population, we will not know before 20–30 years from now the exact consequences of ART-induced premature atherosclerosis on cardiovascular endpoints (arterial hypertension, stroke, myocardial infarction, hypoxic pulmonary hypertension, and related morbidity and mortality). Extrapolation from young populations with a similar risk profile and known prevalence of cardiovascular morbidity and mortality later in life suggest that they may be important.[7]

Key questions to be resolved are, what detrimental events at specific stages of the *in vitro* development induce epigenetic alterations that lead to cardiovascular dysfunction, what are the underlying mechanisms, and what are the means that may allow these alterations to be prevented/restored. What are the mechanisms by which ART facilitates pathological events during foetal life that increase the risk for cardiovascular disease in the offspring, and are there ways to prevent these events? Are there ART procedures that have less detrimental long-term cardiovascular effects than others? To attain these aims, there is an urgent need to oblige providers to fully document ART procedures and to initiate long-term studies on cardiovascular outcome. Last but not least, it should be noted here that ART represents a unique opportunity to study effects, underlying mechanisms and possibilities for prevention/reversal of epigenetically induced alterations of the phenotype that are expected to be of importance beyond the field of ART.

Assisted reproductive technology emerges as an important risk factor for long-term health with potentially major socio-economic consequences. Society and politics need to engage in a debate on the future of ART and the consequences of its exponential growth for public health. Profit-driven initiatives to promote social freezing in presumably fertile young women appear hardly defendable at this time, as is medicine's deliberate abandon of its fundamental principle of doing no harm (to future children). What is needed now is to understand the mechanisms underpinning ART-induced alteration of the cardiovascular phenotype, improve the procedure, and establish its long-term safety, and, while awaiting the results, use ART parsimoniously.

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