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Protocol

Study protocol for a Developmental Epidemiological Study of Children born through Reproductive Technologies (DESCRT)

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ABSTRACT

STUDY QUESTIONS: The primary objective of this study is to determine what parental factors or specific ART may influence the risk for adverse cardiometabolic outcomes among children so conceived and their parents. The secondary objective of this study is to prospectively examine the effects of infertility or ART on the intrauterine environment, obstetric and neonatal outcomes.

WHAT IS KNOWN ALREADY: Pregnancies conceived with ART are at an increased risk of being affected by adverse obstetric and neonatal outcomes when compared to spontaneously conceived (SC) pregnancies among fertile women. Small cohort studies have suggested ART-conceived children may have a higher risk of long-term cardiometabolic disturbances as well. Currently, few studies have compared long-term cardiometabolic outcomes among ART-conceived children and non-IVF treated (NIFT) children, to children conceived spontaneously to parents with infertility (subfertile parents).

STUDY DESIGN, SIZE, DURATION: The Developmental Epidemiological Study of Children born through Reproductive Technologies (DESCRT) is a prospective cohort study that aims to: establish a biobank and epidemiological cohort of children born to subfertile or infertile parents who either conceived spontaneously (without assistance) or used reproductive technologies to conceive (all offspring were from couples assessed and/or treated in the same institute); prospectively examine the effects of infertility or ART on the intrauterine environment, obstetric and neonatal outcomes; and determine what parental factors or ART may influence the cardiometabolic risk of children so conceived. Pregnancies and resultant children will be compared by mode of conception, namely offspring that were conceived without medical assistance or SC or following NIFT, IVF with fresh embryo transfer or frozen embryo transfer (FET), and by fertilization method (conventional versus ICSI). DESCRT has a Child group evaluating long-term outcomes of children as well as a Pregnancy group that will compare obstetric and neonatal outcomes of children conceived since the commencement of the study. Recruitment started in May of 2017 and is ongoing. When the study began, we estimated that ~4000 children would be eligible for enrollment.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Eligible participants are first-trimester pregnancies (Pregnancy group) or children (Child group) born to parents who were evaluated at an infertility center in the University of California, San Francisco, CA, USA who were SC or conceived after reproductive treatments (NIFT, IVF ± ICSI, FET). Children in the Child group were conceived at UCSF and born from 2001 onwards. In the Pregnancy group, enrollment began in November of 2017.

The primary outcome is the cardiometabolic health of offspring in the Child group, as measured by blood pressure and laboratory data (homeostatic model assessment for insulin resistance (HOMA-IR), oral glucose disposition). There are several secondary outcome measures, including: outcomes from parental survey response (assessing parent/child medical history since delivery—incidence of cardiometabolic adverse events), anthropomorphic measurements (BMI, waist circumference, skinfold thickness), and laboratory data (liver enzymes, lipid panel, metabolomic profiles). In the Pregnancy group, outcomes include laboratory assessments (bhCG, maternal serum analytes, soluble fms-like tyrosine kinase-1 (sFLT-1), and placental growth factor (PlGF)) and placental assessments (placental volume in the second and third trimester and placental weight at delivery). Importantly, aliquots of blood and urine are stored from parents and offspring as part of a biobank. The DESCRT cohort is unique in two ways. First, there is an extensive amount of clinical and laboratory treatment data: parental medical history and physical examination at the time of treatment, along with ovarian reserve and infertility diagnosis; and treatment specifics: for example, fertilization method, culture O_2 status, embryo quality linked to each participant. These reproductive data will aid in identifying explanatory variables that may influence the primary cardiometabolic outcomes of the offspring—and their parents. Second, the DESCRT control group includes pregnancies and children SC from parents with subfertility, which may help to assess when infertility, as opposed to reproductive treatments, may be affecting offspring cardiometabolic health.

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WHAT DOES THIS MEAN FOR PATIENTS?

Reproductive technologies have helped millions of people build their families. Some studies have suggested children born from reproductive technologies may have a higher risk for some health conditions such as high blood pressure and diabetes. Overall, few studies have looked at the long-term health outcomes for the millions of children born with the assistance of reproductive technologies. The few studies that have examined a relation between reproductive technologies and the health of resultant children, do not commonly distinguish between whether it is the reproductive technology or the infertility that is the more important influence on health outcomes. A study of the developmental children born through reproductive technologies (called DESCRT) aims to understand what specific factors (e.g. diagnosis or fertility treatments or both) influence the health of children born to infertile parents.

We are recruiting children born to infertile parents who, by chance, were spontaneously conceived (SC), were born after IVF followed by fresh embryo transfer or frozen embryo transfer (FET); were born after non-IVF treatments (NIFT) such as ovulation induction or ovarian stimulation agents (e.g. clomiphene citrate, letrozole or gonadotropins) with timed intercourse or intrauterine insemination (IUI); or following IUI without ovarian stimulation. Broadly considering the three conception groups, SC, NIFT, or IVF, we will evaluate the resultant children for markers of the heart and overall health by using blood tests and measurements of the child (e.g. height, weight and skin-fold thickness). We are also looking at how the placenta, the organ that develops to feed a growing fetus, may grow differently or use nutrients differently in pregnancies conceived with fertility treatments.

There are potentially many factors that influence the long-term health of children. In some cases, the treatment for infertility may have a more significant effect but in other scenarios it may be some aspect of the infertility itself that is a more important influence on childhood health. Not only will DESCRT begin to answer these questions, but also we will develop resources for future studies, by our group and others. Children born through the pregnancy cohort may be enrolled in the child cohort. As part of future investigations, we intend to send parents additional questionnaires on a regular basis to maintain interest and gather long-term data on growing families. Additionally, we are storing blood and urine samples to investigate questions that may arise in the future.

Introduction

Globally, a staggering one in seven couples of reproductive age experiences infertility. Thanks to ART and non-IVF treatments (NIFT), millions of people have been able to achieve their dreams of having a family (Dyer *et al.*, 2019; Fauser, 2019). While ART can successfully treat infertility, it is also associated with an increased risk of adverse maternal and neonatal outcomes, such as pre-eclampsia, small for gestational age (SGA) infants, and may portend long-term consequences for the offspring (Arends *et al.*, 2005; Hansen *et al.*, 2013; Pinborg *et al.*, 2013). Understanding whether underlying parental infertility or the infertility treatments impact parental and offspring well-being is critically important for the millions of people who utilize, or are conceived as a result of, ART.

Current data are conflicting on whether ART influences maternal or offspring health beyond the perinatal period. For example, Yeung *et al.* compared 968 singletons conceived with ART to 2471 children conceived spontaneously and reported no differences in growth patterns or other developmental milestones up to 3 years of age (Yeung *et al.*, 2016). Other small prospective studies demonstrated children conceived with ART have an increased incidence of hypertension and insulin resistance when compared to spontaneously conceived (SC) children (Law *et al.*, 1993; Ceelen *et al.*, 2008; Gkourogianni *et al.*, 2014; Meister *et al.*, 2018). In particular, a Nordic study suggested ART-conceived adults had an increased risk of cardiovascular disease and type 2 diabetes. However, this study did not find an association between specific ART treatments and outcomes that could explain these findings (Norrman *et al.*, 2021). Current studies on ART and offspring cardiometabolic health are relatively limited in size, duration of follow-up, and differ in the control groups used and in the types of outcomes assessed (Table 1). To address the questions of the long-term impact of infertility treatments on the infertile couple and the resultant offspring, it is imperative to use well-controlled, robust, and prospectively collected data.

To date, offspring cardiometabolic outcomes seem to have the strongest potential for an association with parental infertility and ART treatments but the etiology of these associations is unclear. However, animal data support some biologic plausibility. Bloise *et al.*, (2012) demonstrated murine embryos obtained by IVF showed altered placental growth relative to SC embryos (Bloise *et al.*, 2012). Although IVF placentae were larger, they were less efficient at nutrient transport, with altered regulation of several genes involved in nutrient and cortisol metabolism (sodium-coupled neutral amino acid transporter 1, 2, and 4 (*SNAT1*, 2, and 4); glucose transporter 1 and 3 (*GLUT1* and 3); H19, insulin like growth factor 2 and P0 (*Igf2* and *Igf2P0*); and 11β hydroxysteroid dehydrogenase (*11βHSD*)). It is possible that alterations in placental metabolism may impact postnatal and lifelong cardiometabolic health. Aljahdali *et al.*, (2020) compared SC mice to IVF conceived mice cultured in varying environments and demonstrated that prolonged embryo culture was associated with adverse cardiovascular outcomes in the adult male mice. Additionally, mice conceived from a cleavage stage embryo transfer had an increased risk of adverse metabolic outcomes in

Table 1. Summary of studies comparing offspring cardiometabolic health in cohorts that were conceived following ART versus controls.

Author	Study	Treatment group(s)	Control Group	Study Outcomes	Location
Scherrer *et al.* (2012)	Vascular Dysfunction in Offspring of Assisted Reproduction Technologies	IVF ± ICSI, FET	Spontaneously conceived—fertile	Ejection fraction, left ventricular muscle mass, systemic and pulmonary vascular function	Switzerland
Huang *et al.* (2021)	Growing Up in Singapore Towards healthy Outcomes (GUSTO)	ART	Spontaneously conceived—possibly subfertile	Cardiometabolic outcomes (anthropometry, blood pressure, serum metabolic biomarkers, and cord tissue DNA methylation)	Singapore
This study	Developmental Epidemiological Study of Children born through Reproductive Technologies (DESCRT)	NIFT, IVF ± ICSI, FET	Spontaneously conceived—subfertile	Cardiometabolic outcomes (Anthropometric, Blood Pressure, Laboratory Assessments etc.)	Bay Area, United States

FET: frozen embryo transfer; NIFT: non-IVF treatment.

adulthood. It is also possible that because cardiometabolic conditions occur with a higher frequency among people seeking ART, these parental conditions may independently influence the health of offspring (Murugappan *et al.*, 2019). Women experiencing ovarian aging, which can be detected with elevated FSH levels or decreased anti-Müllerian hormone (AMH) levels, have higher lipid profiles and an increased risk of cardiovascular disease (Chu *et al.*, 2003). Maternal cardiovascular disease may lead to *in utero* programming that may also predispose offspring to cardiovascular disease (Palinski, 2014). Although maternal age may directly affect pregnancy and birth outcomes, other etiologies for infertility may track with long-term health outcomes for parents and offspring as well.

At this point, we do not know whether infertility, or its treatments, may predispose offspring or their parents to an increased risk of cardiometabolic disease. A major limitation of currently available studies is that the control group is often represented by children SC from fertile parents. It is possible, in addition to the ART, that infertility *per se* could influence health outcomes of the offspring or their parents, and this could be better evaluated by including children who were conceived spontaneously from infertile parents (subfertile parents) (Magnus *et al.*, 2021). Furthermore, data on health outcomes of children conceived with NIFT (i.e. ovulation induction and/or IUI) are conspicuously lacking in the literature. Thus, a significant proportion of children with infertile parents are conceived with NIFT and are excluded from assessments of their long-term health. It has been proposed that there is potentially a 'common underlying mechanism' that encompasses ovarian aging, infertility, and the specific treatments utilized, and may be linked to the health of the parent, pregnancy, and of offspring (Yeung and Druschel, 2013). The Developmental Epidemiological Study of Children born through Reproductive Technologies (DESCRT) was designed to disentangle these relationships and provide a resource for future investigations on the relations between infertility, fertility treatments, and parental and offspring health.

Outcomes

DESCRT has three AIMS, as described below:

AIM I: to establish a biobank and epidemiological cohort of children born to subfertile or infertile parents who conceived spontaneously or used reproductive technologies to conceive.
AIM II: to determine what parental factors or specific ART may influence the risk for adverse cardiometabolic outcomes among children so conceived and their parents.
AIM III: to prospectively examine the effects of infertility or ART on the intrauterine environment, obstetric, and neonatal outcomes.

The primary outcome of DESCRT is the prevalence of cardiometabolic dysregulation among children born from NIFT and ART compared to SC children born to subfertile parents. We will assess this by looking at the following measures: homeostatic model assessment for insulin resistance (HOMA-IR), oral glucose disposition, blood pressure, and specific metabolomics patterns in offspring in the DESCRT cohort (Matsuda and DeFronzo, 1999) (Table 2).

Materials and methods

Study design

DESCRT is a cohort study that includes collection of patient clinical data from electronic medical records (EMRs), information on lifestyle and environmental factors from multiple questionnaires, offspring anthropometric and laboratory assessments from in-person visits, and biobanking of samples from children and parents. We will also assess the impact of infertility and its treatments on pregnancy and intrauterine development by following people that recently conceived. This will involve sonographic and laboratory assessment of fetal and placental growth, and collection and assessment of placental function among a subset of participants. Ultimately, we will assess several cardiometabolic parameters over the span of children's lives from conception to early adulthood (Fig. 1).

Cohort development

The DESCRT cohort is maintained through a single large university program focused on reproductive health. The Center for Reproductive Health (CRH) at the University of California, San

Table 2. Summary of DESCRT primary, secondary and exploratory outcomes.

			Outcome*
Child group	Primary outcomes	Laboratory	• Composite insulin sensitivity index • Oral glucose tolerance test • HOMA-IR • Oral glucose disposition
		Anthropomorphic	• Blood pressure
	Secondary outcomes	Laboratory	• Lipid profile • Uric acid • AST/ALT • Metabolomic profile (7- to 9-year-old children)
		Anthropomorphic	• BMI, weight, height • Skinfold thickness • Bio-electrical impedance analysis • Blood pressure • Waist circumference • Tanner stage
		Historical	*Collected from parental questionnaire at enrollment* • Reported parent and child cardiometabolic dysregulation (obesity, hypertension, glucose intolerance, or diabetes)
Pregnancy Group	Exploratory prospective outcomes	Laboratory	*Collected for pregnancy detection* • bhCG levels at 10–14 days after ovulation and repeated every 2 days, twice then 2 and 4 weeks passed a pregnancy diagnosis *Collected at 6 and 8 weeks* • PlGF and sFLT-1 *Collected at 11-14 weeks* • First trimester screen labs: PAPP-A and bHCG • PlGF and sFLT levels *Collected at 15–20 weeks* • Quad screen: AFP, bhCG, estriol, inhibin PlGF, and sFLT levels
		Placental (subgroup analysis)	Collected at 18–22 weeks • Placental volume at anatomy scan and third trimester when indicated Collected at delivery • Placental weight • Placental expression of SNAT1, 2 and 4; GLUT1 and 3; H19, Igf2, Igf2P0, and 11βHSD measured with RT-PCR

* Primary and secondary outcomes are collected from the DESCRT Child cohort when the participant is age 4 years or older. 11βHSD: 11β hydroxysteroid dehydrogenase; AFP: alpha fetoprotein; AST/ALT: aspartate and alanine transaminase; bhCG: beta human chorionic gonadotropin; GLUT1 and 3: glucose transporter 1 and 3; HOMA-IR: homeostatic model assessment for insulin resistance; Igf2 and Igf2P0: insulin like growth factor 2 and P0; PlGF: platelet growth factor; PAPP-A: pregnancy-associated plasma protein-A; SNAT1, 2, and 4: sodium-coupled neutral amino acid transporter 1,2 and 4; sFLT-1: soluble FMS-like tyrosine kinase-1.

Francisco (UCSF) has grown since 2001 to provide over 2000 fresh IVF cycles/year. Beginning in 2008, we collected all clinical data using an EMR. Additionally, laboratory data for IVF cycles between 2001 and 2008 were retrospectively added to the EMR making the information on all IVF cycles available, starting with 2001. This database contains detailed information regarding oocyte/embryo development, including details such as oxygen tension, specific media preparations, use of micromanipulation, cleavage stage versus blastocyst culture, and fresh versus frozen transfer.

Accessible population

This cohort includes pregnancies and the resultant children born to parents who conceived after an initial consultation for infertility or procreative management (e.g. fertility assistance for same-sex couples or preimplantation genetic testing for monogenic conditions (PGT-M)) at UCSF CRH starting in 2001 as well as their parent(s). Eligible children may have been conceived after:

- SC to infertile couples.
- NIFT such as ovulation induction (OI) or ovarian stimulation (with the use of letrozole, clomiphene citrate, or gonadotrophins) with or without IUI. Additionally, IUI may have been performed with or without ovarian stimulation. For people using donor sperm for IUI, the choice to use ovarian stimulation was determined by the provider and not required.
- IVF with or without ICSI and a fresh transfer with autologous or donated gametes.
- Frozen embryo transfer of embryos conceived using either conventional insemination or ICSI with autologous or donated gametes.

Compared to several prior studies exploring the influence of ART on offspring health, DESCRT will include a cohort of children conceived spontaneously with parents experiencing infertility (subfertile) and specifically examine cardiometabolic outcomes (Table 2). The number of months trying to conceive is not a prespecified criteria to define a parent as infertile; however, the majority of our patients experience infertility as defined by Zegers-Hochschild *et al.*, wherein they have experienced 12 months or longer of regular unprotected intercourse and have not established a clinical pregnancy, whether or not they ultimately receive infertility treatment (Zegers-Hochschild *et al.*, 2017). We collect data on the months trying to conceive as part of the demographic data (see *Data Collected* below) and can address this in analysis. Patients who presented for infertility care but who conceived without NIFT or ART are defined as subfertile and

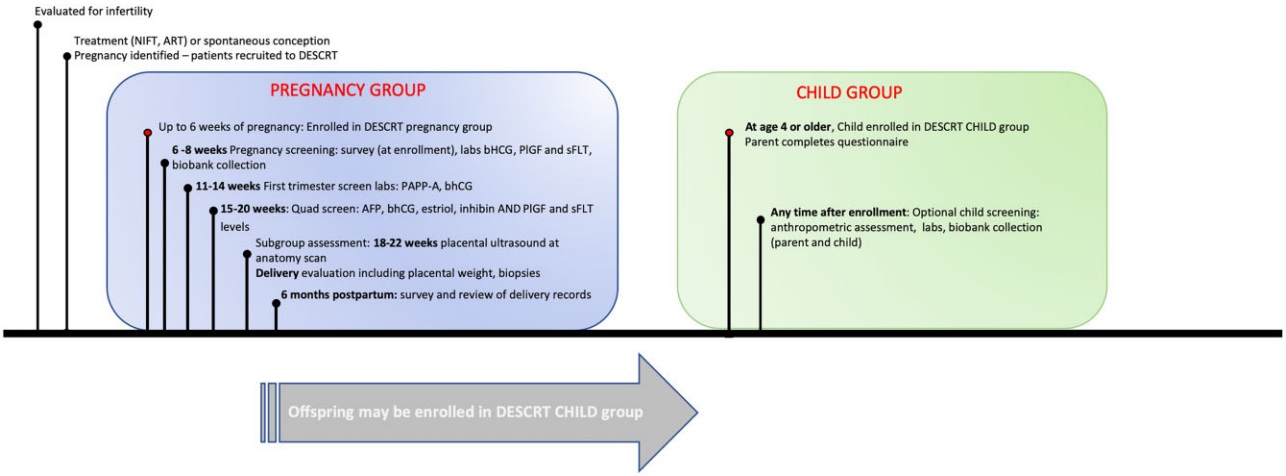

Figure 1. Timeline of enrollment and assessment in DESCRT. Timecourse of participant enrollment and participation in the DESCRT study for the Pregnancy and Child Groups. DESCRT: Developmental Epidemiological Study of Children born through Reproductive Technologies.

their children define the 'spontaneously conceived' SC group. To maximize our ongoing cohort recruitment, we utilize two approaches:

- *Child group*: All children conceived after parental consultation at the UCSF CRH are considered eligible. Children who were either conceived spontaneously or after treatment at UCSF and were born starting in 2001 at 24 weeks of gestation or greater, are eligible to participate in the study. Children aged 4 years and older are invited to complete the cardiometabolic assessment (see *Data collected* below).

Children conceived using third party reproduction (donor egg, sperm, or a gestational carrier) are included if third party information was accessible and all participants consented to the study. When possible, both parents in heterosexual couples and both egg and sperm sources in all cases are included in the study.

- *Pregnancy group*: All patients who conceive after an initial consultation at the UCSF CRH, who are within the first 6 weeks of pregnancy, are invited to enroll in the study at the time of pregnancy confirmation (ultrasound at approximately 6 weeks demonstrating a gestational sac and yolk sac). Enrollment started in November 2017 and is ongoing. The offspring from pregnancies that were enrolled in the Pregnancy cohort are eligible to enroll in the Child cohort upon birth.

For both cohorts, the primary exclusion criterion is that offspring could not have been conceived with assistance from a clinic other than our clinic.

Data collected

We collect detailed information on the following factors:

- Infertility evaluation, duration, diagnosis, and treatment
- Parental medical history at the time of conception and at the time of interview
- Environmental exposures around conception and at the time of interview (for the Child group)
- Sociodemographic and occupational data
- Childhood health (for Child group)
- Biospecimens:
  - Blood and urine at 6 weeks for pregnant people (Pregnancy group)
  - Placenta and cord blood at delivery (Pregnancy group)
  - Blood from children and parents and coparents (Child group, age 4 years and older).

Child group

Table 2 and Figs 1 and 2 include relevant information for the Child group. Childhood history is obtained from pediatric records including growth charts and health history including any hospitalizations. Parents are surveyed about their health during pregnancy and delivery, mode of delivery, neonatal outcomes including birthweight, childhood and parental health (from when the child was born until enrollment), as well as environmental exposures via an extensive questionnaire (Supplementary Data File S2). Data are also extracted from the parental EMRs when available. We encourage parents to bring their children (after age 4 years) for an in-person evaluation to assess their cardiometabolic health. During this assessment, fasting blood and anthropomorphic measurements are collected. We obtain additional blood for biobanking from both enrolled children and their parent(s). Developmental milestones assessed include Tanner staging, and an assessment of medical or psychological conditions.

Anthropomorphic measurements include BMI, measurements of fat composition (skinfold thickness and/or bio-electrical impedance analysis results), blood pressure, and waist circumference. Laboratory assessment includes fasting glucose, insulin, and a lipid profile including total cholesterol (TC), triglycerides, low-density lipoprotein, and high-density lipoprotein (HDL). Additional markers include uric acid, as a marker of cardiovascular risk and metabolic syndrome in children, and alanine transaminase (ALT) as a marker of non-alcoholic fatty liver disease and metabolic syndrome (Ford *et al.*, 2007; Wicklow *et al.*, 2012). Serum samples are additionally collected for metabolomic assessment using mass spectroscopy in a sub-group of prepubertal children aged 7–9 years, to be performed by Metabolon, Inc. (Morrisville, NC, USA) (Evans *et al.*, 2009).

Biobank collection includes whole blood and serum samples, and a urine sample stored at −80°C for future use.

Pregnancy group

Table 2 and Figs 1 and 3 include relevant information for the Pregnancy group. People who conceive after a consultation at the UCSF Center for Reproductive Health, either spontaneously or with treatment assistance at UCSF, are considered eligible for the Pregnancy group. Pregnancies that result in a miscarriage,

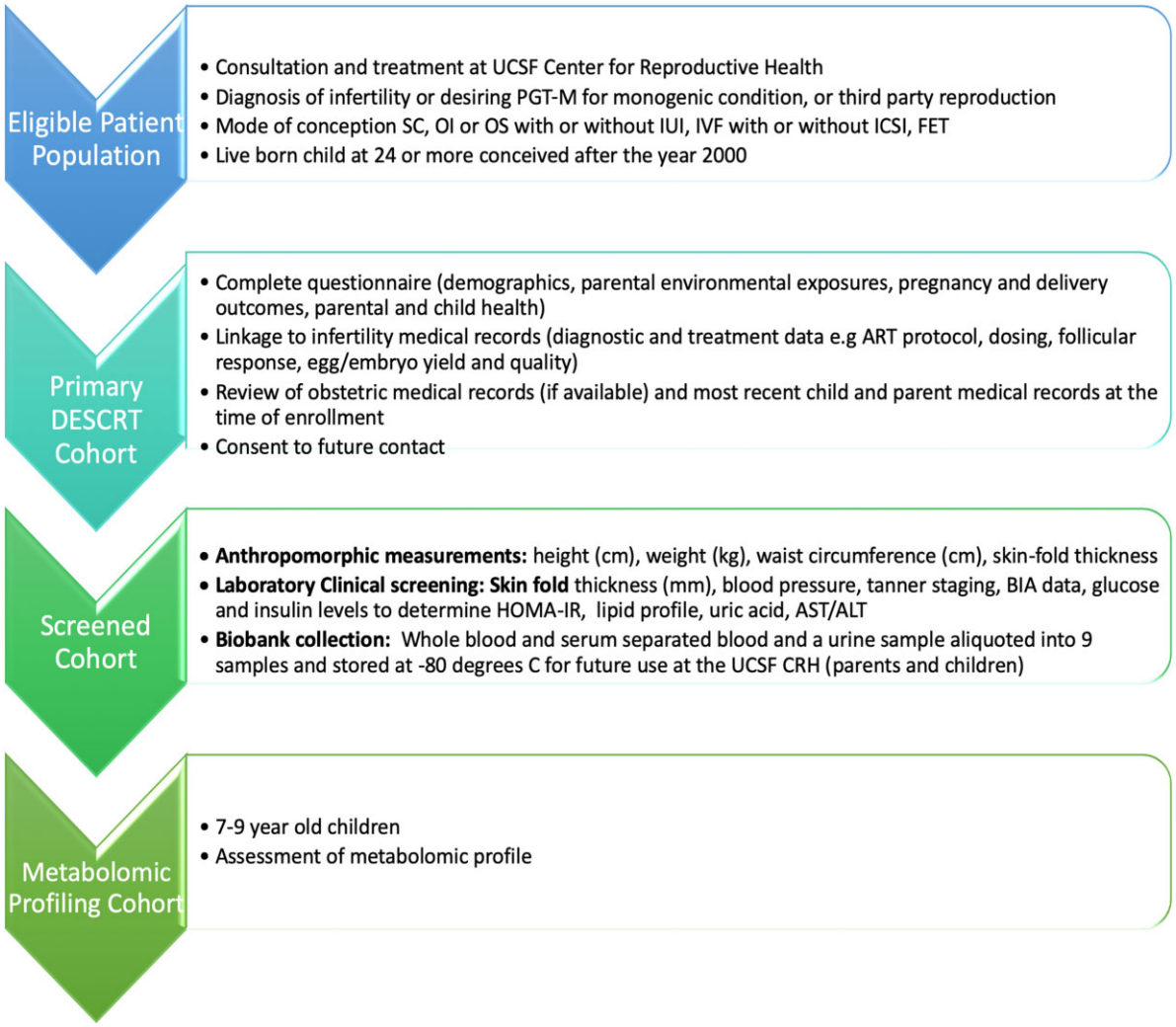

Figure 2. Flow of participant assessment for Child group. Flow of participant assessment for Child group: PGT: preimplantation genetic testing; SC: spontaneous conception; OI: ovulation induction; OS: ovarian stimulation; FET: frozen embryo transfer; BIA: bioelectrical impedance analysis; HOMA-IR: homeostatic model assessment for insulin resistance; AST/ALT: aspartate aminotransferase/alanine aminotransferase.

ectopic pregnancy, or stillbirth (birth occurring after 24 weeks) are maintained in the database.

Participants are enrolled in the study after they demonstrate an adequate rise in bHCG levels and prior to their second pregnancy scan (at ~8 weeks). This does allow for some patients to be enrolled at the time of their first pregnancy ultrasound. The Pregnancy group is followed from enrollment in the first trimester through delivery, at which time they are offered continued enrollment in the developing cohort of children (Child group). For all patients in our care, we collect bhCG levels at multiple time points (2 weeks after ovulation, and repeated 2 days later or approximations of this timing after embryo transfer). We also collect blood for later assessment of soluble fms-like tyrosine kinase (sFLT) and platelet growth factor (PlGF) as well as biobanking at the first and second pregnancy ultrasounds (~6 and 8 weeks). At each timepoint for data collection, our research coordinators extract data from the participant medical record; send REDCap surveys electronically; or connect with the participant in person, by telephone or email when indicated.

Placental assessment

We collect serum analytes by abstracting results of patients integrated screening (first trimester pregnancy associated plasma protein (PAPP-A), and second trimester alpha fetoprotein (AFP), hCG, estriol, inhibin A). Integrated screening is organized through the California Prenatal Screening Program and the vast majority of state residents participate (Flessel and Lorey, 2011). Among a subset of participants who receive care at UCSF, we collect additional sFLT and PlGF samples at 11–14 weeks and again at 16–22 weeks. When participants deliver at UCSF, placental volume is assessed at the anatomy scan and at third trimester ultrasounds (if indicated). At delivery, placental weight is obtained and placental biopsies are collected. In the future, expression of several transporter proteins shown to be dysregulated in mouse models of ART (*SNAT1*, 2, and 4; GLUT1 and 3; Igf2 and Igf2P0; and 11βHSD) will be measured (Bloise *et al.*, 2012).

Perinatal and neonatal outcomes

Participants are surveyed about their health during pregnancy and delivery, mode of delivery, and neonatal outcomes, including birthweight and neonatal health, in an extensive follow-up questionnaire that is sent to participants 6 months after their expected due date (Table 2 and Supplementary Data File S2). Accuracy of data is verified by chart review.

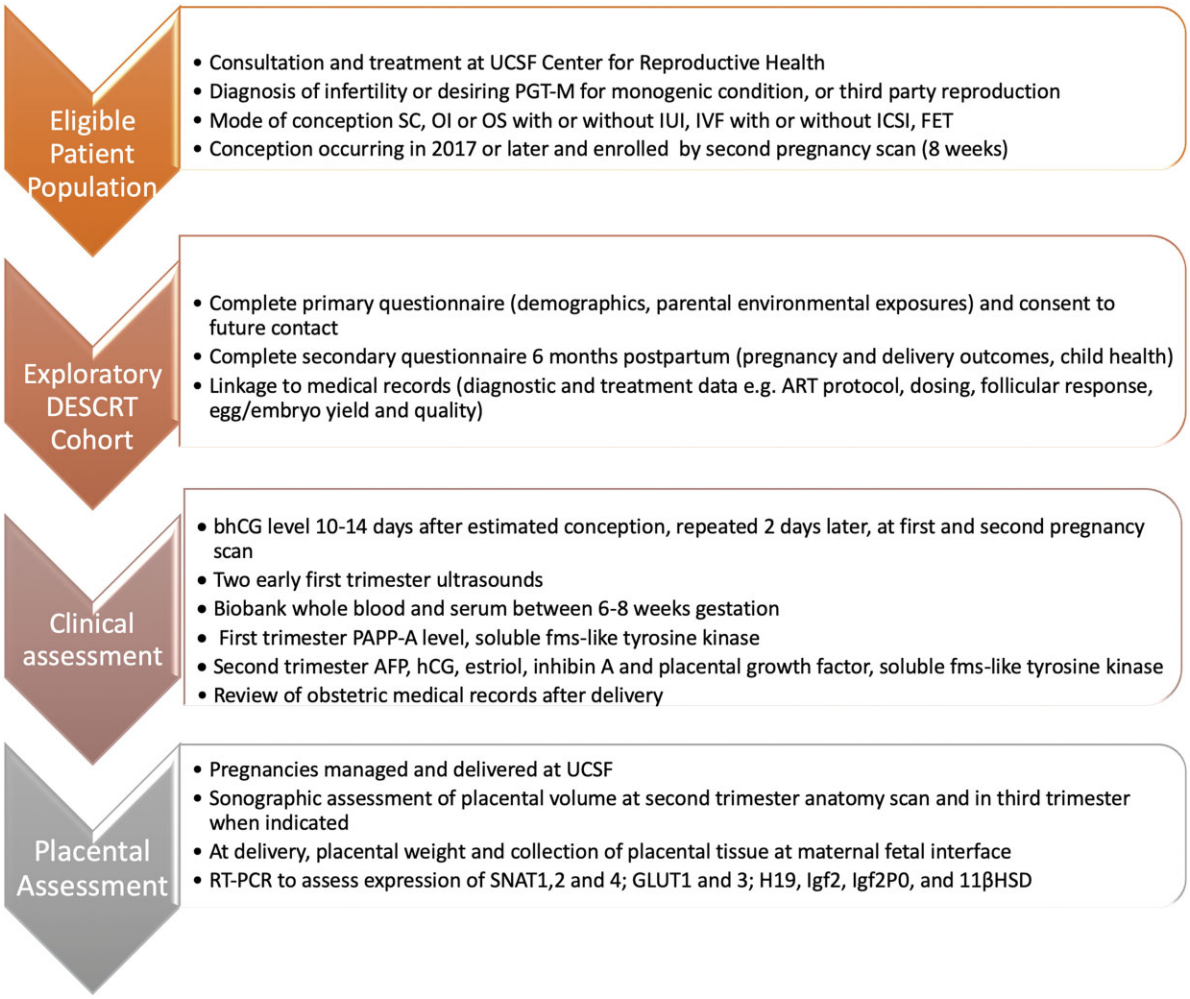

Figure 3. Flow of participant assessment for Pregnancy group. Flow of participant assessment for pregnancy group: PGT: preimplantation genetic testing; SC: spontaneous conception; OI: ovulation induction; OS: ovarian stimulation; FET: frozen embryo transfer; PAPP-A: pregnancy associated plasma protein A; AFP: alpha fetoprotein.

Ethics approval

The DESCRT study has been approved by the University of California, San Francisco Institutional Review Board (IRB 16-20474).

Study requirements

Acquisition of clinical data from EMRs

The following information is abstracted in the study database from EMRs:

- Demographic information including race, ethnicity, age, primary and secondary infertility diagnoses, duration of infertility, gravidity, and parity, markers of ovarian reserve (FSH, estradiol, and/or AMH level)
- Treatment, including treatment type, medication regimen, dosing, follicular response and, when applicable, ART outcomes such as egg yield, embryo yield, and quality at 1, 3, and 5 to 6 days after fertilization
- ART laboratory, including media type, gas mixture to the incubator, technique-related data (e.g. culture media, fertilization technique etc.), egg/embryo yield, embryo quality, transfer on Day 3 versus blastocyst stage, specific incubator type (table top versus big box) etc. A detailed list of ART laboratory covariates is provided in Supplementary Data File S1.
- Clinical laboratory: resultant beta-hCG levels, and pregnancy outcomes, i.e. number of pregnancies (defined as gestational sac and yolk sac and location of pregnancy)
- Pregnancy and delivery outcomes, including peripartum records and last antenatal note, delivery report and discharge summary for reports of pregnancy complications, delivery complications, assessment of whether the offspring was born alive or stillborn, birthweight and sex, whether or not child was hospitalized in neonatal intensive care unit (NICU), and length of NICU stay. Data that cannot be abstracted from the EMR is collected by contacting the parent directly. Our internal EMR is reviewed to assess pregnancies ending in miscarriage or are ectopic that would not be detected in the aforementioned review.
- Childhood outcomes: pediatric records, specifically the most recent annual pediatric exam, are reviewed. When outcomes of interest are identified, supplemental records may be requested to confirm diagnoses

Supplementary Data File S1 provides the full list of abstracted data.

Development and administration of the study questionnaires

Separate questionnaires have been designed for Child and Pregnancy groups to gather data on mediating and confounding

variables that could influence cardiometabolic outcomes of offspring born after ART. These include environmental factors (including diet) or parental behavior. These questionnaires also serve to gather supportive data on pregnancy, neonatal, and childhood outcomes that are not gathered in the medical records review. Each questionnaire gathers detailed demographic data including level of education, income, employment status, occupation, medical history, family medical history of the primary parent, and any involved co-parent. Racial and ethnic groups are defined in accordance with the US Office of Management and Budget standards (Office of Management and Budget, 1997).

The questionnaires contain several questions about the medical and psychiatric health of parents and enrolled children (Child group), developmental milestones, and educational attainment (Supplementary Data File S2). We included multiple subquestionnaires querying diet and lifestyle as well as school performance based upon previously validated questionnaires (Supplementary Data File S3).

These questionnaires have been used in previous studies assessing the efficacy of a multidisciplinary childhood weight loss clinic (Madsen *et al.*, 2009). To ascertain tobacco and alcohol use, we modified questions from the National Adult Tobacco Survey developed by the Centers for Disease Control Office on Smoking and Health along with the Food and Drug Administration and implemented in 2013 (Surveillance Team/Epidemiology Branch/Office on Smoking and Health/National Center for Chronic Disease Prevention and Health Promotion/Centers for Disease Control and Prevention, n.d.). To understand alcohol use in our population, we utilize abbreviated questions from the National Institute on Drug abuse quick screen (PATH Study, 2014).

Several studies have demonstrated a relation between *in utero* environmental exposures and childhood metabolic outcomes (Rich *et al.*, 2015; Russ and Howard, 2016; Wang *et al.*, 2016). In particular, several studies have demonstrated a potential link between certain chemical exposures and obesity (Heindel *et al.*, 2022). To adjust for environmental exposures that might influence neonatal and childhood cardiometabolic outcomes, we queried participants about several common products identified as potentially concerning environmental exposures by the UCSF Pregnancy Exposures to Environmental Chemicals (PEEC) study (Gerona *et al.*, 2016).

For the Child group questionnaire, we also utilize the Family Nutrition and Physical Activity (FNPA) screening tool developed by Ihmels *et al.* to understand the environmental and nutritional behaviors that may independently influence the cardiometabolic outcomes of interest (Ihmels *et al.*, 2009a). The instrument was developed with the Academy of Nutrition and Dietetics (formerly the American Dietetics Association) and validated with 1085 responses from parents of first graders in an urban school district in the USA. It is a 20-item questionnaire with questions related to family nutrition and activity such as 'How often does your family encourage your child to be physically active?' scored from 1 (never/almost never) to 4 (very often/always). Scores could range from 20 to 80. The total score was negatively correlated with child BMI (Ihmels *et al.*, 2009a). Importantly, FNPA scores were not associated with cardiovascular risk scores (HDL cholesterol ratio (TC:HDL), mean arterial pressure, and waist circumference) (Yee *et al.*, 2011). Furthermore, Ihmels *et al.* demonstrated that the FNPA score may be an independent predictor of changes to BMI over the course of 1 year in their urban cohort of first graders (Ihmels *et al.*, 2009b).

Parents of children enrolled in the Child DESCRT group complete their questionnaire at enrollment (Figs 1 and 2). In the Pregnancy group, intended parents complete a preliminary questionnaire to ascertain demographics at enrollment (Figs 1 and 3). They are asked to complete a second questionnaire 6 months after the estimated due date that queries health outcomes from their pregnancy and their child or children (Figs 1 and 3).

Biobank

For both arms of the study, we have developed a biobank wherein we collect whole blood and serum in multiple 1 ml aliquots, along with urine, and when applicable, placental tissue and cord blood for future use. Blood samples are stored in a −80°C freezer on site at the UCSF Center for Reproductive Health and are available for use by other investigators by contacting the primary investigator of this study in co-ordination with the UCSF Biospecimen Services Program. Biobank samples will be associated with the parental treatment data and pregnancy and childhood outcomes, making this a unique resource for answering future questions about the role of infertility and ART on childhood health. UCSF is a well-recognized biorepository center with nationally funded HIV, placental, and endometrial tissue banks. Additionally, the UCSF CRH has maintained an IVF biobank for over 15 years including serum, follicular fluid, and semen. Policies and procedures are in place for specimen processing and for linkage to clinical de-identified data (Office of Research/Department of Pathology, 2021).

Potential independent risk factors

We have collected information on parental medical history, infertility diagnoses, years of infertility, and prognosis as measured by ovarian reserve, maternal and paternal age, semen quality, etc. Underlying infertility may influence the treatments administered and mediate offspring outcomes.

Potential biases

We are cognizant of potential causes for bias and have instituted appropriate checks and controls wherever possible to mitigate these biases.

Selection bias: all eligible participants are invited to participate through multiple modalities (email, telephone, mail). However, it is possible that parents who choose to enroll their children in the study are systematically different from the general eligible population of patients who conceived after encountering our clinic. They may either feel positively about their overall experience with fertility care (thus possibly good prognosis parents) or feel that their children are healthy. Conversely, parents with concerns regarding their child's health may preferentially enroll. Furthermore, it is likely that participants with fertility issues who were able to access care are different from the general population of people experiencing infertility (Goisis *et al.*, 2020). The overall prevalence of cardiometabolic dysfunction among children born to subfertile or infertile parents may thus be higher, or lower, than that demonstrated in the DESCRT cohort. To address this potential bias, we ask those who refuse to participate in the study to complete a short questionnaire to ascertain basic sociodemographic characteristics. We will compare this information to EMR information from our center regarding treatment undertaken, between those who participated in the study and those who declined participation.

Information (measurement) bias: it is possible that a potential recall bias, a type of information bias, may influence some of our results, particularly in the reporting of pregnancy and neonatal complications. However, medical records of children will be reviewed to confirm parental report. Additionally, our primary outcome, namely the cardiometabolic health of offspring from sub-fertile and infertile couples, will be established through anthropometric and laboratory data collected prospectively.

Confounding: it is possible those with a more severe underlying infertility issue may require more aggressive treatment. Thus, outcomes that appear associated with progressive complexity of treatment may still be caused by severity of the underlying infertility. Such confounding by indication occurs when both the disease that forms the indication and its severity are the potential confounders. Both factors should be controlled to prevent the possibility of residual confounding. To address this potential pitfall, we designed the study to include patients who SC after the initial visit to our clinic and could serve as a natural comparison group. We would further assess the effects of this confounding by indication by examining its effects in multivariable models with propensity score matching. The propensity score is the probability that a patient receives a specific treatment based on his or her characteristics and the clinical indications determined by the treating physician. This probability is used to match patients receiving the treatment of interest with those receiving the comparison treatment in order to control confounding by balancing potential confounding factors between these groups (Kyriacou and Lewis, 2016). Although we made a tremendous effort to collect a wide variety of data, it is possible that there could be additional confounding factors for which the data were not collected (residual confounding). We will develop directed acyclic graphs to assess causal pathways and better understand relations between different variables of interest.

Mediating variables: through our efforts to gather a broad selection of data, we hope to not only address confounding factors but also identify potential mediating variables. As an example, we collect data on pregnancy complications, such as pre-eclampsia, which may be associated with usage of ART and also impact offspring outcomes including their personal risk of cardiometabolic disease. Mediation analysis will performed in subsequent studies.

Data management

All treatment data for patients at the UCSF CRH are maintained in an electronic database (IDEAS; Mellowood Inc, Toronto, ON, Canada). Treatment data are linked to the DESCRT database via REDCap. All questionnaires are administered electronically through REDCap. Prospective data collection from clinical screening or laboratory results are entered into the REDCap database manually.

Data quality assurance

To verify the accuracy of manual data entry, we will select a sample of data and conduct double-entry of all fields (coded and free-text). All errors will be resolved through manual inspection and the data entry procedures revised to avoid them in the future.

Data validation

To verify validity of collected data in the DESCRT database, we will conduct multiple checks, including verification of validity of ranges for continuous data, missing information, logical checks for dates, etc.

Handling of missing data

It is now widely recognized that the routinely used complete-case analysis—which excludes subjects with any missing variable—can reduce the accuracy of coefficients in regression analyses, and also could be severely biased when the data are not a completely random sample of the full data (thus not missing completely at random) (Tchetgen, 2009). We propose to use weighted estimating equations, which can provide a powerful framework to perform regression analysis, while appropriately accounting for covariate data missing at random but not necessarily missing completely at random (Tchetgen, 2009). For variables with a small amount of missing data (<3%), we will use the multiple imputation method, which recently has been shown to produce interval estimates covering near the nominal 95% of the true risk interval (Karahalios *et al.*, 2012). For variables with larger amounts of missing data, we will use recently developed methods of indirect adjustment and the parametric g-formula (Edwards *et al.*, 2014; Richardson *et al.*, 2014).

For prospectively collected data, multiple staff are involved at each step adding a layer of security to ensure we have the most complete data possible. For pediatric anthropomorphic and specimen collection, at times children may express anxiety around certain tests. However, our screening exam is performed at the UCSF pediatric clinical research center (PCRC), which has been specifically designed with children in mind and is managed by providers with a background in administering care to the pediatric population. Parents are informed about the PCRC and their experience if they express hesitations as well.

Discussion

DESCRT is the largest single-site, US study following a cohort of children conceived with different fertility treatments, which includes detailed data on interventions, and early pregnancy and long-term health outcomes of offspring using standardized screening methods. Although other epidemiological cohorts exist, few have focused on the specific type of infertility interventions, analyzed parental factors, and contained prospectively collected data compared to an adequate subfertile control group, to robustly assess how these variables might influence cardiometabolic health (Table 1) (Ponjaert-Kristoffersen *et al.*, 2005; Middelburg *et al.*, 2009; Scherrer *et al.*, 2012; Pinborg *et al.*, 2013; Luke *et al.*, 2017; Hargreave *et al.*, 2019; Huang *et al.*, 2021; Mitter *et al.*, 2021; Norrman *et al.*, 2021; Penova-Veselinovic *et al.*, 2021; Richmond *et al.*, 2022). Importantly, unlike most other established cohorts, our control group will be represented by children conceived spontaneously by subfertile parents who had a diagnosis of infertility after having undergone a formal consultation at our center. The Growing Up in Singapore Towards healthy Outcomes (GUSTO) cohort is similar to the DESCRT cohort in that they aim to include children conceived from subfertile parents and assess cardiometabolic outcomes (Huang *et al.*, 2021). However, the depth of detail regarding of diagnostic, prognostic, and treatment data are limited in comparison to DESCRT. Our control group will allow us to more precisely assess the role of interventions while controlling for the diagnosis of infertility. The depth and breadth of data collected through DESCRT, such as infertility diagnoses,

intervention details including specific medications and dosage, embryo culture, obstetric, and neonatal outcomes, will aid in our understanding of the effects of important confounders and mediators on the association between treatments for infertility and long-term health outcomes for parents and offspring.

Cardiometabolic outcomes in offspring may be influenced by preconception, conception, and *in utero*, post-natal, or childhood environmental factors, as stated by the developmental origin of health and disease hypothesis (Kumaran *et al.*, 2017). Furthermore, parental health, particularly a personal history of hypertension, diabetes, metabolic syndrome, or advanced age, may influence the risk of related cardiometabolic conditions in offspring (Gaillard *et al.*, 2014; Eberle *et al.*, 2020). Increased maternal age is associated with an increased risk of infertility, abnormal lipid profiles, and cardiovascular disease that may influence offspring health (Chu *et al.*, 2003). Further assessments of a potential relation between infertility, its treatments, and future parental cardiovascular health could have significant impacts on how people with infertility are monitored in the future.

Both animal studies and preliminary observational data in humans raise the spectre that ART may directly impact cardiometabolic health in offspring. Mice embryos cultured *in vitro* may have atypical placental growth in pregnancy and less efficient transport of nutrients (Bloise *et al.*, 2012). Furthermore, they also demonstrated differences in trophectoderm cell proliferation when compared to SC mice (Giritharan *et al.*, 2010). After delivery and into adulthood, some ART-conceived mice demonstrate that prolonged culture exposure may be associated with an increased risk of cardiovascular and metabolic disease in adulthood (Rexhaj *et al.*, 2013; Chen *et al.*, 2014; Donjacour *et al.*, 2014; Feuer *et al.*, 2014). In humans, IVF pregnancies are known to have an increased risk of pre-eclampsia, which may be predicted by levels of soluble FMS-like tyrosine kinase (sFLT-1), a split product of vascular endothelial growth factor that inhibits angiogenesis (Andrietti *et al.*, 2016). Placental function is clearly associated with birthweight, and SGA is associated with increased risk of type 2 diabetes and cardiovascular disease later in life (Mericq *et al.*, 2017). There are ample reasons to be concerned that ART techniques may influence the long-term cardiometabolic health of the offspring, possibly mediated through placental changes, developmental programming, or epigenetic marks that could be impacted by culture technique (Santos *et al.*, 2010).

Should the severity of metabolic dysregulation correlate with the degree of reproductive interventions (SC versus NIFT or ART), this may suggest an independent influence of infertility treatment. However, should no differences be discerned between SC children and the remainder of the cohort, yet all children show some increased prevalence of metabolic dysregulation, it may suggest that infertility itself may influence the risk for adverse cardiometabolic outcomes. An association of offspring metabolic dysregulation with markers of ovarian aging, regardless of treatment modality, would also confirm our hypothesis.

Importantly, it is possible that infertility or its treatments may also impact future parental health. Current data on the influence of infertility on future maternal health are conflicting. In a secondary analysis of data from the Study of Women's Health Across the Nation, a history of infertility was not associated with the development of cardiometabolic outcomes 7 years later (Cairncross *et al.*, 2021). In contrast, in a prospective assessment of women with infertility in the Women's Health Initiative, a history of infertility was associated with an increased risk of heart failure (Lau *et al.*, 2022). By collecting data on parental history of cardiometabolic disease, DESCRT may be able to discern if infertility itself influences parental health.

The main goal of DESCRT is to assemble a cohort of children conceived after infertility treatment and follow them longitudinally for long-term health outcomes. We plan to continue collecting additional health outcomes data in future studies. Additionally, the DESCRT biobank, which contains the blood, urine, and tissue samples from hundreds of parents and children linked to extensive demographic, intervention, and outcomes data, will be invaluable to future exploration of long-term outcomes of infertility or its treatments on offspring. This biobank will be available to qualified research teams globally. Likewise, there will be opportunities to study the long-term health outcomes of the infertile parents, both in terms of impact of an infertility diagnosis on long-term health and the potential impact of the interventions themselves.

DESCRT has several strengths. By providing cardiometabolic and developmental data on a large cohort of children born to parents with subfertility or infertility, DESCRT may be able to discern the influence of infertility, its treatments and the environment on the health of offspring.

Study strengths of DESCRT include: a cohort of children born since 2001 with long follow-up (up to 20 years); a large sample size that would allow analyses within subgroups of treatment; similar characteristics of the patient population coming to one hospital; uniform data collection; and dyads and triads of parents and children to evaluate the joint effects of infertility and intervention. Offspring born from pregnancies enrolled in the Pregnancy group are eligible to enroll in the Child group upon birth, and we encourage this. DESCRT is positioned to have a future cohort of children with detailed, prospectively collected pregnancy data as well as follow-up assessment of their cardiometabolic health.

This study also has limitations, which include: skewed recruitment; laboratory complexity; certain design elements; and dealing with coronavirus disease (COVID)-related obstacles. Thus far, we have found limited acceptance in the clinical screening program for the Child group. Some parents have expressed concerns related to child discomfort with phlebotomy, whereas other parents have found it difficult to co-ordinate screening with child and parental schedules. Furthermore, for the years 2020–2022, the COVID-19 pandemic complicated enrollment and completion of in-person visits for the Child group. Additionally, tracing families has become complicated, insomuch as we are recruiting children that may have been conceived over two decades ago. Importantly, it is possible that socioeconomic factors could also influence offspring health. In our patient population, a slight majority of patients (52–69%) had insurance to cover fertility evaluation or treatment from 2017 to 2022. In California, fertility evaluations are often covered by insurance but such policies are not required to cover treatment and many patients self-pay for treatment. We collect socioeconomic data from parents in our study questionnaires and intend to adjust for this during our analyses. However, although our population is likely comparable to other patients and offspring in the San Fransisco Bay Area, this may not be true compared to other populations of infertile people in the USA or globally.

In terms of the prospective collection of placental tissue in a subset of patients, we have found that having a research team member available at the time of delivery can be challenging. This process was further complicated by the COVID-19 pandemic, which limited access to the Labor & Delivery suite of non-essential personnel (such as the research team). Because tissue

collection is time sensitive, only a proportion of eligible patients have been able to contribute to our placental tissue studies.

Although our study is well-positioned to address the impact of infertility treatments on offspring in an infertile or subfertile population, our ability to assess the independent impact of infertility on offspring is somewhat limited as the vast majority of children in our cohort of children are conceived by parent(s) that have fertility issues. A subset of children were conceived with IVF plus PGT-M, which does provide us with an opportunity to compare the children of presumably fertile parents to the rest of the cohort. Furthermore, many of our outcomes are well defined in the general child population (e.g. BMI) and will allow us to make reasonable assessments of the influence of infertility on offspring metabolic health.

Ultimately, DESCRT is uniquely positioned to address the impact of infertility and its treatments on the long-term cardiometabolic health of offspring. We anticipate that the DESCRT cohort and biobank will prove to be a valuable resource for future investigations at the intersection of ART and offspring health and well-being.

Supplementary data

Supplementary data are available at *Human Reproduction Open* online.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Authors' roles

A.J.A. was involved in database development, recruitment, preliminary data-analysis, and the development of manuscripts. M.I.C. was the primary investigator for the study, was involved in study design, ethics approval, project management, and manuscript development and editing. L.Z. was a co-primary investigator for the study, involved in study design, instrument development, recruitment, data collection, and analysis and manuscript development and revision. P.R. was a study co-investigator involved in the primary analysis and manuscript revision. R.H.L. was involved in initial study design and revision, review of clinical lab data on the offspring, as well as manuscript preparation and review.

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Conflict of interest

A.J.A. is shareholder of Carrot and consultant for Flo Health. The other authors have no conflicts of interest.

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