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Vascular Health of Couples Undergoing Assisted Reproductive Technology and their Offspring: Insights from the Munich heARTerY Study

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List of abbreviations

ART	Assisted reproductive technology
CHD	Coronary heart disease
CVD	Cardiovascular disease
GIFT	Gamete intrafallopian transfer
GnRH	Gonadotropin-releasing hormone
HICs	High-income countries
ICMART	International Committee Monitoring Assisted Reproductive Technologies
ICSI	Intracytoplasmic sperm injection
IVF	In vitro fertilization
LMICs	Low- and middle-income countries
PCOS	Polycystic ovary syndrome
PID	Pelvic inflammatory disease
POF	Premature ovarian failure
TESE	Testicular sperm extraction
TRT	Testosterone replacement therapy
WHO	World Health Organization
ZIFT	Zygote intrafallopian transfer

List of publications

1. **Li P**, Langer M, Vilsmaier T, Kramer M, Sciuk F, Kolbinger B, et al. Vascular health of fathers with history of intracytoplasmic sperm injection. Aging Male. 2024;27(1):2360529.

2. **Li P**, Langer M, Vilsmaier T, Kramer M, Sciuk F, Kolbinger B, et al. Vascular Health of Females with History of Assisted Reproductive Technology. J Cardiovasc Dev Dis. 2024;11(2).

3. Oberhoffer FS, Langer M, **Li P**, Vilsmaier T, Sciuk F, Kramer M, et al. Vascular function in a cohort of children, adolescents and young adults conceived through assisted reproductive technologies-results from the Munich heARTerY-study. Transl Pediatr. 2023;12(9):1619-33.

4. **Li P**, Haas NA, Dalla-Pozza R, Jakob A, Oberhoffer FS, Mandilaras G. Energy Drinks and Adverse Health Events in Children and Adolescents: A Literature Review. Nutrients. 2023;15(11).

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11. **Li P**, Mandilaras G, Jakob A, Dalla-Pozza R, Haas NA, Oberhoffer FS. Energy Drinks and Their Acute Effects on Arterial Stiffness in Healthy Children and Teenagers: A Randomized Trial. J Clin Med. 2022;11(8).

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13. Bačová M, **Li P**, Arnold L, Dalla-Pozza R, Haas NA, Oberhoffer FS. Cardiovascular Care of Turner Syndrome Women in Germany: Where Do We Stand?-Results from an Online Patient Survey. Healthcare (Basel). 2022;10(3).

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15. **Li P**, Bačová M, Dalla-Pozza R, Haas NA, Oberhoffer FS. Prevalence of Bicuspid Aortic Valve in Turner Syndrome Patients Receiving Cardiac MRI and CT: A Meta-Analysis. Congenital Heart Disease. 2022;17(2).

16. **Li P**, Jiang J, Xi Q, Yang Z. An ADAMTS13 mutation that causes hereditary thrombotic thrombocytopenic purpura: a case report and literature review. BMC Med Genomics. 2021;14(1):252.

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1. Contribution to the publications

1.1 Contribution to paper I

- Methodology
- Software
- Validation
- Formal analysis
- Investigation
- Data curation
- Writing – original draft preparation
- Writing – review and editing
- Visualization
- Project administration

1.2 Contribution to paper II

- Collection and assembly of data
- Data analysis and interpretation
- Manuscript writing
- Final approval of manuscript

1.3 Contribution to paper III

- Methodology
- Software
- Validation
- Formal analysis
- Investigation
- Data curation
- Writing – original draft preparation
- Writing – review and editing
- Visualization
- Project administration

2. Introductory summary

2.1 Background

Infertility generally refers to male or female reproductive disorders, defined as the inability to conceive or become pregnant after one year or even more time of regular unprotected intercourse [1]. The World Health Organization (WHO) estimates that 48.5 million couples globally and 186 million ever-married females in developing countries suffer from infertility [2, 3]. The numbers can be greatly underestimated due to cultural and social factors.

Infertility can be classified as either primary or secondary. Primary infertility refers to individuals who have never conceived with a global prevalence estimated at 9.6% [4]. Secondary infertility refers to the inability to conceive after having had at least one prior pregnancy with a prevalence of 6.5% [4]. The prevalence of infertility differs little across countries with different income levels with an estimated prevalence of 17% in both high-income countries and low- and middle-income countries (4).

The significant impacts on the health and well-being of infertility include not only involuntary childlessness but also the psychological burden on individuals and families and the economic burden of related treatments [5]. In addition, the decline in fertility has a direct impact on population and social structures and poses challenges to the formulation of corresponding population policies [6]. More importantly, the pathogenesis of infertility is complex and has not been fully elucidated. It affects not just the reproductive system but is often accompanied by the development of complex diseases of other system such as cardiovascular diseases (CVD) or other health issues [7, 8].

To cope with the challenges brought by infertility, assisted reproductive technology (ART) has emerged. ART has greatly alleviated the burden of infertility on individuals, families and society. However, due to the relatively short time of its application, few studies have investigated the overall health of individuals and families using ART technology. Existing research tended to focus more on the cardiovascular phenotype of offspring conceived through ART, but the results are often vague and contradictory.

2.2 Female infertility

In 2010, the global prevalence of primary infertility among females aged 20-44 was assumed to be 1.9% and 10.5% of females had experienced secondary infertility [2].

2.2.1 Pathophysiology of female infertility

Different factors may lead to female infertility, which can be categorized as follows:

- Tubal disorders: damaged, blocked or scarred fallopian tubes keep sperm from getting to the egg or block the passage of the fertilized egg into the uterus. Potential reasons include untreated sexually transmitted infections that lead to pelvic inflammatory disease (PID), which accounts for the most common infectious cause of female infertility [9, 10]. Complications of unsafe abortion, puerperal sepsis or abdominal/pelvic surgery including ectopic pregnancy surgery may also damage the tubal function and lead to fallopian tube blockage [10].
- Uterine disorders: uterine infertility is related to conditions of space-occupying lesions or reduced endometrial receptivity, which prevents the fertilized egg from implanting. The main causes are inflammatory, mostly endometriosis which is frequently an idiopathic uterine disease [1]; congenital abnormalities such as uterine septum [11]; benign such as uterine fibroids.
- Ovulation disorders: 25% of female infertility is caused by infrequent or no ovulation. Ovulation disorders can be detailed into four categories [9]: a. hypogonadotropic hypogonadal anovulation (hypothalamic amenorrhea); b. normogonadotropic normoestrogenic anovulation: the most common type is polycystic ovary syndrome (PCOS), which accounts for about 80% of all cases of anovulation [12]; c. hypergonadotropic hypogonadal anovulation (premature ovarian failure, POF), refers to natural menopause in female before the age of 40 [1]; d. hyperprolactinemic anovulation: most commonly associated with pituitary adenoma.

2.2.2 Female infertility and CVD

CVD is the primary cause of mortality in both females and males, accounting for one-third of all deaths worldwide [13]. Growing evidence shows that the pathophysiology of CVD in females differs from males, suggesting sex differences in CVD etiology. Female-specific reproductive factors such as premature menopause, adverse pregnancy outcomes and infertility are considered to be associated with increased CVD morbidity and mortality [14-17].

For example, research on the long-term cardiovascular health of PCOS females has gathered attention due to its common clinical features such as insulin resistance, obesity and hyperandrogenism. Wild et al. showed that CVD risk factors such as diabetes, hypertension, hypercholesteremia were more prevalent in PCOS females compared to control peers [18]. Mani et al. revealed that PCOS females are at an elevated risk of experiencing Type 2 diabetes (T2D), myocardial infarction and angina [19]. A retrospective study conducted in western Australia also found that PCOS was associated with subsequent more prevalent diabetes, obesity, cerebrovascular disease and ischemic heart disease [20].

In contrast, there are studies suggesting PCOS females might not be at a higher risk of developing CVD or CVD mortality, even with a higher BMI, which questions PCOS as a later-life CVD risk factor [21-23]. Meanwhile, the European Society for Reproductive Medicine and Embryology stated that the evidence on the links between PCOS and CVD are limited.

As for endometriosis, another major cause of female infertility, it has been confirmed that inflammatory factors are increased in the peripheral blood of females with endometriosis, suggesting that endometriosis may increase the risk of CVD [24]. An earlier prospective cohort study of the Nurses' Health Study II (one of the largest and longest running investigation of factors that influence females' health) also suggests that females with endometriosis might represent a higher risk group of having (coronary health disease) CHD, particularly at a young age [25]. Consistent with this, it also suggests that endometriosis is linked to a higher risk of developing hypercholesterolemia and arterial hypertension [26].

In the context of the Framingham Heart Study, Mahalingaiah et al. observed an increasing in both BMI and waist circumference among females experiencing general infertility, without specific diagnoses. They suggested that this trend might contribute to increased incidences of obesity and diabetes in infertile premenopausal females.[27]. In a study investigating the relationship between females with unexplained infertility and CVD, Verit et al. found that among 65 unexplained infertile females, blood lipid profiles were elevated, suggesting that females with unexplained infertility may potentially be at increased risk of CVD [28]. Consistent with these findings, another cross-section study conducted by Gleason et al. found that U.S. females who reported infertility were 1.83 times more likely to develop a cardiovascular event than females who had never reported infertility, suggesting that the infertility experiences at any stage during a female's reproductive years might be potentially associated with cardiovascular health issues later in life. [29]. The study by Murugappan et al. showed that infertile females had an increased risk of atherosclerotic CVD compared with their fertile control peers. Nulliparous female, especially those who have experienced miscarriage, are at a much higher risk of developing atherosclerotic CVD [30]. These findings suggest that infertility may be associated with atherosclerotic CVD risk in these more extreme infertility phenotypes. Recently, a large prospective cohort study demonstrated a significant positive correlation between infertility and the development of heart failure with preserved ejection fraction and appears to be independent of other traditional CVD risk factors [31].

Although female infertility is gradually considered an indicator of overall health, its role as a reproductive factor has been underrecognized in terms of cardiovascular risk. The exact link between female infertility and CVD remains unclear and sometimes the evidence can be contradictory.

2.3 Male infertility

Due to cultural factors, survey bias and the challenges in defining male infertility, it remains difficult to accurately determine the prevalence of infertile males [32]. According to a large retrospective epidemiological study, Agarwal et al. estimated that the global prevalence of male infertility ranges from 2.5% to 12% [33].

2.3.1 Pathophysiology of male infertility

Male infertility can be caused by pre-testicular, testicular, and post-testicular factors [34]:

Pre-testicular infertility: hormonal imbalance is probably the most prevalent cause of pre-testicular infertility. Any drug, tumor or disease affecting the pituitary or hypothalamus can cause male infertility by altering the secretion of gonadotropin-releasing hormone (GnRH) or causing gonadotropin and testosterone deficiency, such as hypogonadotropinemia and hyperprolactinemia [35]; obesity and excessive exercise, as well as exogenous testosterone supplementation are also causes of hormonal imbalance [36]. In addition, untreated celiac disease may also lead to pre-testicular infertility [37].

Testicular infertility: mainly caused by impaired testicular function and sperm production, such as orchitis caused by epidemic mumps, testicular tumors and varicocele [38, 39]. Genetic and chromosomal abnormalities such as Klinefelter's syndrome, Y chromosome deletion, and USP26 enzyme deficiency are also important causes of testicular infertility [40].

Post-testicular infertility: refers to obstruction of the genital tract caused by injury or infection of the genital tract resulting in a failure to ejaculate semen, such as prostatitis, gonorrhea and/or chlamydia infection or following bladder neck surgery [35]; cystic fibrosis gene carriers may also suffer from infertility due to the possibility of congenital bilateral vas deferens absence [40].

2.3.2 Male infertility and cardiovascular disease

Although the connection between individual’s overall health and male infertility has received widespread attention [41], research has mainly focused on the risk of testicular and prostate cancer, with less attention paid to the risk of non-malignant chronic diseases such as CVD; and similar to research on female infertility and CVD risk, the existing evidence is also contradictory.

One theory that has reached a relatively high level of consensus is that testosterone levels in infertile male play an important role of the development of CVD. Endogenous testosterone concentrations in male are inversely associated with CVD and all-cause mortality [42]. Low levels of testosterone have been proven to predispose male to develop T2D and metabolic syndrome [43]. Oh et al. demonstrated that decreased testosterone levels were related to an increased risk of T2D in males and a decreased risk of T2D in females [44], which is also consistent with our discussion of cardiovascular risk factors for female infertility above. Additionally, a two-year randomized controlled trial conducted in Australia demonstrated that testosterone replacement therapy (TRT) reduced the proportion of participants with T2B [45]. However, the supplementation of exogenous testosterone remains controversial. A recent clinical trial in the United States found that TRT did not improve glycaemic control in males with hypogonadism and pre-diabetes or diabetes [46].

Low levels of endogenous testosterone have also been associated with atherogenic dyslipidemia. Several studies have found that high levels of endogenous testosterone concentrations in male are associated with a more favorable profile of CVD risk factors including a higher high-density lipoprotein and a lower apolipoprotein B/A1 ratio [47, 48]. Meanwhile, studies have also shown a negative correlation between testosterone levels and triglycerides and total cholesterol which are considered the risk factors for CVD [49, 50]. Interestingly, however, an analysis from the Framingham Heart Study found no connection between plasma lipids level and testosterone concentrations in elderly males [51].

Recent data also suggest that impaired semen quality is also associated with dyslipidemia and T2B[52-54]. Schisterman et al. showed in a prospective cohort study that elevated serum total cholesterol, free cholesterol, and phospholipid levels may affect semen parameters, especially sperm head morphology[54]. Ghsemi et al. demonstrated that the zinc and magnesium contents in the seminal plasma of diabetic males, which were positively correlated with sperm motility and morphology, were lower than those in the diabetic group [55]. Moreover, males diagnosed with hypertension were shown to have reduced semen volume, sperm motility and total sperm count compared to males with normal blood pressure [56]. Eisenberg et al. found a higher rate of semen abnormalities in males with hypertension, peripheral vascular and cerebrovascular disease, and non-ischemic heart disease [57].

Notably, obesity is another important link in the association between male infertility and CVD, similar to female infertility. Obesity can not only directly cause erectile dysfunction [58], but also alter sperm morphology and motility resulting in infertility [59]. Additionally, obesity may affect male reproduction by causing hormonal disturbances leading to a lower testosterone levels through systemic inflammation and oxidative stress [60].

The relationship between male fertility and cardiovascular health and even overall health is very complex, and there are many confounding factors such as smoking, alcohol consuming, diet, exercise level and other lifestyle factors [61, 62]. Whether infertility specifically causes elevated risk of CVD or whether the two are causally related is still a topic worth discussing.

2.4 Assisted Reproductive technology

Individuals or couples struggling with infertility tend to seek medically supported fertility treatments, of which ART is a widely chosen option. ART refers to a range of medical procedures that involve the manipulation of human gametes or zygotes and these procedures can be categorized into zygote intrafallopian transfer (ZIFT), gamete intrafallopian transfer (GIFT), in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), etc [63].

The birth of Louise Brown in 1978 represented the first successful application of IVF, which has since become one of the most common ART methods and is widely used around the world. The IVF procedure normally begin with ovarian stimulation to retrieve the oocyte. The oocyte is then fertilized with sperm in the laboratory. The fertilized embryo is transferred to the uterus after three to five days [64]. The subsequent introduction of GIFT and ZIFT technologies further enriched the options for ART. Afterward in 1992, the successful application of ICSI enabled more couples to find a solution that suited their situation [65]. ICSI is considered a component of IVF and is more commonly used for male infertility while the technology allows a single live sperm to be injected directly into the egg [66].

Based on the latest preliminary global report by the International Committee for Monitoring Assisted Reproductive Technologies (ICMART), approximately 3200 thousand ART cycles were performed from 78 countries [67]. The performed ART cycles maintain growth in most of the regions such as Europe, North America and Asia [67]. In 2018, around 660 thousand ART-deliveries with around 770 thousand ART-newborns were conducted [67].

2.4.1 ART and cardiovascular health of females

As every new technology brings exciting developments, accompanying concerns and public controversies also rise simultaneously. These concerns may generally involve ethical, social, legal and medical aspects and one of the concerns is that the technique itself might pose a long-term cardiovascular risk for infertile females. During the treatment of

ART, females often have to utilize hormonal superovulation medications such as Clomiphene citrate, Gonadotropins, GnRH to stimulate follicle development and induce ovulation [68]. The application of these hormonal medications might have a potential influence on the cardiovascular system and might increase the risk of developing thrombosis [69-71]. Notably, certain conditions caused by using ovulation inducing medication such as ovarian hyperstimulation syndrome; bone loss due to GnRH agonists or even the development of ovary cancer share some common pathways with the development of CVD [72-74]. In addition, multiple pregnancy appears to be more common in females who have undergone ART than in those who conceive spontaneously, possibly due to the nature of technologies applied, particularly IVF, where multiple embryos are often implanted to increase the chance of pregnancy, which could sometimes lead to multiple pregnancy [75]. Studies have shown that twin pregnancies is the independent risk factor of maternal preeclampsia while preeclampsia is associated with the increased risk of future incidents of developing CVD such as heart failure and coronary heart disease and stroke [76, 77].

Despite the speculation and assumption, sparse research has investigated the association of ART with long-term CVD risk in females and the data remain inconclusive. A large cohort study conducted in Sweden found that females who underwent IVF had an increased risk of developing hypertension and stroke, whereas another Canadian study found that females who delivered after ART treatment were not at increased risk of CVD [78, 79].

2.4.2 ART and cardiovascular health of males

As mentioned above, ICSI has been chosen by an increasing number of infertile males to improve fertilization rates due to its technical characteristics [80]. It may even be the only option for most infertile males to become the genetic father of their children, given the limited effects of empirical drug and IVF treatment [81].

Compared to females, it is technically much easier for males to obtain gametes. In addition to natural sperm retrieval methods, the main sperm retrieval procedures include testicular sperm extraction (TESE), which involves obtaining sperm directly from a small biopsy of testicular tissue by dissection, micro-TESE, testicular sperm aspiration without incision, microsurgical epididymal sperm aspiration and percutaneous epididymal sperm aspiration [82].

Eliveld et al. found in a meta-analysis a transient but statistically significant decrease in total testosterone levels in males undergoing TESE, suggesting that male may be at risk of transient hypogonadism following TESE, but there is insufficient evidence to show whether males actually develop clinical symptoms in the setting of a decrease in serum testosterone levels [83]. A small study in the Netherlands found that males with infertility experience psychosocial problems as a result of infertility and ICSI treatment [84], which may also potentially contribute to the development of CVD.

Although we have discussed the risk and possible causes of cardiovascular disease in infertile males in the previous chapter, data on the cardiovascular health of males undergoing ICSI, especially over a longer period of time, are still not available.

2.4.3 ART and cardiovascular health of offspring

It is assumed that ART might have adverse effects on the long-term health outcomes in its offspring. Particularly negative cardiovascular health effects are presumed. The human ART population is still at a relatively young age and the long-term effects of ART-induced changes on cardiovascular health remain largely unclear. ART procedure involves a sequence of handlings on early embryos, which might be particularly susceptible to external disturbances and stimulations. Because of the epigenetic change and environmental influences during fetal development, it is assumed that offspring conceived by ART may be at an increased risk of premature CVD [85]. Scherrer et al. suggested that offspring conceived through ART display generalized vascular dysfunction represented by endothelial dysfunction within circulatory system [85].The same team reevaluated the vascular function of the same study cohort and they found that ART-induced early-onset vascular degeneration sustained in healthy adolescents and young adults without other identifiable classical CVD risk factors five years following the initial evaluation, [86]. In animal models, endothelial dysfunction, increased arterial stiffness, arterial hypertension and even shortened lifespan have also been observed in ART mice [87]. Although the study by Scherrer et al. denied that changes in cardiovascular phenotypes in ART participants are attributable to genetic contributors, but rather related to the ART procedure itself [85, 86], parental genetic factors still cannot be ignored. Infertile couples with underlying compromised cardiovascular health might potentially pass on risk factors to their offspring [88]. One the other hand, some studies did not present the unfavorable cardiovascular outcomes in ART offspring. Wijs et al. demonstrated in their study that most cardiometabolic and vascular health measures of ART adolescents are similar or even more favorable, than those of age-matched control peers conceived spontaneously [89]. Recently, a multi-cohort analysis focusing on cardiovascular and metabolic outcomes in ART-conceived offspring demonstrated no significant differences in the alterations in cardiometabolic outcomes between ART offspring and their control peers [90].

Considering the younger age of the human ART population, most studies have focused on children, with few studies on adolescence and beyond. At the same time, many studies focus on only one or a few cardiovascular parameters per study and long-term health cardiovascular outcome data is limited.

2.5 Study summary

To overcome the above-mentioned scarcity and inconsistency of health data regarding ART individuals and their offspring, we therefore conducted a single-center retrospective cohort study, the Munich heARTerY study (Assisted Reproductive Technologies and their effects on heart and arterial function in Youth). This study included couples who have undergone ART and their offspring within the greater Munich area as well as age- and sex-matched spontaneous control families. We conducted detailed assessments of all participants, including physical examination, medical history, reproductive process, diet, physical exercise and vascular health and performed statistical analysis accordingly.

This cumulative dissertation “Vascular Health of Couples Undergoing Assisted Reproductive Technology and their Offspring: Insights from the Munich heARTerY Study” aims to explore the potential vascular outcomes of the heARTery study from three perspectives: female, male and offspring and to comprehensively analyze and discuss the corresponding results.

As a result, our studies did not show significant alterations in vascular function in females or males who underwent ART, and vascular function did not degenerate more severely in ART offspring than in spontaneous controls. However, the potential higher prevalence of elevated Lp(a) in both ART females and offspring warrants further attention, as it might contribute to impaired vascular health in this cohort. To enhance the precision of cardiovascular risk stratification, large-scale, multicenter trials with expanded ART sample sizes are required in the future. Moreover, future studies should not be limited to focusing solely on the phenotype of the ART subjects; instead, studies should delve deeper into genotypes to explore the potential genetic association between the application of ART, infertility and vascular health.

3. Paper I

Journal of
Cardiovascular Development and Disease

Article

Vascular Health of Females with History of Assisted Reproductive Technology

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Abstract: The use of assisted reproductive technologies (ART) for the treatment of infertility is gaining popularity. Limited data on the overall vascular health of females with history of ART are available. This pilot study aimed to investigate the overall vascular health of females with history of ART compared to individuals who conceived spontaneously. The assessment of overall vascular health included the measurement of brachial blood pressure, central blood pressure, and pulse wave velocity, as well as the evaluation of the arterial stiffness and carotid intima-media thickness (cIMT) of the common carotid arteries. Conventional blood lipids including lipoprotein a (Lp(a)) were also determined. In total, 45 females with history of ART and 52 females who conceived spontaneously were included (mean age: 47.72 ± 5.96 years vs. 46.84 ± 7.43 years, $p = 0.525$). An initial comparison revealed a significantly higher prevalence of elevated Lp(a) in ART females ($p = 0.011$). However, after multiple comparison correction, the significant result disappeared ($p = 0.132$). Within the cohort of ART females, no significantly higher cardiovascular risk was detected regarding vascular function. The potentially higher prevalence of elevated Lp(a) in ART females must be further investigated in future studies, as it might contribute to the impaired reproductive process in this cohort.

Keywords: infertility; assisted reproductive technologies; females; vascular health

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1. Introduction

Infertility refers to the inability of a person or a couple to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [1]. Infertility remains highly prevalent, with approximately 48 million couples worldwide suffering from it [2]. Several conditions can cause female infertility such as tubal factor infertility, ovulatory dysfunction, and endometriosis; however, in 40% of cases, the underlying cause remains uncertain [3]. People affected by infertility may seek a fertility treatment, from which assisted reproductive technologies (ART) are widely chosen. According to the latest report of the International Committee for Monitoring Assisted Reproductive Technology, more than 3 million ART cycles were performed in 2018 [4]. Different approaches to ART exist, including in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), and gamete intrafallopian transfer (GIFT) [5].

In the past, adverse health effects of ART on the offspring's cardiovascular health were reported [6–8]. It was suggested that children born via ART have increased cardiovascular morbidity, but the pathophysiological reasons behind this remain unclear [9]. In addition, it has been proposed that infertile females might display an increased cardiovascular risk [10,11]. Murugappan et al. demonstrated that compared to their fertile peers, infertile females display a higher risk of atherosclerotic cardiovascular disease [10]. Moreover, Farland et al. found that females with a history of infertility have a 1.13-times higher risk of developing cardiovascular diseases compared to controls [11]. Females who suffer from infertility and thus seek ART treatment could potentially pass down certain cardiovascular risk factors to their offspring. However, to the best of our knowledge, limited data on the overall vascular health of females with history of ART are available.

Therefore, we conducted a pilot study aiming to investigate the overall vascular health of females with history of ART compared to individuals who conceived spontaneously.

2. Materials and Methods

2.1. Ethical Approval

This study was approved by the Ethics Committee of the Medical Faculty of LMU Munich on 27 December 2020 (Ethikkommission der Medizinischen Fakultät der Ludwig-Maximilians-Universität München, Pettenkoferstraße 8a, 80336 Munich, Germany; approval number: 20-0844). The study was conducted following the Declaration of Helsinki. Prior written informed consent was obtained from all study participants.

2.2. Study Design

Females who were treated at the Division of Gynecological Endocrinology and Reproductive Medicine, Division of Obstetrics and Gynecology, University Hospital, LMU Munich (Munich, Germany) and successfully conceived a child between 1995 and 2017 with the help of ART were informed in writing about the ongoing study. Age-matched individuals who conceived spontaneously were enrolled by public calls (e.g., flyers and posters in schools, sports clubs, etc.) within the greater Munich area (Germany).

As this pilot study was part of the Munich heARTerY-study (assisted reproductive technologies and their effects on heart and arterial function in youth), inclusion and exclusion criteria focused primarily on the pediatric cohort [12–16]. To assess whether parents with history of ART displayed an increased cardiovascular morbidity, no exclusion criteria were applied for the parental cohort.

Study participants were examined at the Division of Pediatric Cardiology and Intensive Care, University Hospital, LMU Munich (Munich, Germany) from May 2021 to March 2022.

2.3. Medical History, Physical Examination, Course of Pregnancy and Birth, Level of Education

The self-reported medical history of each study participant, focusing on the presence of cardiovascular disease (e.g., arterial hypertension, disorders of glucose, and/or lipid metabolism), was assessed. In addition, self-reported smoking status and the regular use of medication were evaluated. All study participants underwent a physical examination. Body weight (kg), height (cm), waist circumference (cm), and hip circumference (cm) were measured in all subjects. The ratio of waist to hip circumference was determined. In addition, body mass index (BMI, kg/m^2) was calculated. The weight classification was defined as follows: underweight if BMI < 18.5 kg/m^2, normal weight if BMI $\geq$ 18.5 kg/m^2 but <25 kg/m^2, overweight if BMI $\geq$ 25 kg/m^2 but <30 kg/m^2, and obese if BMI $\geq$ 30 kg/m^2 [17]. The following data regarding the course of pregnancy and birth were obtained from electronic and/or maternity records: conception mode (IVF, ICSI, GIFT, spontaneous conception), maternal age at birth (years), BMI at conception (kg/m^2), presence of multiple pregnancy, weeks of gestation (weeks), maternal blood pressure during pregnancy $\geq$ 140/90 mmHg. Self-reported maternal educational level was determined according to the German education system: no school leaving qualification (0), lower secondary school leaving certificate (1), intermediate secondary school leaving certificate (2), general qualification for university entrance (3), completed apprenticeship (4), completed university degree (5).

2.4. Adherence to the Mediterranean Diet

High adherence to the Mediterranean diet has a positive effect on cardiovascular morbidity [18]. To assess participants' adherence to the Mediterranean diet, the validated

14-item Mediterranean diet assessment tool developed by Martínez-González et al. was translated into German and applied [19]. According to Martínez-González et al., a score ≤ 7 was considered low adherence to the Mediterranean diet and a score > 7 high adherence to the Mediterranean diet [19].

2.5. Level of Physical Activity and Sedentary Behavior

To determine the level of physical activity in study participants, the German version of the Global Physical Activity Questionnaire (GPAQ) officially provided by the World Health Organization (WHO) was used [20]. Picture cards were presented for each activity type [20]. Total and recreational metabolic-equivalent (MET) minutes per week were calculated according to GPAQ recommendations [20]. Adult subjects met WHO recommendations if $\geq$600 total MET-minutes per week were achieved [20]. Furthermore, study participants were asked how many times per week muscle-strengthening activities were performed and how much time was spent per day with sedentary activities.

2.6. Vascular Function

2.6.1. Pulse Wave Analysis

An oscillometric blood pressure device (Mobil-O-Graph®, IEM GmbH, Aachen, Germany) was utilized to measure brachial systolic blood pressure (SBP, mmHg), brachial diastolic blood pressure (DBP, mmHg), mean arterial pressure (MAP, mmHg), heart rate (HR, bpm), central SBP (cSBP, mmHg), central DBP (cDBP, mmHg), augmentation index averaged to a heart rate of 75 bpm (AIx@75, %), and pulse wave velocity (PWV, m/s). Elevated SBP and DBP were present if SBP $\geq$ 130 mmHg and DBP $\geq$ 85 mmHg [21]. Cuff sizes were chosen based on participants' right upper arm circumference. Study participants were asked to remain in a supine and calm position $\geq$ 5 min before and during the examination. Three consecutive measurements were performed and averaged.

2.6.2. Sonography of the Common Carotid Artery

Sonography of both common carotid arteries (CCA) was performed by one investigator for all study participants using either a Philips iE33 xMatrix or a Philips Epiq 7G ultrasound device (Philips Healthcare, Amsterdam, The Netherlands). During the examination, study participants were asked to remain in a supine position, and the neck was extended to a 45° angle and turned to the opposite side of examination [22]. Offline analysis was conducted by one investigator.

Peak Circumferential Strain, Peak Strain Rate and Arterial Distensibility

The area directly below the carotid bifurcation was examined in the short-axis view using a 3–8 MHz sector array transducer (Philips Healthcare, Amsterdam, The Netherlands). Three consecutive loops were recorded under three-lead ECG tracing and transferred to a separate workstation (QLAB Cardiovascular Ultrasound Quantification Software, version 11.1, Philips Healthcare, Amsterdam, The Netherlands) for offline analysis. The software's SAX-A function was utilized. The vascular region of interest (ROI) was manually adjusted to precisely track the vessel's wall and to avoid tracking of the perivascular tissue. Pixels of the vascular ROI were then tracked two-dimensionally over the cardiac cycle. Peak circumferential strain (CS, %) and peak strain rate (SR, 1/s) were determined manually. To improve data validity, the average of three measurements was computed for each CCA side. Arterial distensibility ($\text{mmHg}^{-1} \times 10^{-3}$) was calculated using the following formula [23]:

$$\text{Arterial Distensibility} = \frac{2 \times \text{Peak Circumferential Strain}}{\text{Systolic Blood Pressure} - \text{Diastolic Blood Pressure}}$$

In addition, CS, SR, and arterial distensibility of the right and left CCA were averaged.

Carotid Intima-Media Thickness

At the level of carotid bifurcation, both CCAs were evaluated in long-axis view utilizing a 3–12 MHz linear array transducer (Philips Healthcare, Amsterdam, The Netherlands). Three consecutive loops were recorded under three-lead ECG tracing and transferred to a separate workstation (QLAB Cardiovascular Ultrasound Quantification Software, version 11.1, Philips Healthcare, Amsterdam, The Netherlands) for offline analysis. At end-diastole (R wave in ECG), the carotid intima-media thickness (cIMT, mm) was assessed semi-automatically for each side. The ROI was set proximal to the carotid bifurcation and the length was adjusted to 10 mm. Three measurements were performed on each side, and the average cIMT for the right and left CCA was calculated. Moreover, the mean cIMT value of both CCAs was assessed.

Stiffness Index β

The abovementioned sonographic study protocol was applied. M-mode examinations of both CCAs were performed in long-axis view under three-lead ECG tracking using a 3–12 MHz linear array transducer (Philips Healthcare, Amsterdam, The Netherlands). The end-diastolic diameter (dD, mm) and end-systolic diameter (sD, mm) of both CCAs were measured offline on a separate workstation (IntelliSpace Cardiovascular Ultrasound Viewer, Philips Healthcare, Amsterdam, The Netherlands).

Stiffness index β was defined as [23]:

$$\text{Stiffness Index } \beta = \frac{\ln\left(\frac{\text{SBP}}{\text{DBP}}\right)}{\Delta D/dD}$$

2.7. Blood Lipid Profile

To evaluate the blood lipid profile, total cholesterol (TC, mg/dL), low-density lipoprotein cholesterol (LDL-C, mg/dL), high-density lipoprotein cholesterol (HDL-C, mg/dL), non-high-density lipoprotein cholesterol (non-HDL, mg/dL), triglycerides (mg/dL), and lipoprotein a (Lp(a), mg/dL) were assessed. A fasting period of $\geq$4 h was requested before blood drawing. The presence of elevated conventional blood lipids was defined according to adult recommendations [24,25]. A Lp(a) $\geq$ 50 mg/dL was defined as increased [26].

2.8. Statistical Analysis

As this was a pilot study, a prior sample size calculation was not feasible. A chi-square test was applied to compare nominal data. Continuous parameters were tested for normality using the Kolmogorov–Smirnov test and the Shapiro–Wilk test. In case of normal distribution, an unpaired t-test was used. For non-normally distributed continuous variables, the Mann–Whitney U test was utilized. Normally distributed data are presented as means $\pm$ standard deviation (SD) and non-normally distributed data as medians (interquartile range (IQR)). To control the false discovery rate (FDR), the Benjamini–Hochberg (BH) procedure was applied for multiple comparison correction. For data analysis, SPSS 26 (IBM SPSS Statistics for Windows, version 26.0, IBM Corp., Armonk, NY, USA) was used. A $p < 0.05$ was considered statistically significant.

3. Results

3.1. Patient Characteristics

A total of 46 ART females and 52 controls were initially recruited for this study. One subject in the ART group was excluded due to insufficient data assessment. In total, 45 ART females and 52 individuals who conceived spontaneously were included in the final analysis.

Mean age was 47.72 $\pm$ 5.96 years in ART females and 46.84 $\pm$ 7.43 years in controls ($p = 0.525$). The two groups did not differ significantly in anthropometric variables, weight classification, smoking status, or educational level (Table 1).

Table 1. Patients' characteristics.

Variable	ART (n = 45)	Control (n = 52)	p-Value	p-Value Adjusted
Age (years)	47.72 ± 5.96	46.84 ± 7.43	0.525	0.735
Bodyweight (kg)	64.50 (60.90–77.93)	65.15 (59.30–74.18)	0.332	0.719
Height (cm)	167.86 ± 7.14	168.25 ± 6.56	0.782	0.876
BMI (kg/m^2)	23.41 (21.43–28.90)	23.03 (21.60–25.27)	0.506	0.735
Underweight (n (%))	1 (2.22)	1 (1.92)	0.724	0.845
Normal weight (n (%))	27 (60.00)	37 (71.16)		
Overweight (n (%))	8 (17.78)	7 (13.46)		
Obese (n (%))	9 (20.00)	7 (13.46)		
Waist-hip ratio	0.85 (0.82–0.91)	0.84 (0.80–0.88)	0.175	0.663
Smoking (n (%))	4 (8.89)	5 (9.62)	1	1
Maternal educational level	4.00 (3.00–5.00)	5.00 4.00–5.00)	0.188	0.663
Course of pregnancy and birth				
Maternal age at birth (years)	38.45 ± 3.64	31.85 ± 3.99	<0.001 ***	0.028 *
BMI at conception (kg/m^2) [1]	22.22 (20.23–24.25)	21.42 (20.19–22.82)	0.277	0.705
Multiple pregnancy (n (%))	11 (24.44)	2 (3.85)	0.003 **	0.042 *
Weeks of gestation (weeks) [2]	39.00 (36.00–40.00)	39.00 (38.00–40.00)	0.101	0.663
Maternal blood pressure during pregnancy ≥ 140/90 mmHg [3]	0 (0)	3 (12.5)	0.246	0.689
Medical history				
Arterial hypertension (n (%))	6 (13.33)	4 (7.69)	0.506	0.735
Dyslipidemia (n (%))	4 (8.89)	7 (13.46)	0.479	0.735
Glucose metabolism disorder (n (%))	2 (4.44)	0 (0)	0.213	0.663
Thyroid disease (n (%))	9 (20)	10 (19.23)	0.924	0.995
History of thrombosis (n (%))	2 (4.44)	1 (1.92)	0.595	0.756
History of pulmonary embolism (n (%))	1 (2.22)	0 (0)	0.464	0.735
History of questionable transient ischemic attack (n (%))	1 (2.22)	0 (0)	0.464	0.735
History of cancer (n (%))	0 (0)	2 (3.85)	0.497	0.735
Medication				
Antihypertensive medication (n (%))	1 (2.22)	3 (5.77)	0.621	0.756
Lipid-lowering medication (n (%))	1 (2.22)	3 (5.77)	0.621	0.756
L-thyroxine (n (%))	9 (20)	10 (19.23)	0.924	0.958
Antidiabetic medication (n (%))	2 (4.44)	0 (0)	0.213	0.663
Blood thinners (n (%))	2 (4.44)	0 (0)	0.213	0.663
Hormone replacement therapy (n (%))	5 (11.11)	0 (0)	0.019 *	0.177
Oral contraceptives (n (%))	3 (6.67)	1 (1.92)	0.334	0.719

ART, assisted reproductive technologies; BMI, body mass index. [1] 33 ART females and 36 control subjects were included in the analysis. [2] 41 ART females and 48 control subjects were included in the analysis. [3] 21 ART females and 24 control subjects were included in the analysis. Maternal educational level was assessed according to the German education system: no school leaving qualification (0), lower secondary school leaving certificate (1), intermediate secondary school leaving certificate (2), general qualification for university entrance (3), completed apprenticeship (4), completed university degree (5). Data are presented as means ± SD for normally distributed parameters and as medians (IQR) for non-normally distributed parameters. Nominal data are presented as n (%). Benjamini–Hochberg procedure was applied for adjusting p-value. * $p < 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

A total of 34 ART females conceived successfully with the help of ICSI, 10 with the help of IVF, and 1 with the help of GIFT. Regarding the course of pregnancy and birth, maternal age at birth and the presence of multiple pregnancy were significantly higher in the ART cohort compared to controls (Table 1).

Self-reported medical history was not significantly different between ART females and their peers (Table 1).

3.2. Diet Quality, Level of Physical Activity, and Sedentary Behavior

The two groups did not differ significantly in diet quality, level of physical activity, or sedentary behavior (Table 2).

Table 2. Diet quality, level of physical activity, and sedentary behavior.

Variable	ART (n = 45)	Control (n = 52)	p-Value
MEDAS	6.49 ± 2.29	6.87 ± 2.28	0.420
Total MET-min per week [1]	2880.00 (1350.00–8490.00)	3300.00 (925.00–5670.00)	0.843
Recreational MET-min per week [1]	900.00 (240.00–2070.00)	1080.00 (375.00–1680.00)	0.935
Muscle strengthening activities (times/week)	0 (0–1)	0 (0–0)	0.534
Sedentary behavior (hours/day)	6.01 ± 3.53	6.70 ± 2.73	0.281

ART, assisted reproductive technologies; MEDAS, Mediterranean diet adherence score; MET, metabolic-equivalent. Data are presented as means ± SD for normally distributed parameters and as medians (IQR) for non-normally distributed parameters. Nominal data are presented as n (%). [1] 44 ART females were included in the analysis.

3.3. Vascular Function

Vascular function did not show a significant difference between ART females and controls (Table 3).

Table 3. Vascular function.

Variable	ART (n = 45)	Control (n = 52)	p-Value
Pulse wave analysis			
SBP (mmHg)	123.49 ± 15.50	120.94 ± 12.35	0.371
Elevated SBP (n (%))	11 (24.44)	13 (25)	0.950
DPB (mmHg)	79.29 ± 10.71	78.01 ± 8.70	0.518
Elevated DBP (n (%))	12 (26.67)	12 (23.08)	0.683
MAP (mmHg)	99.51 ± 12.36	97.64 ± 9.87	0.411
cSBP (mmHg)	118.33 ± 14.48	115.05 ± 11.38	0.215
cDBP (mmHg)	80.29 ± 10.87	79.01 ± 8.85	0.526
Heart rate (bpm)	61.42 ± 7.98	60.71 ± 8.76	0.680
AIx@75 (%)	17.85 ± 11.05	20.41 ± 12.60	0.293
PWV (m/s)	6.93 ± 0.96	6.78 ± 0.91	0.424
Sonography of the common carotid artery			
CS (%) [1]	7.18 ± 2.30	7.30 ± 2.19	0.791
SR (1/s) [1]	1.58 (1.30–2.08)	1.58 (1.27–2.02)	0.917
Arterial distensibility ($mmHg^{-1} \times 10^{-3}$) [1]	333.31 ± 106.93	347.98 ± 116.06	0.526
Stiffness index β [2]	5.87 (3.97–8.97)	5.24 (3.59–8.11)	0.438
cIMT (mm)	0.55 (0.50–0.65)	0.55 (0.51–0.60)	0.783

ART, assisted reproductive technologies; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; AIx@75, augmentation index averaged to a heart rate of 75 bpm; PWV, pulse wave velocity; CS, peak circumferential strain; SR, peak strain rate; cIMT, carotid intima-media thickness. [1] 44 ART females and 51 control subjects were included in the analysis. [2] 50 control subjects were included in the analysis. Data are presented as means ± SD for normally distributed parameters and as medians (IQR) for non-normally distributed parameters. Nominal data are presented as n (%).

3.4. Blood Lipid Profile

An initial comparison revealed a significantly higher prevalence of elevated Lp(a) in ART females (p = 0.011). However, after multiple comparison correction, this significant result no longer remained (p = 0.132) (Table 4). The remaining blood lipid profile did not display significant alterations between the two groups (Table 4).

Table 4. Blood lipid profile.

Variable	ART (n = 45)	Control (n = 52)	p-Value	p-Value Adjusted
TC (mg/dL)	196.00 (180.00–225.00)	189.00 (175.50–214.00)	0.441	0.722
Increased TC (n (%))	20 (44.44)	23 (44.23)	0.983	0.983
LDL-C (mg/dL)	121.16 ± 24.33	115.98 ± 30.76	0.366	0.722
Increased LDL-C (n (%))	24 (53.33)	24 (46.15)	0.481	0.722
HDL-C (mg/dL)	69.40 ± 18.47	68.81 ± 17.44	0.871	0.983
Decreased HDL-C (n (%))	5 (11.11)	6 (11.54)	0.947	0.983
Non-HDL-C (mg/dL)	124.00 (110.50–156.00)	122.00 (105.75–140.75)	0.399	0.722
Increased Non-HDL-C (n (%))	22 (48.89)	21 (40.38)	0.400	0.722
Triglycerides (mg/dL)	81.00 (59.50–99.50)	76.50 (56.00–121.75)	0.876	0.983
Increased Triglycerides (n (%))	4 (8.89)	8 (15.38)	0.333	0.722
Lp(a) (mg/dL) [1]	12.00 (5.00–55.75)	7.00 (5.00–17.00)	0.110	0.66
Increased Lp(a) (n (%)) [1]	10 (27.78)	4 (7.69)	0.011 *	0.132

ART, assisted reproductive technologies; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; non-HDL-C, non-HDL cholesterol; Lp(a), lipoprotein (a). Data are presented as means ± SD for normally distributed parameters and as medians (IQR) for non-normally distributed parameters. Nominal data are presented as n (%). Benjamini–Hochberg procedure was applied for adjusting p-value. [1] 36 ART females were included in the analysis. * $p < 0.05$.

4. Discussion

This pilot study investigated overall vascular health in 45 ART females and 52 controls who conceived spontaneously. Interestingly, the results of this study did not reveal significant differences in overall vascular function between ART females and controls. Initially, a significantly higher prevalence of elevated Lp(a) was observed in ART females. However, after multiple comparison correction, this significant result disappeared. Special care was taken to adequately match both groups by age, as well as by diet quality, physical activity, and sedentary behavior. The two groups did not differ significantly in anthropometric variables, weight classification, smoking status, educational level, or self-reported medical history. Maternal age at childbirth was significantly higher in the ART group than in the control group. There is evidence that as age increases, the mother's cardiovascular system may be less able to adapt to pregnancy, putting the child at higher risk of adverse pregnancy outcomes, which could potentially harm the cardiovascular function of the offspring [27,28]. In addition, the prevalence of multiple pregnancy was significantly higher in the ART group. This may be due to the ART procedure itself, as multiple embryos are often transferred at once. The literature suggests that one in five IVF cycles results in a multiple pregnancy [29]. Even though no significant difference in gestational hypertension was found in our study, multiple pregnancy still resulted in a higher rate of peripartal morbidities, such as preeclampsia [30].

To assess overall vascular function, different non-invasive methodologies were applied, including the measurement of brachial blood pressure, central blood pressure, and PWV via an oscillometric blood pressure device. Additionally, arterial stiffness and cIMT of the CCA were assessed via sonography in this study.

Female infertility can be caused by a variety of factors such as ovulation disorders, hormonal imbalances, structural alterations of the reproductive system, chronic illnesses, lifestyle, and age [2,31]. Some studies have demonstrated that female infertility and cardiovascular dysfunction might have potential associations [32,33]. A recent prospective cohort study of more than 100,000 participants with 28 years of follow-up demonstrated that females with a history of infertility (12 months of trying to conceive without success, including individuals who subsequently conceived) may show an increased risk of facing coronary heart disease later in life compared to fertile controls [11]. The study observed that cardiovascular risk correlated inversely with age of first infertility diagnosis. Interestingly, the authors revealed that cardiovascular risk may be restricted to infertility-related ovulation disorders such as polycystic ovary syndrome (PCOS) and endometriosis [11]. Another cross-sectional study including over 700 female participants from the USA (age range: 20–59 years) demonstrated that experiencing infertility at any point within the female reproductive window may be linked with later-life cardiovascular morbidity [34].

In contrast to the abovementioned studies, we did not find a significant difference in vascular function between individuals who conceived through ART and control subjects who conceived spontaneously. One of the reasons for this might be that our study solely focused on females who had successfully conceived offspring with the help of ART, while other studies focused on females with infertility who did not conceive successfully even when an ART history was potentially present [11,32]. Therefore, we may have examined individuals with "less-pronounced" infertility. Additionally, the subjects in our cohort displayed a younger age compared to the study participants of Farland et al. [11]; hence, vascular abnormalities might have not become detectable yet in our study.

Initially, a significantly higher prevalence of elevated Lp(a) was observed in ART females. However, after multiple comparison correction, this significant result disappeared. Lp(a) has a similar structure to low-density lipoprotein (LDL) and is characterized by the binding of apolipoprotein(a) (apo (a)) to apolipoprotein B100 [35]. Circulating Lp(a) levels are largely determined by the *LPA* gene encoding apo(a) and are therefore not significantly affected by age, sex, physical activity, and diet [36]. Elevated Lp(a) levels are present in 10% to 30% of the population and increase cardiovascular risk [37–39]. Limited data on the relationship between Lp(a) and infertility are available. However, some studies suggest a link between elevated Lp(a) levels and female disorders that are associated with infertility, such as endometriosis and PCOS. Crook et al. investigated the blood lipid profile of 29 females with endometriosis and 29 healthy females and found that the Lp(a) levels in females with endometriosis were significantly higher [40]. Furthermore, Swetha et al. demonstrated that the prevalence of elevated Lp(a) levels was significantly higher in PCOS patients [41].

The exact pathophysiological mechanisms linking elevated Lp(a) levels and female infertility are still largely unknown and require further investigation. Potentially, elevated Lp(a) levels disturb the regulation of female sex hormones, such as estrogen and progesterone, and lead to irregular menstrual cycles, anovulation, and ultimately decreased fertility [42,43]. Moreover, it was shown that elevated Lp(a) levels induce inflammatory responses via interleukin 6 (IL-6), which could potentially alter cardiovascular, as well as reproductive, function [44].

With the widespread use of ART, it is of particular concern whether the offspring of females with history of ART may inherit certain cardiovascular risk factors associated with female infertility, such as altered Lp(a) levels. Interestingly, some studies have suggested that ART offspring may have a higher risk of vascular dysfunction [6,45]. Therefore, further studies investigating the role of maternal risk factors in cardiovascular morbidity of ART offspring are required.

Notably, the causes of female infertility can be complex and are often multifactorial. Although there are shared risk factors between the onset of cardiovascular disease and infertility (e.g., obesity, arterial hypertension, diabetes) [46–49], research on the association between female infertility and increased cardiovascular risk is sparse. Hence, in the future, more studies are required addressing such pathophysiological interactions.

4.1. Limitations

4.1.1. Study Design and Study Population

The present study was a single-center study in Germany. The participants were recruited in the greater Munich area, and therefore the results might be restricted by cultural factors, socioeconomic status (SES), and ethnic homogeneity, due to selection bias. Leischik et al. pointed out that the spectrum of diseases is different in countries with different levels of development. In developed countries, non-communicable diseases, especially cardiovascular diseases, have received much attention, and differences in SES can lead to health inequalities. People with higher SES tend to have better health outcomes than those with lower SES [50]. Moreover, different ecosystems and the physical environment affect health performance, including the cardiovascular system [50]. The sample size of this pilot study could be regarded as relatively small. Our ART group included different types of ART, including IVF, ICSI, and GIFT, which resulted in a relatively heterogeneous study sample. This manuscript does not address data on the number of ART cycles in the examined females or the type of embryo transfer (fresh vs. frozen) conducted. Some patient characteristics were obtained via questioning the participants. Hence, some response bias might be present. Females with history of ART were more frequently on hormone replacement therapy, which could have altered the results on vascular function [51]. We did not acquire data on an individual's menstrual cycle, which could potentially alter cardiovascular function. Furthermore, we recruited relatively young ART females, and therefore some cardiovascular changes might have not become detectable yet. The causes of infertility are complex. Hormonal, genetic, and psychological factors, as well as the potential influence of male infertility, were not addressed in this study. One in four females with infertility suffer from hormonal imbalances and disorders causing anovulation [52]. In approximately 10% of cases, genetic abnormalities are present [53]. Moreover, psychological distress may be also related to the development of female infertility [54]. Hence, prospective multicenter studies are needed to validate the results demonstrated in this study and enable precise cardiovascular risk stratification of females with history of ART.

4.1.2. Methodology

As this was a pilot study, a prior power analysis was not feasible. We applied the BH procedure to reduce FDR and this might have lowered the ability to find significant results and reduce statistical effects. This pilot study suggests Lp(a) as a significant cardiovascular risk factor in ART females. To draw more definitive conclusions, a separate study focusing solely on Lp(a) should be executed in the future. In this study, we applied a wide variety of methodologies to detect vascular dysfunction. Although carotid-femoral PWV (cfPWV) measurement is considered the noninvasive gold standard of arterial stiffness assessment, it has not been widely used in clinical practice, because the procedure is time-consuming and requires special equipment. We therefore applied oscillometric PWV measurement, as it has a satisfying agreement and consistency with the abovementioned gold standard, making it an easy as well as suitable screening tool for cardiovascular risk determination [55,56]. To the best of our knowledge, two-dimensional speckle tracking (2DST) is a new technique for measuring arterial stiffness and has not yet been validated. However, several studies have suggested that 2DST may be a useful tool for the noninvasive assessment of arterial stiffness compared to the current gold standard of measuring cfPWV [57,58]. The assessment of cIMT and 2DST parameters was not conducted blindly, which could have resulted in some bias. However, various vascular parameters (e.g., brachial blood pressure, PWV) were measured automatically by the respective devices. To reduce intermeasurement variability in this study, the mean of three measurements was calculated to improve data validity. To reduce interobserver variability, ultrasound images were acquired and analyzed offline by a single investigator.

5. Conclusions

This pilot study investigated the overall vascular health of females with history of ART compared to individuals who conceived spontaneously. No significant differences in vascular function were displayed between the two groups. The potentially higher prevalence of elevated Lp(a), a cardiovascular risk factor, among females with history of ART might contribute to an impaired reproductive process and play an important role in the etiology of female infertility. As female infertility has been linked with an increased cardiovascular risk within the literature, subjects suffering from infertility might profit from a cardiovascular screening to identify risk factors at an early stage and therefore improve their overall outcome. In the future, multicenter studies with a larger sample size, as well as a long-term follow-up design, are needed for a more precise cardiovascular risk stratification of infertile females.

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4. Paper II

Original Article

Vascular function in a cohort of children, adolescents and young adults conceived through assisted reproductive technologies—results from the Munich heARTerY-study

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Background: Over 8 million individuals worldwide have been conceived through assisted reproductive technologies (ART). There is conflicting evidence on the cardiovascular health of ART offspring. This study aimed to investigate vascular function in a cohort of children, adolescents and young adults conceived through ART compared to spontaneously conceived peers.

Methods: Anthropometric variables, diet quality, level of physical activity and sedentary behavior were assessed. An extensive evaluation of vascular function was conducted. Blood pressure as well as endothelial function were evaluated. Carotid intima-media thickness was recorded sonographically. Blood draws were taken to determine blood lipids as well as HbA1c.

Results: In total, 66 ART subjects conceived through in vitro fertilization (IVF) or intracytoplasmic sperm injection and 86 spontaneously conceived peers were included in this observational cohort study. Both groups were similar in age [11.31 (8.10–18.00) *vs.* 11.85 (8.72–18.27) years, P=0.373]. ART subjects displayed a significantly higher body fat percentage [19.30% (15.80–26.02%) *vs.* 15.91% (13.21–21.00%), P=0.007]. Both groups did not differ significantly in diet quality, physical activity, sedentary behavior, and vascular function. Blood lipids and HbA1c were comparable between both groups. ART subjects showed significantly lower levels of triglycerides compared to spontaneously conceived peers. The prevalence of lipoprotein (a) [Lp(a)] ≥50 mg/dL tended to be higher within the ART cohort. Vascular function did not deteriorate more profoundly with age in ART subjects than in spontaneously conceived peers.

Conclusions: The results of the current study do not indicate a significantly lower vascular function in a cohort of children, adolescents and young adults conceived through ART compared to spontaneously conceived peers. Future studies should address the prevalence of elevated Lp(a) levels in infertile individuals who sought ART treatment. In addition, more studies evaluating body fat percentage as well as cardiovascular morbidity in adult ART subjects are required. For a more precise cardiovascular risk stratification, multi-center studies with larger ART sample sizes, preferably at adult age, are required in the future.

Keywords: Assisted reproductive technologies (ART); vascular function; children; adolescents; adults

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Introduction

Infertility affects millions of couples worldwide (1). On July 25th 1978, Louise Brown was the first child conceived with the help of in vitro fertilization (IVF) (2). Since then, assisted reproductive technologies (ART) have been widely used to treat infertility (3). It is assumed that over 8 million individuals have been conceived through ART worldwide (4). Today, more than 2% of all European infants are conceived through ART (3). With 6.2%, Denmark holds the highest proportion of ART infants per national birth within Europe (3).

In the past, several studies suggested distinct vascular alterations, such as increased blood pressure, pulse wave velocity and carotid intima-media thickness (cIMT) as well as lower endothelial function, in ART children and adolescents (5-8). In the literature, multiple causes are proposed to be involved in the vascular pathophysiology of ART offspring being the ART procedure itself, the intrauterine environment, parental risk factors and lifestyle habits (9). In contrast to these results, a recent study by Halliday *et al.* did not detect significant vascular differences in 193 ART adults compared to spontaneously conceived controls (10). These results may indicate that vascular changes in the ART offspring are only transiently present during childhood and "vanish" later in life (10).

Highlight box

Key findings

- The results of the current study do not indicate a significantly lower vascular function in a cohort of children, adolescents and young adults conceived through assisted reproductive technologies (ART).

What is known and what is new?

- There is conflicting evidence on the cardiovascular health of ART offspring.
- An extensive vascular evaluation in a cohort of children, adolescents and young adults conceived through ART was conducted in this study.

What is the implication and what should change now?

- For a more precise cardiovascular risk stratification, multi-center studies with larger ART sample sizes, preferably at adult age, are required in the future.

As a rising number of children are conceived through ART, the potentially increased vascular morbidity remains of great concern for families and society. Regarding the currently ambiguous data situation, further studies are required to investigate the potential impact of ART on the offspring's cardiovascular health.

The aim of this study was to assess whether ART subjects develop vascular alterations compared to spontaneously conceived peers over their lifespan. We therefore conducted a single center observational cohort study, which included an ART cohort composed of children, adolescents, and young adults. We present this article in accordance with the STROBE reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-23-67/rc).

Methods

Ethical approval

This study received ethical approval (No. 20-0844) on the 27th of December 2020 by the Ethics Committee of the Medical Faculty of LMU Munich (Munich, Germany). The study was conducted in accordance with the ethical standard of the Declaration of Helsinki (as revised in 2013). Prior written consent was obtained from all study participants. In minor study participants, prior written consent was additionally received from parents or legal guardians.

Study design and study population

The Munich heARTerY-study (Assisted Reproductive Technologies and their effects on heart and arterial function in Youth) was a single center observational cohort study. Between May 2021 and March 2022, individuals conceived through ART were enrolled in collaboration with the Division of Gynecological Endocrinology and Reproductive Medicine, Department of Obstetrics and Gynecology, University Hospital, LMU Munich (Munich, Germany). For this study, ART subjects conceived solely through IVF or intracytoplasmic sperm injection (ICSI) were included. In contrast to previous publications of our departments, subjects conceived through gamete intrafallopian transfer (GIFT) were excluded. While GIFT can be considered a conventional ART procedure, it does not include *in vitro* cultivation as fertilization happens in the natural environment of the fallopian tubes (11). Healthy spontaneously conceived peers matched in age and gender without known cardiovascular diseases were enrolled through public calls within the greater Munich area. To evaluate the influence of age on vascular function, subjects of different developmental stages (children, adolescents, adults) were included. All study participants were examined at the Division of Pediatric Cardiology and Intensive Care, University Hospital, LMU Munich (Munich, Germany).

Assessment of anthropometric variables

Bodyweight (kg) and height (cm) were determined. In addition, body mass index (BMI, kg/m^2) was measured. The following weight classification was defined for adult study participants: underweight if BMI <18.5 kg/m^2, normal weight if BMI ≥18.5 but <25 kg/m^2, overweight if BMI ≥25 but <30 kg/m^2 and obese if BMI ≥30 kg/m^2. In minor study participants, weight classification was determined in accordance with BMI percentiles (P.) provided by Kromeyer-Hauschild *et al.*: underweight if BMI <10 P., normal weight if BMI ≥10 P. but <90 P., overweight if BMI ≥90 P. but <97 P. and obese if BMI ≥97 P. (12). A skinfold caliper was utilized to measure skinfold thickness in study participants (Harpenden Skinfold Caliper, Baty International, UK). In all subjects, the skinfold thickness was measured three consecutive times and an average was calculated. For adult subjects, the gender-dependent, three-site skinfold protocol of Jackson *et al.* was utilized to assess body fat percentage (BFP, %) (13,14). In minors, the right triceps skinfold thickness and the right subscapular skinfold thickness were measured according to Neuhauser *et al.* (15). BFP was estimated in minor subjects using formulas established by Slaughter *et al.* (16).

Medical history, course of pregnancy and birth, maternal level of education, clinical examination

The assessment of medical history, smoking status and regular use of medication was performed. The following data regarding the course of pregnancy and birth was assessed by evaluating clinical records and by interviewing parents: birth weight (g), gestational age (week), maternal age at birth (years), case of multiple pregnancy, maternal BMI at conception (kg/m^2), presence of gestational diabetes, maternal blood pressure during pregnancy ≥140/90 mmHg. Maternal level of education was defined based on the German education system: no school leaving qualification [0], lower secondary school leaving certificate [1], intermediate secondary school leaving certificate [2], general qualification for university entrance [3], completed apprenticeship [4], completed university degree [5]. Additionally, all study participants underwent a physical examination.

Adherence to Mediterranean diet

High adherence to the Mediterranean diet was shown to positively influence cardiovascular health (17-19). To evaluate adherence to the Mediterranean diet in adult study participants, the validated 14-item Mediterranean diet assessment tool established by Martínez-González *et al.* was translated into German and applied (17). For minors, the validated KIDMED test established by Serra-Majem *et al.* was translated into German and utilized (20). For both questionnaires a score ≥8 was defined as high adherence to the Mediterranean diet (17,20).

Level of physical activity and sedentary behavior

The German version of the Global Physical Activity Questionnaire (GPAQ) provided by the World Health Organization (WHO) was utilized to assess the level of physical activity in study participants ≥18 years of age (21). Picture cards were shown for each activity type (21). Total and recreational Metabolic-Equivalent-(MET)-minutes per week were calculated according to GPAQ recommendations (21). Further, adult study participants were asked how many times muscle strengthening activities are performed per week. Adult subjects met WHO recommendation if ≥600 total MET-minutes per week were accomplished (21).

To assess the level of physical activity in study participants <18 years of age, subjects were asked how much time is spent per day on moderate and/or vigorous physical activities. Moreover, minor subjects were asked how many times vigorous, muscle strengthening and/or bone strengthening activities are performed per week. For each activity type, picture cards were shown. Pediatric subjects met WHO recommendations if (I) an average of ≥60 minutes per day was achieved for moderate and/or vigorous activities and (II) vigorous, muscle strengthening and/or bone strengthening activities were performed ≥3 times per week (22).

Sedentary behavior was defined as time spent sitting (22). Picture cards visualizing different examples of sedentary behavior (e.g., sitting on the train, driving the car, sitting while working or doing homework, watching TV) were presented to all study participants. Study participants were then asked how much time (min) is spent per day with such sedentary activities.

Vascular function

Pulse wave analysis

An oscillometric blood pressure device (Mobil-O-Graph®, IEM GmbH, Germany) was utilized to measure brachial systolic blood pressure (SBP, mmHg), brachial diastolic blood pressure (DBP, mmHg), mean arterial pressure (MAP, mmHg), heart rate (HR, bpm), central SBP (cSBP, mmHg), central DBP (cDBP, mmHg) and augmentation index averaged to a heart rate of 75 bpm (AIx@75, %). Cuff sizes were selected to match the right upper arm circumference. Study participants were asked to remain in a supine and calm position ≥5 minutes before and during the measurements. To enhance data validity, three consecutive measurements were executed and averaged as recommended by the European Society of Cardiology/European Society of Hypertension (23). In subjects ≥16 years of age, SBP was elevated if ≥130 mmHg and DBP if ≥85 mmHg (23). In subjects <16 years of age, elevated SBP and/or DBP was present if ≥90 P. of a reference population in Germany (15).

Endothelial function

The reactive hyperemia index (RHI), a marker of endothelial function (24), was measured using the EndoPAT™2000 device (Itamar Medical, Israel) and its corresponding software [version 3.7.2.(2.0)]. A fasting period ≥4 hours and an alcohol abstinence ≥24 hours prior to study participation was required. The examination was performed in a quiet and temperature-controlled room. Study participants were asked to remain in a supine and calm position for ≥15 minutes prior to as well as during the entire examination. The measurement consisted of a 5-minute baseline recording period, a 5-minute occlusion period and a 5-minute post occlusion period. A cuff was positioned on the right upper arm during occlusion period. The cuff was inflated between 200–300 mmHg in adult subjects and ≥60 mmHg above SBP in pediatric subjects for complete blood flow cessation.

Intima-media-thickness of the common carotid artery (CCA)

A Philips iE33 xMatrix or a Philips Epiq 7G ultrasound device (Philips Healthcare, The Netherlands) with a 3–12 MHz linear array transducer was used to image both CCAs in long axis view at bifurcation level. During sonography, subjects were asked to remain in a supine position while extending their neck up to a 45° angle and turning it to the contralateral side of examination (25). Under constant three-lead ECG tracking, three consecutive loops were recorded. The loops were then transferred to a separate workstation (QLAB cardiovascular ultrasound quantification software, version 11.1, Philips Healthcare, The Netherlands) for further analysis. At end-diastole (R wave in ECG), the carotid intima-media thickness (cIMT, mm) was evaluated semiautomatically for both sides individually. Proximal to the carotid bifurcation, the 10 mm long region of interest was set. An average of three measurements was calculated for both cIMT individually. CCA sonography and offline analysis was conducted by one investigator.

Cardiometabolic risk profile

To evaluate the cardiometabolic risk profile, total cholesterol (TC, mg/dL), low-density lipoprotein cholesterol (LDL-C, mg/dL), high density lipoprotein cholesterol (HDL-C, mg/dL), non-high density lipoprotein cholesterol (non-HDL, mg/dL), triglycerides (mg/dL), apolipoprotein A1 (Apo A1, mg/dL), apolipoprotein B (Apo B, mg/dL), lipoprotein (a) [Lp(a), mg/dL], HbA1c (%) and plasma homocysteine level (µmol/L) was measured. Prior to blood drawing, a fasting period of ≥4 hours was requested. The presence of elevated blood lipids was defined according to adult and pediatric recommendations (26-30). An HbA1c ≥5.7% and a plasma homocysteine level >12 µmol/L was considered to be increased in all study participants, independent of age (31,32).

Primary and secondary outcome variables

For this study, data on vascular function and cardiometabolic risk profile were considered as primary outcome variables. Data on anthropometric variables, medical history, course of pregnancy and birth, maternal level of education, diet quality, level of physical activity and sedentary behavior were defined as secondary outcome variables.

Statistical analysis

For data analysis, SPSS 28 (Release Date 2021, IBM SPSS Statistics for Windows, version 28.0, IBM Corp., Armonk, NY, USA) was used. The chi-square test was applied to compare nominal data. Continuous parameters were tested for normality using the Kolmogorov-Smirnov test and the Shapiro-Wilk test. In case of normal distribution, the unpaired *t*-test was used. For non-normally distributed continuous variables the Mann-Whitney-*U* test was utilized. For correlation analysis of normally distributed variables, the Pearson's correlation coefficient was applied. For correlation analysis of non-normally distributed variables, the Spearman's correlation was used. By using the Cocor software, z-scores were generated enabling the statistical comparison of correlations (33). A range between −1.96 and 1.96 at a 95% confidence level was defined as a normal Z-score level. Normally distributed data is presented as mean ± standard deviation (SD) and non-normally distributed data as median [interquartile range (IQR)]. A P<0.05 was regarded as statistically significant.

Results

Patient's characteristics

In total, 70 ART subjects and 86 spontaneously conceived peers were recruited for this study. Within the ART group, one patient was excluded due to history of T-cell lymphoma, one due to history of heart surgery, one due to GIFT and one due to the incomplete data assessment. The final analysis included 66 ART subjects (50 ICSI, 16 IVF) and 86 spontaneously conceived peers.

Within the ART group, one subject presented with long QT syndrome, one with bicuspid aortic valve, one with questionable history of myocarditis, one with history of hypercholesterolemia and one with hypothyroidism. Three ART subjects used oral contraceptives, one L-thyroxine and one methylphenidate. Six control subjects were on oral contraceptives, one on bisoprolol due to chronic migraine and one on methylphenidate. Three ART subjects and 2 controls were smoking (P=0.653).

There were no significant differences in age [11.31 (8.10–18.00) *vs.* 11.85 (8.72–18.27) years, P=0.373] and sex (females 57.58% *vs.* 51.16%, P=0.432) between the ART and the control group. The mean age was 12.61 years (absolute range: 4.41–24.38 years) within the ART group and 13.43 years (absolute range: 4.34–26.05 years) within the control group. Twenty-nine ART subjects and 31 controls were <10 years of age. Twenty-one ART subjects and 33 controls were between ≥10 and <18 years of age. Sixteen ART subjects and 22 controls were ≥18 years of age.

Anthropometric variables, including bodyweight, body height, BMI and weight classification, did not display significant differences between both groups. ART subjects showed, compared to spontaneously conceived peers, a significantly higher BFP.

Regarding the course of pregnancy and birth, ART subjects demonstrated a significantly lower birth weight as well as gestational age. Maternal age at birth and the prevalence of multiple pregnancy were significantly higher in the ART group. The remaining variables, including maternal BMI at conception, prevalence of gestational diabetes, prevalence of maternal blood pressure during pregnancy ≥140/90 mmHg as well as maternal educational level, were not significantly different between both groups.

Detailed information on patient's characteristics is given in *Table 1*.

Adherence to Mediterranean diet, level of physical activity and sedentary behavior

ART subjects and spontaneously conceived peers did not differ significantly in adherence to Mediterranean diet, level of physical activity and sedentary behavior. This was the case for adult as well as for pediatric subjects. *Table 2* summarizes data on adherence to Mediterranean diet, level of physical activity and sedentary behavior for adult as well as for pediatric subjects.

Vascular function

No significant differences in vascular function were demonstrated between ART subjects and spontaneously conceived peers. AIx@75 tended to be higher within the ART group, however, did not reach statistical significance. *Table 3* visualizes data on vascular function for both groups.

Cardiometabolic risk profile

Blood draws were taken in 65 ART subjects and 83 spontaneously conceived peers for the assessment of cardiometabolic risk profile. ART subjects displayed significantly lower levels of triglycerides compared to spontaneously conceived peers. The remaining blood lipids, HbA1c and homocysteine did not demonstrate significant differences between both groups. Interestingly, the prevalence of Lp(a) ≥30 and ≥50 mg/dL tended to be higher within the ART group, however, did not reach statistical significance. *Table 4* summarizes data on cardiometabolic

Table 1 Patients' characteristics

Variable	ART (n=66)	Control (n=86)	P value
Age (years)	11.31 [8.10–18.00]	11.85 [8.72–18.27]	0.373
Female	38 (57.58)	44 (51.16)	0.432
Body weight (kg)	36.95 [23.30–58.60]	42.70 [29.05–59.25]	0.213
Body height (cm)	145.00 [124.50–166.25]	157.00 [133.38–170.25]	0.085
BMI (kg/m^2)	16.77 [15.04–20.86]	17.66 [15.43–21.05]	0.459
Underweight	4 (6.06)	6 (6.98)	1.00
Normal weight	57 (86.36)	74 (86.04)	
Overweight	5 (7.58)	6 (6.98)	
Obese	–	–	
Body fat percentage (%)[1]	19.30 [15.80–26.02]	15.91 [13.21–21.00]	0.007**
Course of pregnancy and birth			
Birth weight (g)[2]	2,985.00 [2,362.50–3,240.00]	3,440.00 [3,210.00–3,670.00]	<0.001***
Gestational age (weeks)[3]	38.00 [36.00–39.50]	39.00 [38.00–40.00]	<0.001***
Maternal age at birth (years)[4]	35.41±3.74	33.07±4.10	<0.001***
Multiple pregnancy	21 (31.82)	2 (2.32)	<0.001***
Maternal BMI at conception (kg/m^2)[5]	22.49 [20.39–24.81]	21.38 [20.24–22.72]	0.100
Gestational diabetes[6]	3 (5.36)	3 (4.23)	1
Maternal blood pressure during pregnancy ≥140/90 mmHg[7]	0 (0)	3 (6.38)	0.289
Maternal educational level[8]	4 [3–5]	5 [4–5]	0.241

Data is presented as mean ± SD for normally distributed parameters and as median [IQR] for non-normally distributed parameters. Nominal data is presented as n (%). **, P≤0.01; ***, P≤0.001. [1], 85 control subjects were included in the analysis. [2], 64 ART subjects and 79 control subjects were included in the analysis. [3], 61 ART subjects and 77 control subjects were included in the analysis. [4], 65 ART subjects were included in the analysis. [5], 46 ART subjects and 61 control subjects were included in the analysis. [6], 56 ART subjects and 71 control subjects were included in the analysis. [7], 28 ART subjects and 47 control subjects were included in the analysis. [8], 44 ART subjects and 52 control subjects were included in the analysis. Maternal educational level was assessed according to the German educational system: no school leaving qualification [0], lower secondary school leaving certificate [1], intermediate secondary school leaving certificate [2], general qualification for university entrance [3], completed apprenticeship [4], completed university degree [5]. ART, assisted reproductive technologies; BMI, body mass index; SD, standard deviation; IQR, interquartile range.

risk profile in detail for both groups.

Correlation analysis

By conducting a correlation analysis, the effect of age on vascular function was investigated within the ART and the control group. Observed Z-scores (Z_{obs}) ranged between 0.40 and 1.54, indicating no significant differences between the correlations of both groups (*Table 5*).

Discussion

The present study included 66 ART subjects and 86 spontaneously conceived peers. Special care was taken to match both groups by age and gender as well as lifestyle factors (e.g., diet quality, level of physical activity, sedentary behavior). In contrast to previous studies (5,6,9), we were not able to display significant differences in vascular function between ART subjects and spontaneously conceived peers. To investigate the influence of age on

Table 2 Adherence to Mediterranean diet, level of physical activity and sedentary behavior

Variable	ART (n=66)	Control (n=86)	P value
Adult study participants	n=16	n=22	
MEDAS	6.06±2.49	7.27±1.67	0.081
Total MET (min/week)[1]	5,574.67±4,244.85	4,439.64±2,411.40	0.360
Recreational MET (min/week)[2]	1,920.00 (675.00–5,160.00)	1,600.00 (720.00–2,790.00)	0.570
Muscle strengthening activities (times/week)[1]	1.00 (0.00–2.00)	2.00 (0.00–3.00)	0.433
Sedentary behavior (min/day)	440.63±159.02	409.09±139.35	0.520
Minor study participants	n=50	n=64	
KIDMED	6.24±2.33	6.92±2.11	0.105
Moderate and/or vigorous physical activities (min/day)[3]	90.00 (60.00–127.50)	90.00 (60.00–120.00)	0.258
Vigorous, muscle strengthening and/or bone strengthening activities (times/week)	3.00 (2.00–5.00)	3.00 (2.00–4.00)	0.986
Sedentary behavior (min/day)[4]	360.00 (270.00–420.00)	420.00 (300.00–480.00)	0.134

Data is presented as mean ± SD for normally distributed parameters and as median (IQR) for non-normally distributed parameters. [1], 15 ART subjects were included in the analysis. [2], 14 ART subjects were included in the analysis. [3], 49 ART subjects and 63 control subjects were included in the analysis. [4], 49 ART subjects were included in the analysis. ART, assisted reproductive technologies; MEDAS, Mediterranean diet adherence score; MET, metabolic-equivalent; KIDMED, Mediterranean diet quality index for children and adolescents; BMI, body mass index.

Table 3 Vascular function

Variable	ART (n=66)	Control (n=86)	P value
SBP (mmHg)	113.74±12.10	113.22±8.96	0.768
Elevated SBP	17 (25.76)	14 (16.27)	0.151
DPB (mmHg)	64.33 (59.00–72.00)	63.50 (59.00–71.25)	0.873
Elevated DBP	6 (9.09)	6 (6.98)	0.632
MAP (mmHg)	87.00 (80.75–94.00)	86.00 (81.00–93.00)	0.929
cSBP (mmHg)[1]	99.50±12.69	98.45±10.45	0.588
cDBP (mmHg)[1]	66.00 (61.00–73.17)	65.50 (61.00–73.00)	0.936
Heart rate (bpm)	74.17±13.17	72.74±13.39	0.513
AIx@75 (%)[1]	19.18±11.80	15.54±11.09	0.054
Endothelial function			
RHI[2]	1.48 (1.21–1.92)	1.47 (1.21–1.97)	0.818
Intima-media thickness of the common carotid artery			
cIMT (mm)[3]	0.44±0.03	0.44±0.03	0.965

Data is presented as mean ± SD for normally distributed parameters and as median (IQR) for non-normally distributed parameters. [1], 65 ART subjects were included in the analysis. [2], 58 ART subjects and 81 control subjects were included in the analysis. [3], 62 ART subjects and 83 control subjects were included in the analysis. ART, assisted reproductive technologies; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; AIx@75, augmentation index averaged to a heart rate of 75 bpm; RHI, reactive hyperemia index; cIMT, intima-media thickness of the common carotid artery; SD, standard deviation; IQR, interquartile range.

Table 4 Cardiometabolic risk profile

Variable	ART (n=65)	Control (n=83)	P value
TC (mg/dL)	169.89±29.06	167.51±26.63	0.604
Increased TC	10 (15.38)	11 (13.25)	0.712
LDL-C (mg/dL)	92.38±24.04	94.92±22.79	0.514
Increased LDL-C	8 (12.31)	10 (12.05)	0.962
HDL-C (mg/dL)	68.00 (54.00–78.00)	62.00 (55.00–74.00)	0.166
Decreased HDL-C	4 (6.15)	3 (3.61)	0.700
Non-HDL-C (mg/dL)	100.43±25.87	103.24±25.30	0.508
Increased non-HDL-C	7 (10.77)	4 (4.82)	0.213
Triglycerides (mg/dL)	60.00 (43.00–85.50)	73.00 (53.00–103.00)	0.036*
Increased triglycerides	4 (6.15)	13 (15.66)	0.072
Apo A1 (mg/dL)	157.00 (140.50–171.50)	148.00 (137.00–172.00)	0.287
Decreased Apo A1	0 (0)	0 (0)	–
Apo B (mg/dL)	77.58±17.61	78.43±18.15	0.775
Increased Apo B	3 (4.62)	6 (7.22)	0.732
Lp(a) (mg/dL)	6.00 (5.00–33.00)	6.00 (5.00–13.00)	0.377
≥30	16 (24.62)	11 (13.25)	0.076
≥50	13 (20.00)	8 (9.64)	0.073
HbA1c (%)[1]	5.18±0.37	5.27±0.28	0.088
≥5.7%[1]	9 (13.85)	6 (7.32)	0.194
Homocysteine (μmol/L)[2]	9.17±2.35	9.03±3.06	0.777
Homocysteine >12 μmol/L[2]	8 (16.33)	12 (17.39)	0.879

Data is presented as mean ± SD for normally distributed parameters and as median (IQR) for non-normally distributed parameters. Nominal data is presented as n (%). *, P<0.05. [1], 82 control subjects were included in the analysis. [2], 49 ART subjects and 69 control subjects were included in the analysis. ART, assisted reproductive technologies; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; non-HDL-C, non-HDL cholesterol; Apo A1, apolipoprotein A1; Apo B, apolipoprotein B; Lp(a), lipoprotein (a); SD, standard deviation; IQR, interquartile range.

vascular function, subjects at different developmental stages (children, adolescents, adults) were included to conduct a correlation analysis. The results of the current study do not indicate that the vascular system of ART individuals ages more profoundly compared to the one of spontaneously conceived controls.

Cardiovascular function in the ART offspring

Comparison to previous findings

Despite ART being used to treat infertility for over 40 years, there is still limited data on the health outcome of its offspring. To date, data on the long-term cardiovascular outcome of ART individuals is relatively sparse and rather inconsistent. A well-known Swiss study of Scherrer *et al.* described distinct vascular alterations visualised by a generalized endothelial dysfunction, an increased blood pressure and arterial stiffness as well as an elevated cIMT in ART children (6). A follow-up study of the authors confirmed the persistence of these vascular alterations in adolescent ART individuals (5). Moreover, the findings of systemic and pulmonary vascular dysfunction in a cohort of 65 ART singletons (mean age: 11.10±2.40 years) reinforces the conjecture of an elevated cardiovascular risk within this cohort (7). The assessment of cardiovascular health in a cohort study including 382 children who were conceived

Table 5 Correlation analysis between age and vascular function

Variable	ART (n=66)		Control (n=86)		Z_{obs}
	r	P value	r	P value	
SBP	0.706	<0.001***	0.634	<0.001***	0.78
DPB	0.644	<0.001***	0.509	<0.001***	1.22
MAP	0.712	<0.001***	0.632	<0.001***	0.88
cSBP[1]	0.774	<0.001***	0.715	<0.001***	0.79
cDBP[1]	0.644	<0.001***	0.467	<0.001***	1.54
Heart rate	−0.422	<0.001***	−0.495	<0.001***	0.55
AIx@75[1]	−0.387	0.001***	−0.443	<0.001***	0.40
Endothelial function					
RHI[2]	0.703	<0.001***	0.627	<0.001***	0.78
Intima-media thickness of the common carotid artery					
cIMT[3]	0.282	0.027*	0.191	0.083	0.56

[1], 65 ART subjects were included in the analysis. [2], 58 ART subjects and 81 control subjects were included in the analysis. [3], 62 ART subjects and 83 control subjects were included in the analysis. *, P<0.05; ***, P≤0.001. ART, assisted reproductive technologies; Z_{obs}, observed Z-score; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; cSBP, central systolic blood pressure; cDBP, central diastolic blood pressure; AIx@75, augmentation index averaged to a heart rate of 75 bpm; RHI, reactive hyperemia index; cIMT, intima-media thickness of the common carotid artery.

through ART and 382 control subjects (mean age: 7.20±1.21 *vs.* 7.20±1.21 years) supports the perception of abnormal vascular health in ART individuals (34). The examined ART subjects displayed elevated blood pressure as well as distinct changes in their left ventricular structure compared to spontaneously conceived controls (34). In a review and meta-analysis by Guo *et al.* the cardiovascular health of 2,112 IVF/ICSI subjects and 4,096 spontaneously conceived peers was investigated (9). A significantly higher blood pressure, an increased vessel wall thickness as well as a decreased cardiac diastolic function was detected within the IVF/ICSI cohort (9).

In contrast, a study conducted by Halliday *et al.* could not confirm the above-mentioned findings in a large cohort of young ART adults (10). Compared to spontaneously conceived peers, the authors could not find significant differences in vascular function measured by blood pressure, pulse wave velocity and cIMT. Metabolic markers, such as conventional blood lipids, fasting blood glucose and fasting insulin, were not significantly altered between both groups (10). A population-based cohort study, including 122,429 ART subjects and 7,574,685 spontaneously conceived peers from Norway, Sweden, Finland, and Denmark, reinforces these findings (35). No significant differences in the risk for cardiovascular disease (e.g., ischemic heart disease, cardiomyopathy, heart failure, cerebrovascular disease) were demonstrated between both groups (35). Another study by Shiloh *et al.* compared the number of hospitalizations due to cardiovascular disease (e.g., valvular disorders, arterial hypertension, cardiac arrhythmias, ischemic heart disease) between a pediatric IVF group (n=2,603), a pediatric ovulation induction group (n=1,721) and a pediatric control group (n=237,863) (36). Between groups, no significant differences in hospitalizations due cardiovascular disease were found (36). The cohort study of Wijs *et al.* including 163 ART subjects and 1,457 controls (age range: 13–21 years) did also not confirm the hypothesis of an impaired cardiometabolic health in ART offspring (37). Blood lipids, glucose, insulin, arterial stiffness and blood pressure were evaluated and mostly did not show any statistical differences (37). In some parameters, such as BMI, waist circumference and arterial stiffness, the ART group displayed an even more favourable profile (37). In accordance with the above-mentioned findings as well as with previous publications of our departments, the results of the current study do not indicate significant impairments of cardiovascular function in a cohort of children, adolescents and young adults conceived after using ART compared to spontaneously conceived peers (11,38).

Pathophysiological considerations

Increased oxidative stress levels

The health of the ART offspring has been an omnipresent discussion since its introduction in 1978 (39). It is assumed that some undesired health consequences are linked with the ART procedure itself as increased oxidative stress levels were demonstrated (40). The elevated oxidative levels may be driven by various factors during the ART procedure (e.g., cryopreservation, pH fluctuations, temperature fluctuations, culture media) as well as a lack of natural antioxidant mechanisms (40). In addition, women who suffer from infertility as well as mothers of an advanced age tend to have higher oxidative stress levels (40,41). In accordance with literature (42), ART mothers of the current study were significantly older when giving birth. Moreover, pregnancy complications as well as perinatal risk factors associated with ART (e.g., hypertensive disorders, gestational diabetes, prematurity) are linked with increased oxidative stress levels and are more frequently linked with ART pregnancies (40,43). As the cardiovascular system is one of the first to mature during fetal development, it is particularly sensitive to altered environmental stimuli (40). Epigenetic modifications due to elevated oxidate stress levels can result in vascular dysfunction at adult age (40). Within the last years, updated ART protocols (e.g., improved handling of oocytes, reduced exposure to atmospheric oxygen concentrations, modified culture media) have led to a better management of oxidative stress levels (41). Potentially, this might partially explain the discrepancies found in literature regarding the cardiovascular morbidity of the ART offspring.

Pregnancy complications and perinatal risk factors

The fetal origins hypothesis suggests that the intrauterine environment plays a crucial role during fetal development (44,45). If the fetus is exposed to an adverse intrauterine environment (e.g., maternal hypertensive disorder, gestational diabetes, maternal excess weight, prematurity, multiple pregnancy), an increased morbidity might be present in later life (44,45). Interestingly, a higher prevalence of pregnancy complications and perinatal risk factors can be observed after the use of ART. A meta-analysis of Qin *et al.* reported higher incidences of maternal hypertension [relative risk (RR): 1.30], gestational diabetes (RR: 1.31), preterm birth (RR: 1.71), very preterm birth (RR: 2.12) and small for gestational age (RR: 1.35) within ART cohorts compared to spontaneously conceived peers (43). Preeclampsia belongs to the group of maternal hypertensive disorders and is numerously described in pregnancies following ART (46). Women who underwent ART have a 1.71-fold higher risk of preeclampsia than those who conceived spontaneously (46). Individuals who were exposed to preeclampsia in-utero show higher SBP and DBP compared to peers (47). In the current study, no significant differences in maternal BMI at conception, prevalence of gestational diabetes, or maternal blood pressure during pregnancy ≥140/90 mmHg were displayed between both groups. Multiple pregnancy occurs in one of five IVF cycles and was also more present in the examined ART cohort (42,48). In accordance with literature (43,49), ART subjects displayed a significantly lower gestational age and birth weight in comparison to spontaneously conceived peers in this study. This needs to be addressed as prematurely born children show an elevated risk for arterial hypertension, excess weight as well as glucose and lipid metabolism disorders (50,51).

Parental cardiovascular morbidity

The literature suggests that individuals who suffer from infertility present an increased cardiovascular morbidity. Murugappan *et al.* revealed that postmenopausal women with a history of infertility show a moderately higher risk for atherosclerotic cardiovascular disease compared to peers (52). A cross-sectional analysis among 744 women in the United States evaluated the association between self-reported infertility and cardiovascular events (53). Interestingly, the authors found that women with a history of infertility exhibit 1.83 higher odds of having experienced a cardiovascular event (53). Men with infertility or with semen abnormalities also display a higher risk for cardiovascular disease including arterial hypertension, peripheral vascular disease and ischemic heart disease (54,55). A Danish study demonstrated a strong association between sperm concentration and subsequent hospitalization for cardiovascular disease in a cohort of 4,712 men seen for infertility (56). Potentially, couples who suffer from infertility and thus seek ART treatment pass down certain cardiovascular risk factors to their offspring.

Elevated Lp(a) levels are suggested to be a risk factor for atherosclerotic cardiovascular disease (27). Around 90% of an individual's Lp(a) level is inherited (27). A study by Krause *et al.* identified elevated Lp(a) levels as a risk factor for unexplained recurrent miscarriage in Caucasian women (57). In the current study, the presence of Lp(a) ≥50 mg/dL tended to be higher within the examined ART cohort.

Moreover, Vlachopoulos *et al.* demonstrated that children conceived through IVF display significantly higher Lp(a) levels compared to children conceived through ICSI and spontaneously conceived peers (58). To the best of our knowledge, limited data on the prevalence of elevated Lp(a) levels in infertile individuals who sought ART treatment exist. A general Lp(a) screening of such individuals could potentially help identifying families at increased cardiovascular risk. Hence, further research on this matter is required.

Lifestyle factors

Unfavourable lifestyle habits such as poor dietary habits, a low level of physical activity and increased sedentary behavior can contribute to an elevated cardiovascular risk profile (18,21). In this study, diet quality, level of physical activity and sedentary behavior did not differ significantly between both groups. In addition, conventional blood lipids and HbA1c, which can be negatively influenced by poor diet habits, did not show significant differences between ART subjects and controls. Triglycerides were significantly lower in ART study participants which could be due to a potentially lower adherence to the required fasting period ≥4 hours within the control group. While BMI was comparable between both groups, ART subjects displayed a significantly higher BFP compared to spontaneously conceived peers. These findings are in line with results of Ceelen *et al.* who described a disturbed body fat composition in IVF children (59). A population-based cohort study including 122,429 children born after ART and 7,574,685 spontaneously conceived children detected a slightly increased risk of obesity within the ART cohort (35) A recent study by Elhakeem *et al.* suggests that ART individuals demonstrate lower central and total adiposity in childhood but potentially higher levels in adulthood (60). Excess weight and BFP count as important cardiovascular risk factors (61). Moreover, increased BFP is highly associated with arterial hypertension, even if a normal BMI is present (62). Therefore, more studies evaluating BFP as well as cardiovascular morbidity in adult ART subjects are required.

Strengths and limitations

This study was designed as a single center study within Germany and included 66 ART and 86 spontaneously conceived peers. While special emphasis was put on precise age- and gender matching, a generalization of the demonstrated results does not apply. The sample size of the current study can be regarded as adequate. However, ART subjects can display various comorbidities and risk factors (e.g., prematurity, low birth weight) that could potentially impact cardiovascular function. Therefore, larger ART follow-up studies are required in the future. To investigate the influence of age on vascular function, subjects at different developmental stages (children, adolescents, adults) were included to conduct a correlation analysis. Consequently, a large age range was present in both groups. Intentionally, ART subjects with adverse perinatal conditions were included in this study to preserve the "true" cardiovascular risk profile of this cohort. The exclusion of these participants would have substantially reduced the sample size. However, it should be noted that the large age range as well as the inclusion of subjects with adverse perinatal conditions may have influenced the results of the current study.

Data on the course of pregnancy and birth was evaluated retrospectively by screening medical records and interviewing both parents. For some study participants a loss of information was unavoidable as medical records were missing or not fully completed by previous medical professionals. As the present study was not blinded, a potential participation bias cannot be fully ruled out. Nonetheless, parameters of pulse wave analysis and endothelial function were recorded automatically by devices.

For this study, three consecutive office blood pressure measurements were executed and averaged to enhance data validity. However, 24-hour ambulatory blood pressure monitoring is considered the gold standard for the assessment of arterial hypertension and its data should be included in future research. The Mobil-O-Graph® complies to the criteria of the European Society of Hypertension and is therefore recommended as blood pressure device for clinical practice (63). Compared to other devices, it is suggested that the Mobil-O-Graph® underestimates markers of pulse wave analysis (64).

To minimize operator-dependent assessment of endothelial function, the EndoPAT™2000 device was utilized enabling RHI calculation through peripheral artery tonometry (65). However, a study by Allan *et al.* indicates that flow-mediated dilatation might be a more sensitive measure of endothelial function in patients with peripheral arterial disease (66).

A fasting period ≥4 hours and an alcohol abstinence ≥24 hours prior to study participation was required. However, future studies should apply a stricter standardization of diet as well as physical activity ≥12 hours prior to vascular evaluation. Post-exercise hypotension, defined as a decline of SBP and DBP after exercise, can last between 2 and 13 hours after exercise (67). Therefore, an adjustment for this potential cofounder should be applied in future studies.

Modern developments of ART methods and their impact on the offspring's cardiovascular risk profile should be closely observed in the future. For a more precise cardiovascular risk stratification of the ART cohort, larger sample sizes, preferably at adult age, will be required in the future. Therefore, multi-center studies with a longitudinal study design should be established.

Conclusions

The results of the current study do not indicate a significantly lower vascular function in a cohort of children, adolescents and young adults conceived through ART compared to spontaneously conceived peers. Future studies should address the prevalence of elevated Lp(a) levels in infertile individuals who sought ART treatment. In addition, more studies evaluating BFP as well as cardiovascular morbidity in adult ART subjects are required. Ultimately, for a more precise cardiovascular risk stratification, multi-center studies with larger ART sample sizes, preferably at adult age, are required in the future.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of the Medical Faculty of LMU Munich (Munich, Germany; No. 20-0844) on the 27th of December 2020. Informed consent for this study and publication was obtained from all study participants and in minor study participants additionally from parents or legal guardians.

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5. Paper III

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RESEARCH ARTICLE

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Vascular health of fathers with history of intracytoplasmic sperm injection

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ABSTRACT

Objective: Studies suggest that men who undergo assisted reproductive technologies (ART) may have a higher risk of cardiovascular disease; however, limited data on this matter is available. This observational pilot study aimed to investigate the overall vascular health of fathers with history of intracytoplasmic sperm injection (ICSI) compared to fathers whose partners conceived spontaneously.

Methods: Diet quality, physical activity, sedentary behavior as well as overall vascular function including the assessment of pulse wave analysis, intima-media thickness (cIMT), arterial stiffness of the common carotid artery (CCA) and blood lipids, were evaluated.

Results: A total of 34 fathers with history of ICSI and 29 controls (48.49 [46.32 – 57.09] years *vs.* 47.19 [40.62 – 55.18] years, $p = 0.061$) were included. After adjusting for age, no significantly increased cardiovascular risk was detected regarding vascular function.

Conclusions: The results suggest an unaltered cardiovascular risk profile in fathers with history of ICSI. In the future, prospective multicenter studies are required to validate these preliminary results.

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Introduction

Infertility is a disorder of the male or female reproductive system defined as the failure to conceive after at least 12 months or more of regular attempts [1]. According to the World Health Organization (WHO), approximately one in six people globally have experienced infertility at some stage in their lives [2]. It is reported that at least 30 million men worldwide are infertile [3]. Different conditions and diseases can lead to male infertility, such as the obstruction of the reproductive tract, hormonal imbalances, and testicular failure to produce sperm. Lifestyle factors such as smoking, alcohol abuse, or obesity can also negatively affect male fertility. Additionally, exposure to pollutants and toxins can directly harm gametes [4].

Assisted reproductive technologies (ART) opened up new possibilities for couples facing fertility challenges. In 1978, the world's first child was conceived through *in-vitro* fertilization (IVF) [5]. Fourteen years later, the first child was conceived through intracytoplasmic sperm injection (ICSI) [5]. Due to its technical features, which allow a single sperm cell to be directly injected into an oocyte to facilitate fertilization, ICSI is mainly used to treat male infertility [6]. According to the European Society of Human Reproduction and Embryology, more than one million ART treatment cycles were performed as of 2018, of which approximately 40% were ICSI treatments [7].

Increasing evidence suggests that infertility might be related to the development of vascular dysfunction [8–10]. Kasman et al. demonstrated in their retrospective review that infertile men had an elevated risk of developing arterial hypertension, diabetes, dyslipidemia, and heart disease compared to control subjects undergoing vasectomy [11].

Prospective data on vascular function of fathers who suffered from infertility is, however, scarce. Hence, our study aimed to investigate overall vascular

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function of fathers with history of ICSI compared to fathers whose partners conceived naturally.

Methods

Ethical approval

This study was conducted following the Declaration of Helsinki. The Ethics Committee of the Medical Faculty of LMU Munich approved this study on 27 December 2020 (Ethikkommission der Medizinischen Fakultät der Ludwig-Maximilians-Universität München, Pettenkoferstraße 8a, 80336 Munich, Germany; Project number: 20-0844). Prior written informed consent was obtained from all study participants.

Study design

Study participants were recruited in cooperation with the Division of Gynecological Endocrinology and Reproductive Medicine, Division of Obstetrics and Gynecology, University Hospital, LMU Munich (Munich, Germany). Families who successfully conceived a child with help of ICSI treatment were informed in writing about the ongoing study. Families who conceived a child naturally served as controls and were recruited within the greater Munich area (Germany). The study participants were examined at the Division of Pediatric Cardiology and Intensive Care, University Hospital, LMU Munich (Munich, Germany) between May 2021 and March 2022.

Medical history, physical examination, and level of education

Conception types (ICSI *vs.* spontaneous) were recorded. Paternal age at childbirth (years) was calculated. The medical history of each father was reviewed, focusing on the presence of cardiovascular disease (e.g. arterial hypertension, disorders of glucose, and/or lipid metabolism). Smoking status and regular medication intake were assessed. A physical examination was performed on all study participants. Body weight (kg), height (cm), waist circumference (cm), and hip circumference (cm) were measured in all subjects. The waist-to-hip ratio was then calculated. Furthermore, body mass index (BMI, kg/m^2) was calculated. Weight classification was defined as follows: underweight if BMI $< 18.5\,kg/m^2$, normal weight if BMI $\geq 18.5\,kg/m^2$ but $< 25\,kg/m^2$, overweight if BMI $\geq 25\,kg/m^2$ but $< 30\,kg/m^2$, and obese if BMI $\geq 30\,kg/m^2$. The educational level of fathers was determined according to the German education system: no school leaving qualification (0), lower secondary school leaving certificate (1), intermediate secondary school leaving certificate (2), general qualification for university entrance (3), completed apprenticeship (4), and completed university degree (5).

Adherence to the Mediterranean diet

Participants' adherence to the Mediterranean diet was assessed. High adherence to the Mediterranean diet is considered beneficial for cardiovascular health [12]. The validated 14-item Mediterranean diet assessment tool developed by Martínez-González et al. was translated into German and used to assess participants' adherence to the Mediterranean diet [13]. A score $\leq$7 was considered as low adherence to the Mediterranean diet, and a score >7 was considered as high adherence [13].

Level of physical activity and sedentary behavior

Participants' levels of physical activity and sedentary behavior were evaluated. To determine the level of physical activity of study participants, the German version of the Global Physical Activity Questionnaire (GPAQ) provided by the WHO was applied [14]. Corresponding picture cards were presented for each activity type. Total Metabolic-Equivalent (MET)-minutes and recreational MET-minutes per week were calculated based on GPAQ recommendations [14]. Adults with a total of $\geq$600 MET-minutes per week met WHO recommendations [14]. In addition, the study participants were asked how many times per week muscle-strengthening activities were performed and how much time per day (hours/day) they spent with sedentary activities.

Vascular function

Pulse wave analysis

An oscillometric blood pressure device (Mobil-O-Graph®, IEM GmbH, Germany) was used to measure brachial systolic blood pressure (SBP, mmHg), brachial diastolic blood pressure (DBP, mmHg), mean arterial pressure (MAP, mmHg), heart rate (HR, bpm), central SBP (cSBP, mmHg), central DBP (cDBP, mmHg), augmentation index averaged to a heart rate of 75 bpm (AIx@75, %) and pulse wave velocity (PWV, m/s). Cuff sizes were selected based on the participants' right upper arm circumference. Participants were asked to remain supine and still for $\geq$5 minutes before and during the study. Three consecutive measurements were performed and averaged. SBP and DBP were considered elevated if SBP ≥130 mmHg and DBP ≥85 mmHg [15].

Sonography of the common carotid artery

Sonography of both common carotid arteries (CCA) was performed by one investigator for all study participants using either a Philips iE33 xMatrix or a Philips Epiq 7G ultrasound device (Philips Healthcare, Amsterdam, The Netherlands). During the examination, study participants were asked to keep in a supine position with the neck extended to a 45° angle and turned toward the opposite of the examination side [16]. Offline analysis was performed by one investigator.

Peak circumferential strain, peak strain rate, and arterial distensibility

The area directly below the carotid bifurcation was scanned in short-axis view using a 3–8 MHz sector array transducer (Philips Healthcare, Amsterdam, The Netherlands). Three consecutive loops were recorded under three-lead ECG tracking and transferred to a separate workstation (QLAB Cardiovascular Ultrasound Quantification Software version 11.1; Philips Healthcare, Amsterdam, The Netherlands) for offline analysis. The SAX-A function of the software was then utilized. To accurately track the vessel's wall and to avoid the tracking of perivascular tissue, the region of interest (ROI) was manually adjusted. Pixels of the vascular ROI were then tracked in 2D throughout the cardiac cycle. Peak circumferential strain (CS, %) and peak strain rate (SR, 1/s) were determined manually. To improve data validity, an average of three measurements was calculated for each CCA side. Arterial distensibility ($\text{mmHg}^{-1} \times 10^{-3}$) was calculated using the following formula [17]:

$$\text{Arterial Distensibility} = \frac{2 \times \text{Peak Circumferential Strain}}{\text{Systolic Blood Pressure} - \text{Diastolic Blood Pressure}}$$

Additionally, CS, SR, and arterial distensibility of the right and left CCA were averaged.

Carotid intima-media thickness

Both CCAs were scanned in long-axis view using a 3–12 MHz linear array transducer (Philips Healthcare, Amsterdam, The Netherlands) at the level of carotid bifurcation. Three consecutive loops were recorded with simultaneous three-lead ECG tracking and transferred to a separate workstation (QLAB Cardiovascular Ultrasound Quantification Software version 11.1; Philips Healthcare, Amsterdam, The Netherlands) for offline analysis. Carotid intima-media thickness (cIMT, mm) was assessed semi-automatically at end-diastole (R-wave on ECG) on each side. The ROI was set proximal to the carotid bifurcation and the length of ROI was adjusted to 10 mm. To improve data validity, three measurements were taken, and an average was calculated for each CCA individually. Additionally, cIMT of the right and left CCA were averaged.

Stiffness index β

The sonographic study protocol described above was applied. Both CCAs were scanned in long-axis view using a 3–12 MHz linear array transducer (Philips Healthcare, Amsterdam, The Netherlands). M-Mode was applied proximal to the carotid bifurcation. End-diastolic diameter (dD, mm) and end-systolic diameter (sD, mm) of both CCAs were measured offline by a masked investigator (IntelliSpace Cardiovascular Ultrasound Viewer, Philips Healthcare, Amsterdam, The Netherlands).

Stiffness index β was calculated using the following formula [17]:

$$\text{Stiffness Index } \beta = \frac{\ln\left(\frac{\text{SBP}}{\text{DBP}}\right)}{\Delta D/dD}$$

Blood lipid profile

Total cholesterol (TC, mg/dL), low-density lipoprotein cholesterol (LDL-C, mg/dL), high-density lipoprotein cholesterol (HDL-C, mg/dL), non-high-density lipoprotein cholesterol (non-HDL, mg/dL), triglycerides (mg/dL) and lipoprotein a (Lp(a), mg/dL) were measured to assess the blood lipid profile. A fasting period of ≥4 h was required before blood sampling. The presence of elevated conventional blood lipids was defined according to adult recommendations [18,19]. An Lp(a) ≥50 mg/dL was defined as increased [20].

Statistical analysis

Continuous parameters were tested for normality using the Kolmogorov–Smirnov test or the Shapiro–Wilk test. For normally distributed continuous variables, the unpaired *t*-test was used. For non-normally distributed continuous variables, the Mann–Whitney-U test was applied. The chi-square test was used to compare nominal data. Analysis of covariance (ANCOVA) was used to adjust for confounders, such as age. Normally distributed data were presented as mean ± standard deviation (SD) and non-normally distributed data as median (interquartile range [IQR]). IBM SPSS Statistics for Windows version 29.0 (IBM Corp., Armonk, NY) was used for data analysis. A $p < 0.05$ was considered statistically significant.

Results

Patient characteristics

A total of 34 fathers with history of ICSI and 29 control subjects were included in this study.

In the group with history of ICSI, seven subjects had arterial hypertension, four had dyslipidemia, two had glucose metabolism disorders, one had prostate cancer, one displayed with history of transient ischemic attack (TIA) and one had thyroid disease. Seven fathers with history of ICSI were taking antihypertensive medication, one subject was taking lipid-lowering medication, two subjects were taking antidiabetic medication, one subject was taking blood thinners, and one subject was taking L-thyroxine.

In the control group, four subjects had arterial hypertension, three had dyslipidemia, one displayed a history of thrombosis, one displayed with history of pheochromocytoma and one had TIA. Two subjects had thyroid disease. Two subjects were taking antihypertensive medication, two subjects were taking lipid-lowering medication, one subject was taking blood thinners, and two subjects were taking L-thyroxine.

Median age was 48.49 (46.32 – 57.09) years in fathers with history of ICSI and 47.19 (40.62 – 55.18) years in controls ($p = 0.061$). The two groups did not differ significantly in anthropometric variables and smoking status (Table 1). Both groups differed significantly in educational level ($p = 0.017$). Paternal age at birth in fathers with history of ICSI was significantly higher compared to controls ($p < 0.001$). Patients' characteristics are visualized in Table 1.

Diet quality, level of physical activity, and sedentary behavior

Both groups did not differ significantly in diet quality. Compared to controls, fathers with history of ICSI tended to achieve more total MET-minutes per week. Moreover, fathers with history of ICSI were significantly less engaged in sedentary behavior compared to controls ($p = 0.046$). Data on diet quality, level of physical activity, and sedentary behavior are given in Table 2.

Vascular function

PWV and stiffness index β were significantly higher in fathers with history of ICSI ($p = 0.048$, $p = 0.019$). The remaining vascular variables did not differ significantly between both groups (Table 3). After ANCOVA adjustment for age, vascular function variables showed no significant differences between both groups (Table 4).

Blood lipid profile

No differences were found in blood lipid profile between the two groups (Table 5).

Discussion

This pilot study investigated overall vascular health in fathers with history of ICSI. In total, 34 fathers with history of ICSI and 29 controls were enrolled.

Fathers with history of ICSI tended to be older at time of examination. Moreover, they displayed a significantly higher paternal age at birth. While both groups did not differ significantly in anthropometric

Table 1. Patients' characteristics.

Variable	ICSI ($n = 34$)	Control ($n = 29$)	p Value
Age (years)	48.49 (46.32 – 57.09)	47.19 (40.62 – 55.18)	0.061
Bodyweight (kg)	93.67 ± 15.79	90.91 ± 10.83	0.415
Height (cm)	181.86 ± 6.01	183.94 ± 5.15	0.150
BMI (kg/m^2)	28.32 ± 4.62	26.90 ± 3.34	0.174
Underweight (n (%))	0 (0)	0 (0)	0.357
Normal weight (n (%))	9 (26.47)	8 (27.59)	
Overweight (n (%))	14 (41.18)	16 (55.17)	
Obese (n (%))	11 (32.35)	5 (17.24)	
Waist/hip-circumference ratio	0.97 ± 0.05	0.96 ± 0.06	0.723
Smoking status (n (%))[a]	10 (29.41)	5 (17.86)	0.290
Paternal educational level	4.00 (3.00 – 5.00)	5.00 (5.00 – 5.00)	0.017*
Paternal age at birth (years)	38.52 (34.92 – 45.47)	33.53 (30.09 – 35.24)	<0.001***

ICSI: intracytoplasmic sperm injection; BMI: body mass index
[a]28 control subjects were included in the analysis. Paternal educational level was assessed according to the German education system: no school leaving qualification (0), lower secondary school leaving certificate (1), intermediate secondary school leaving certificate (2), general qualification for university entrance (3), completed apprenticeship (4), and completed university degree (5). Data is presented as mean ± SD for normally distributed parameters and as median (IQR) for non-normally distributed parameters. Nominal data is presented as n (%). $^{*}p < 0.05$; $^{***}p \leq 0.001$.

Table 2. Diet quality, level of physical activity, and sedentary behavior.

Variable	ICSI ($n = 34$)	Control ($n = 29$)	p Value
MEDAS[a]	5.85 ± 2.64	5.86 ± 2.48	0.983
Total MET-minutes per week[b]	1950 (930–5005)	1510 (550–2355)	0.050
Recreational MET-minutes per week	480 (150–1290)	720 (270–1500)	0.476
Muscle strengthening activities (times/week)	0 (0 – 0.125)	0 (0–1)	0.711
Sedentary behavior (hours/day)	8.18 ± 3.25	9.74 ± 2.76	0.046*

ICSI: intracytoplasmic sperm injection; MEDAS: Mediterranean diet adherence score; MET: metabolic-equivalent
Data is presented as mean ± SD for normally distributed parameters and as median (IQR) for non-normally distributed parameters. Nominal data is presented as n (%).
[a]33 ICSI subjects were included in the analysis.
[b]32 ICSI subjects and 28 control subjects were included in the analysis.
*$p < 0.05$.

Table 3. Vascular function.

Variable	ICSI ($n = 34$)	Control ($n = 29$)	p Value
Pulse wave analysis			
SBP (mmHg)[a]	127.94 ± 11.38	124.91 ± 12.58	0.329
Elevated SBP (n (%))[a]	13 (38.24)	9 (33.33)	0.692
DPB (mmHg)[a]	86.32 ± 8.12	83.54 ± 8.58	0.201
Elevated DBP (n (%))[a]	18 (52.94)	11 (40.74)	0.343
MAP (mmHg)[a]	105.40 ± 9.21	102.53 ± 9.87	0.246
cSBP (mmHg)[a]	122.38 ± 11.77	119.05 ± 11.99	0.280
cDBP (mmHg)[a]	87.54 ± 8.41	84.69 ± 8.58	0.197
Heart rate (bpm)[a]	58.38 ± 9.53	60.14 ± 8.90	0.465
AIx@75 (%)[a]	10.29 ± 9.47	11.68 ± 13.42	0.652
PWV (m/s)[a]	7.48 ± 0.83	7.01 ± 0.99	0.048*
Sonography of the common carotid artery			
CCA CS (%)[b]	6.61 ± 2.31	7.02 ± 1.99	0.463
CCA SR (1/s)[b]	1.60 ± 0.53	1.84 ± 0.48	0.079
CCA Dis ($mmHg^{-1} \times 10^{-3}$)[c]	319.67 ± 105.95	347.98 ± 99.05	0.294
Stiffness index β[d]	4.97 (3.42 – 7.05)	3.50 (2.86–5.84)	0.019*
cIMT (mm)[e]	0.63 ± 0.11	0.58 ± 0.09	0.056

ICSI: intracytoplasmic sperm injection; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; cSBP: central systolic blood pressure; cDBP: central diastolic blood pressure; AIx@75: augmentation index averaged to a heart rate of 75 bpm; PWV: pulse wave velocity; CCA: common carotid artery; CS: peak circumferential strain; SR: peak strain rate; Dis: arterial distensibility
[a]27 control subjects were included in the analysis. [b]33 ICSI subjects were included in the analysis. [c]33 ICSI subjects and 27 control subjects were included in the analysis. [d]32 ICSI subjects and 26 control subjects were included in the analysis. [e]33 ICSI subjects and 28 control subjects were included in the analysis.
Data is presented as mean ± SD for normally distributed parameters. Nominal data is presented as n (%). *$p < 0.05$.

variables as well as smoking status, paternal educational level was significantly altered between the two cohorts.

This study design accounted for potential confounders, such as diet quality, physical activity, and sedentary behavior. Both groups did not differ significantly in diet quality. However, fathers with history of ICSI were, in comparison to peers, physically more active and engaged significantly less in sedentary behavior.

Overall vascular function was visualized using different non-invasive methodologies including the measurement of brachial blood pressure, central blood pressure, and PWV through an oscillometric blood pressure device. Further, arterial stiffness and cIMT of the CCA were evaluated sonographically.

Interestingly, fathers with history of ICSI demonstrated a significantly higher pulse wave velocity as well as a significantly higher stiffness index β compared to peers. However, these results did not remain significant when adjusted for age.

The assessment of blood lipids revealed no significant alterations between both groups.

Overall vascular function did not display significant differences between both groups suggesting a clinically unaltered cardiovascular risk profile in fathers with history of ICSI.

Associations between male infertility and cardiovascular health

The literature suggests that male infertility might be associated with increased cardiovascular morbidity and mortality: Kasman et al. demonstrated in their retrospective review of insurance data, that infertile men had an elevated risk of developing arterial hypertension, diabetes, dyslipidemia, and heart disease compared to control subjects undergoing vasectomy [11]. A retrospective longitudinal cohort study by Wei et al. investigated the long-term risk of cardiovascular hospitalization in fathers with history of ART [21]. The authors demonstrated that ART was linked with a 24%

elevated risk of paternal cardiovascular hospitalization [21]. In addition, a retrospective cohort study by Eisenberg et al. with 135,903 male subjects revealed that childless men display, compared to fathers, a 17% higher risk of dying from cardiovascular disease [22].

The exact pathophysiological mechanisms explaining the increased cardiovascular morbidity and mortality among infertile men are not yet fully understood. In the following, we want to elaborate on some pathophysiological considerations:

As approximately 15% of the male genome is involved in reproduction [23,24], it looks plausible that other organ systems, such as the cardiovascular one, can be genetically influenced by infertility. Male fertility can be negatively influenced by hormonal imbalances, such as testosterone deficiency [25]. Testosterone deficiency itself has been linked with an increased cardiovascular risk [25,26]. Sexual dysfunctions such as erectile dysfunction are encountered more frequently in male subjects with infertility [27]. The literature suggests that erectile dysfunction is linked with endothelial dysfunction and can be considered as an independent cardiovascular risk factor [25]. A Danish cohort study by Latif et al. investigated the predictive value of semen quality as a biomarker for long-term morbidity in 4712 men [23]. Interestingly, the authors were able to demonstrate that a sperm concentration <15 million/mL was associated with a 40% increased risk for cardiovascular hospitalization [23]. Moreover, poor lifestyle habits (e.g. poor diet quality and low level of physical activity) as well as acquired cardiometabolic risk factors (e.g. excess weight and diabetes) are thought to lower male fertility [28–30]. Further, infertility can have negative effects on male mental health [31]. A systemic review and meta-analysis by Kiani et al. highlighted that depression affects 16.75–18.55% of infertile men [31]. This needs to be taken into consideration as people with depression are more like to develop cardiovascular diseases compared to the general population [32].

To summarize, the pathophysiological mechanisms explaining the increased cardiovascular morbidity and mortality among infertile men are complex and require further research.

Table 4. Age-adjusted vascular function.

Variable	ICSI ($n=34$)	Control ($n=29$)	p Value
SBP (mmHg)[a]	127.66 ± 2.08	125.26 ± 2.34	0.451
DBP (mmHg)[a]	85.74 ± 1.38	84.26 ± 1.56	0.486
MAP (mmHg)[a]	104.97 ± 1.63	103.08 ± 1.83	0.451
cSBP (mmHg)[a]	121.97 ± 2.05	119.57 ± 2.31	0.447
cDBP (mmHg)[a]	86.98 ± 1.42	85.40 ± 1.59	0.469
Heart rate (bpm)[a]	58.16 ± 1.61	60.41 ± 1.82	0.365
AIx@75 (%)[a]	9.45 ± 1.87	12.74 ± 2.11	0.255
PWV (m/s)[a]	7.30 ± 0.07	7.23 ± 0.08	0.491
Sonography of the common carotid artery			
CCA CS (%)[b]	6.76 ± 0.37	6.85 ± 0.39	0.872
CCA SR (1/s)[b]	1.66 ± 0.08	1.77 ± 0.09	0.347
CCA Dis ($mmHg^{-1} \times 10^{-3}$)[c]	323.91 ± 18.05	342.80 ± 20.02	0.493
Stiffness index β[d]	6.31 ± 0.71	4.49 ± 0.79	0.099
cIMT (mm)[e]	0.62 ± 0.02	0.60 ± 0.02	0.406

ICSI: intracytoplasmic sperm injection; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure; cSBP: central systolic blood pressure; cDBP: central diastolic blood pressure; AIx@75: augmentation index averaged to a heart rate of 75 bpm; PWV: pulse wave velocity; CCA: common carotid artery; CS: peak circumferential strain; SR: peak strain rate; Dis: arterial distensibility
The analysis of covariance (ANCOVA) was used to test for significance.
[a]27 control subjects were included in the analysis. [b]33 ICSI subjects were included in the analysis. [c]33 ICSI subjects and 27 control subjects were included in the analysis. [d]32 ICSI subjects and 26 control subjects were included in the analysis. [e]33 ICSI subjects and 28 control subjects were included in the analysis. Data is presented as mean ± SD for normally distributed parameters.

Limitations

In contrast to our expectations, this study did not reveal a significantly lower vascular health in fathers with history of ICSI. Primarily, this might be due to the low sample size, the relatively young age of subjects, the suboptimal age matching, the different educational levels, and differences in physical activity as well as sedentary behavior between both groups.

Table 5. Blood lipid profile.

Variable	ICSI ($n=34$)	Control ($n=29$)	p Value
TC (mg/dL)	205.12 ± 36.52	198.24 ± 40.44	0.481
Increased TC (n (%))	19 (55.88)	14 (48.28)	0.547
LDL-C (mg/dL)	132.38 ± 32.38	124.41 ± 40.36	0.388
Increased LDL-C (n (%))	25 (73.53)	16 (55.17)	0.128
HDL-C (mg/dL)	51.32 ± 13.25	56.24 ± 17.17	0.205
Decreased HDL-C (n (%))	15 (44.12)	10 (34.48)	0.436
Non-HDL-C (mg/dL)	153.74 ± 37.60	142.03 ± 44.04	0.260
Increased Non-HDL-C (n (%))	27 (79.42)	18 (62.07)	0.128
Triglycerides (mg/dL)	121.50 (89.00 – 181.75)	149.00 (82.50 – 189.00)	0.831
Lp(a) (mg/dL)[a]	8.00 (5.00 – 26.25)	9.00 (5.00 – 12.00)	0.895
Increased Lp(a) (n (%))[a]	6 (20.00)	4 (13.79)	0.731

ICSI: intracytoplasmic sperm injection; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; non-HDL-C: non-HDL cholesterol; Lp(a): lipoprotein (a)
Data is presented as mean ± SD for normally distributed parameters and as median (IQR) for non-normally distributed parameters. Nominal data is presented as n (%).
[a]30 ICSI subjects were included in the analysis.

Both groups did not differ significantly in age. However, there was a tendency of an increased age in the group of fathers with history of ICSI. For this matter, vascular parameters were adjusted for age. In addition, median age was relatively low in the examined groups. Further studies need to investigate whether an increased cardiovascular morbidity of fathers with history of ICSI can be detected at advanced age. Unlike reported within the literature, fathers with history of ICSI did not display significant differences in anthropometric variables and acquired cardiometabolic risk factors (e.g. excess weight and dyslipidemia) which could have positively influenced vascular health [11,30]. The differences in paternal educational levels could have posed a bias. In contrast to literature regarding infertile men, our results revealed that fathers with history of ICSI were physically more active and engaged significantly less in sedentary behavior compared to controls. Notably, these factors could have positively influenced vascular function in the cohort of fathers with history of ICSI.

To the best of our knowledge, this was the first study investigating overall vascular health in fathers with history of ICSI compared to peers. As this was a pilot study, a prior power analysis was not feasible. Different non-invasive methodologies were applied to assess vascular function. Limitations on the vascular methodologies used in this study were elaborated on in recent publications of our departments [33,34]. The study design was not longitudinal. Further, the study sample of this pilot study can be considered as relatively low. Participants were recruited by one center and presented with different comorbidities. This study did not address the exact factors leading to male infertility nor baseline markers of male reproductive health (e.g. hormones, sperm morphology, sperm concentration, and genetics) at time of ICSI treatment.

To overcome the above-mentioned limitations, prospective longitudinal multi-centric studies of men with infertility are required in the future.

Conclusion

This pilot study investigated the overall vascular health in fathers with history of ICSI compared to fathers whose partners conceived naturally. Overall vascular function did not display significant differences between both groups suggesting an unaltered cardiovascular risk profile in fathers with history of ICSI. In the future, prospective multicenter studies with a lager study sample are required to validate these preliminary results.

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