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Fetal and postnatal growth following natural conception and IVF treatment

MÅRTEN AGEHEIM

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Abstract
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Abnormal fetal growth is closely related to adverse short-term outcomes as well as negative long-term health consequences. Fetal growth restriction is a common pregnancy complication, and for the detection of fetuses small for gestational age, a reliable reference is vital. Increasing evidence supports that mode of conception has implications for growth. Fresh embryo transfer (ET) is associated with small for gestational age, and frozen ET with large for gestational age. The overall aim of this thesis was to describe optimal fetal growth, as well as pre-and postnatal growth after ART, trying to isolate the direct effects of ART from related factors.

Study I was a prospective multicentre study aiming to construct updated population-based references for fetal growth for the Swedish population from a cohort of 583 low-risk pregnancies. Comparisons were also made with other relevant growth charts.

In Study II, a population-based retrospective cohort study including 10 970 fresh ET, 6520 frozen ET, and 178 518 natural conception (NC) pregnancies, fetal growth after ART was explored with longitudinal statistics. Study III, a prospective longitudinal multicentre study of 82 fresh ET, 175 frozen ET and the 583 NC from Study I as reference, had similar aims. Fetuses after ART in general tended to be larger than NC in early pregnancy and thereafter growing at a slower rate than NC. This was more pronounced after fresh ET and at term mean fetal weight was lower than NC. Frozen ET remained heavier than NC in Study II but in Study III there were no significant differences.

Study IV was a population-based retrospective cohort study of 517 fresh ET, 284 frozen ET and 17 214 NC, examining growth from birth to five years of age. At birth, children after fresh ET were smaller, and those after frozen ET were larger than NC. Most differences were attenuated by 18 months of age.

The new references will in future studies be evaluated for the intended population. Our results support that there is a direct effect of ART on fetal growth. Although differences diminish with age, the differences in growth are known risk factors for future cardiometabolic disease.

Keywords: Fetal Growth, Childhood Growth, Assisted Reproduction, Infertility

Mårten Ageheim, Department of Women's and Children's Health, Obstetrics and Reproductive Health Research, Akademiska sjukhuset, Uppsala University, SE-751 85 Uppsala, Sweden.

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List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Lindström L, Ageheim M, Axelsson O, Hussain-Alkhateeb L, Skalkidou A, Wikström AK, Bergman E. Swedish intrauterine growth reference ranges for estimated fetal weight. *Sci Rep,* 2021 Jun 14;11(1):12464

II. Ageheim M, Skalkidou A, Bergman E, Iliadis S, Lampa E, Lindström L, Oberg AS. Fetal growth after fresh and frozen embryo transfer and natural conception: A population-based register study. *BJOG*, 2024 Aug;131(9):1229-1237

III. Ageheim M, Lindström L, Iliadis S, Lampa E, Baumgart J, Elenis E, Skalkidou A, Bergman E. Fetal growth after fresh and frozen embryo transfer and natural conception: a prospective cohort study. (manuscript)

IV. Ageheim M, Skalkidou A, Lampa E, Iliadis S, Bergman E, Ahlsson F, Lindström L. Postnatal growth after fresh and frozen embryo transfer: a population-based register study. (submitted)

Other relevant publications

Lindström L, Ageheim M, Axelsson O, Hussain-Alkhateeb L, Skalkidou A, Bergman E. Swedish intrauterine growth reference ranges of biometric measurements of fetal head, abdomen and femur. *Sci Rep*. 2020 Dec 31;10(1):22441.

Contents

Abbreviations

AC	Abdominal Circumference
AGA	Appropriate for Gestational Age
aOR	Adjusted Odds Ratio
ART	Assisted Reproductive Technology
BPD	Biparietal Diameter
CI	Confidence Interval
COH	Controlled Ovarian Hyperstimulation
CRL	Crown Rump Length
DAG	Directed Acyclic Graph
EDD	Estimated Date of Delivery
FGR	Fetal Growth Restriction
FL	Femur Length
HC	Head Circumference
ICSI	Intracytoplasmic Sperm Injection
IG	INTERGROWTH-21st Project
IVF	In Vitro Fertilisation
LGA	Large for Gestational Age
LISA	Longitudinal Integrated Database for Health Insurance and Labour Market Studies
LMP	Last Menstrual Period
MAD	Mean Abdominal Diameter
MBR	Medical Birth Register
NC	Natural Conception
NICHD	National Institute of Child Health and Human Development
NPR	National Patient Register
PDR	Prescribed Drug Register
SGA	Small for Gestational Age
SPR	Swedish Pregnancy Register
Q-IVF	National Quality Register for Assisted Reproduction
SD	Standard Deviation
UCMC	Uppsala County Mother and Child Cohort
WHO	World Health Organisation

Introduction

The main topic of this thesis is growth, both prenatal and early postnatal. These different stages of development have many similarities but also distinct differences regarding growth rate and regulation. Size is determined by age and growth rate, and abnormal growth has been shown to carry implications for future health. Being small for gestational age (SGA) is associated with increased risks for adverse perinatal outcomes, and fetal growth restriction is a major cause of perinatal morbidity and mortality.[1,2] Exaggerated growth also comes with increased risks.[3] Postnatal growth velocity can in many cases compensate for differences in birthweight by either a higher or lower growth rate, decreasing some risks, while increasing others. The following sections focus on factors affecting growth, the association between growth patterns and assisted reproduction, the significance of normal or abnormal growth, and the still debated topic of how to detect and manage suspected abnormal growth.

Background

Fetal growth

The foundation of fetal growth is the inherent growth potential, i.e., how a fetus would grow under optimal circumstances. This genetic growth potential is mainly inherited from the parents, but could also be influenced by chromosomal aberrations, or other genetic conditions.[4, 5] External factors then modulate the actual growth, including environmental factors, such as smoking, toxins, and infections. Also, hormonal factors, maternal health, and nutrition play important roles.[6, 7] Maternal size is an important restricting factor, having a larger impact than the father's, on intrauterine growth.[7] Epigenetics, the phenomenon of regulating gene expressions without altering the primary DNA sequence, also influences growth. Different mechanisms, foremost DNA-methylation and demethylation, regulate whether the maternal or paternal genes should be expressed. In many cases, maternal gene expression seems to restrict fetal growth, while paternal genes appear to promote growth.[5]

The placenta plays a crucial role by delivering oxygen and nutrients to the fetus as well as eliminating waste products. It also plays a role as an endocrine organ, producing hormones affecting both the mother and the fetus. There is a complex interaction between the mother, the fetus, and the placenta.[7] In early pregnancy, trophoblast cells invade the decidua causing a gradual remodelling of the spiral arteries to low-resistance vessels, enabling the requirements of the fetus to be met. Abnormal placentation is closely related to early fetal growth restriction and hypertensive disorders.[8] Multiple risk factors have been identified, for instance, chronic hypertension, rheumatic disease, and advanced maternal age.[9]

SGA and FGR

The term SGA was originally used for birthweights and, in contrast to the term "low birthweight", relates weight to gestational age, often defined as below the 10th centile, but definitions vary. In Sweden, SGA is defined as estimated fetal weight (EFW) or birthweight below -2 standard deviations (SD) of the expected mean for gestational age.[10, 11] There is a strong association between SGA and adverse outcomes, and SGA is often used as a proxy for fetal growth restriction (FGR), a condition where the fetus, often due to placental factors, does not reach its growth potential. These terms are similar, but important to

distinguish, as an SGA-fetus is not necessarily growth-restricted. Further, being appropriate for gestational age (AGA) does not exclude FGR.[2] In addition to the varying definitions for SGA and FGR, the acquisition of adequate fetal measurements, the formula by which estimated fetal weight is calculated, and the used reference are of great importance. Cut-off values also have a large impact on the detection rates of growth-restricted fetuses, especially since the purpose is to detect fetuses at risk for adverse outcomes, rather than only focusing on size.

Often a distinction is made between early and late growth restriction, two partly different entities but with overlapping features, and with varying arbitrary cut-offs.

Early-onset growth restriction is closely related to abnormal placentation. Common findings are high resistance flow in the uterine arteries, a gradual increase of umbilical artery resistance, and eventually often late Doppler changes and fetal compromise with changes in cardiotocography (CTG). In many cases the mother has gestational hypertension or preeclampsia and iatrogenic preterm birth is common due to either maternal or fetal indications.[12] Consensus definitions for early and late growth restriction were achieved in 2016, where the delineation was set at 32 weeks of gestation. The diagnostic criteria are different for the two types, as early-onset FGR is more closely related to placental insufficiency.[13] In early growth restriction the diagnosis is often apparent, with severe SGA and abnormal Doppler indices. Management on the other hand is challenging, where the risk for adverse outcomes for both mother and fetus with continued pregnancy must be balanced against the risks associated with being born extremely preterm. Gestational age is an important prognostic factor, and by closely following maternal and fetal well-being, the aim is to determine the optimal timing of delivery.[12]

Late-onset growth restriction (≥32 weeks) is often more subtle, with less apparent associations with placental compromise and maternal hypertensive disorders. A main challenge is to distinguish between a constitutionally small SGA fetus and a growth-restricted fetus that may or may not be SGA. Doppler indices in the uterine and umbilical arteries are typically normal. With advancing gestational age the risks associated with delivery are lower, however still higher for late preterm deliveries than term.[14] Late-onset FGR also constitutes a much larger proportion than early-onset FGR. There are still questions that remain to be answered regarding which parameters to rely on for deciding the optimal timing of delivery.

LGA and macrosomia

Analogous to SGA, the definitions of large for gestational age (LGA) vary, often defined as weight >90th centile, but in Sweden as weight >2 SD above the expected mean for gestational age (97.7th centile). While LGA is dependent on gestational age, the term macrosomia is used for weights over a fixed

cut-off, irrespective of gestational age, often 4000 g or 4500 g are used. LGA and macrosomia are associated with increased risks for both maternal morbidities, such as postpartum haemorrhage and third- or fourth-degree perineal lacerations, as well as neonatal adverse outcomes, including asphyxia and birth trauma.[3] Long-term consequences include increased risks for obesity and metabolic disease but may depend on the underlying aetiology.[15]

Evaluating fetal weight and growth

Fetal size in relation to gestational age can be used to identify fetuses at risk for adverse outcomes. One single weight estimation indicates if the fetus is small, appropriate, or large for gestational age. To evaluate fetal growth over time, serial measurements are needed. Thereby, abnormal growth trajectories can be identified, for example by showing crossing centiles. These assessments can be done in more or less complex manoeuvres, conditioning on the previous value. Hence, a fetus can be suspected to be growth restricted despite being AGA, and accordingly being SGA but without suspicion of FGR.

There are however many methodological factors that need to be considered while evaluating fetal growth. In contrast to postnatal growth, the fetal weight cannot be readily assessed. Different anthropometrics have been used to construct equations for calculating estimated fetal weight. Besides the uncertainties associated with achieving reliable estimates for fetal weight, these must be evaluated in relation to gestational age, using appropriate cut-off centiles. Several studies have demonstrated that the risk for stillbirth is lower when growth restriction is detected antenatally.[1, 16] In general, detection rates for SGA are low, often combined with a rather high false positive rate, which can lead to unnecessary interventions and iatrogenic preterm delivery.[17]

Gestational age assessment

The actual duration of a normal pregnancy is not known. The estimated date of delivery (EDD) can be calculated using Naegele's rule, by adding 280 days to the first day of the last menstrual period (LMP). Assuming a close relationship between size and gestational age, methods for pregnancy dating with ultrasound have been developed. In women with regular menstrual cycles, the performances of the two approaches are comparable, but in cases with large discrepancies between the two methods' estimates, ultrasound dating is more accurate.[18] Pregnancy dating using ultrasound, by crown rump length (CRL) in the first trimester, or biparietal diameter (BPD) or head circumference (HC) in the first or second trimester, is now used for the absolute majority of pregnancies in Sweden.[19] EDD according to first-trimester dating has been shown to have a narrower distribution around the true date of delivery than dating in the second trimester, probably due to less biologic variation in size in early

pregnancy.[20] The equations for gestational age calculation from ultrasound biometric measurements are derived from data on pregnancies in women with regular menstrual periods, why it is somewhat surprising that ultrasound performs better than LMP. A suggested theory is that fetuses that are small already at dating, and hence are attributed a shorter gestational age and later EDD, also grow more slowly and have longer pregnancy durations.[21] This is also relevant considering slight differences in fetal size between male and female fetuses, where ultrasound dating seemingly tends to underestimate gestational age in female fetuses while overestimating male fetuses.[22] Since the exact date of implantation is unknown, the normal duration of pregnancy cannot be determined. When deciding on which references should be used in clinical practice in Sweden, different formulae were evaluated in pregnancies after in vitro fertilisation (IVF), since true gestational age was presumably known based on embryo culture duration and embryo transfer (ET) data.[20] As will be covered in later sections, IVF is associated with both differences in size in early pregnancy and preterm birth, why this approach cannot be considered unproblematic. A reliable estimate of gestational age is of utmost importance for the detection and management of suspected abnormal fetal growth. A recent study showed that if second-trimester dating is applied instead of the preferred first-trimester dating, the detection rate for SGA and FGR would decrease drastically.[23]

Assessment of fetal size and weight

Over the years, numerous formulae for calculating fetal weight have been presented, most commonly derived from measurements of the fetal head, abdomen and femur taken in close proximity to delivery. The formula by Persson and Weldner from 1986,[24] including BPD, mean abdominal diameter (MAD) and femur length (FL), is recommended in the Swedish guidelines.[25] The equations presented by Hadlock et al. from 1985 are commonly used internationally, and include HC, abdominal circumference (AC) and FL, with or without the addition of BPD.[26] These older formulae have, despite often being derived from quite small cohorts, proven to perform well.[27, 28] The performance of the formula from Persson and Weldner has not been evaluated against any of the most used formulae from Hadlock, which include HC. However, according to a study comparing Persson and Weldner with the Hadlock formula including BPD, AC and FL, there are no apparent reasons to abandon it in the Swedish setting.[28] The use of BPD instead of HC can be considered a disadvantage, since in fetuses with dolichocephaly, the smaller BPD results in underestimation of EFW. Equations for estimating fetal weight should ideally be independent of gestational age, a variable not included in the formulae since the sole purpose is to calculate fetal weight. Performances, however, vary with different weight ranges, and gestational ages.[28]

References for size and growth

To evaluate EFW in relation to gestational age, a reliable reference chart is needed. Since preterm birthweights do not represent normal pregnancies, there is a broad consensus that references instead should be based on ultrasound measurements of ongoing pregnancies.[29] The methods used when constructing references for fetal size and growth have evolved and several publications have discussed key concepts for high-quality references.[30, 31] To estimate weight deviations reliably, the reference must also fit the population for which it is used. This is relevant not only for the median but also in the upper and lower ends of the distribution, where uncertainty is the largest. For instance, ideally, ten percent of healthy fetuses should be positioned below the 10th centile. Given a constant cut-off centile for SGA, a reference with too high centiles would classify a larger proportion of fetuses as SGA, thereby risking unnecessary interventions. Correspondingly, too low centiles would misclassify SGA fetuses as AGA, and the increased risks for adverse outcomes would be overlooked.

A prolonged debate concerns whether there are differences in growth and size between different populations due to physiological differences, or if healthy fetuses to healthy mothers grow similarly across the world. There are many examples of differences in distributions of fetal weights between populations and different ethnic groups in the same community,[32] as well as differences in birthweights.[33] If these differences represent physiological differences, or if they reflect pathology is a question still not fully resolved. Indeed, perinatal mortality rates are substantially higher in developing countries where birthweights are lower.[34] Also, within a society there are probably differences in socioeconomy, diet, etcetera between groups of different ethnic backgrounds, making it difficult to draw conclusions regarding the underlying aetiologies.

International references for fetal size and growth

In 2014, the Fetal Growth Longitudinal Study of the INTERGROWTH-21st (IG) was published.[35] An international multicentre study was carried out in eight countries with the aim to produce international growth standards for fetal size and growth. The assumption was that fetal growth in healthy pregnancies is similar in different populations and that a common standard would decrease the risk of underdiagnosing growth-restricted fetuses compared with locally derived growth charts including high-risk pregnancies. The project was prescriptive, i.e., designed to reflect growth under optimal circumstances, thus not being restricted to a specific location. Therefore, strict inclusion criteria were applied ensuring a population with low risk of adverse outcomes, excluding 65% of women screened from entering the study, and exclusions were also made for serious complications arising during pregnancy. Data from all

sites were pooled and universal standards were calculated for individual fetal biometric measurements. Standards were later created for EFW, with a new formula based on HC and AC from ultrasounds within 14 days of delivery.[36]

Another international growth project, the WHO Fetal Growth Charts, was published in 2017.[37] Carried out in ten different countries, also aiming at creating growth references for international use, the approach was somewhat different. The study was descriptive rather than prescriptive. Only healthy pregnant women were included but exclusions were not made for maternal or fetal complications, thereby to a higher degree reflecting the population. References for individual anthropometrics were created, as well as a reference for EFW, using Hadlock's third formula, also evaluating the influence of different maternal factors and fetal sex. The results showed significant differences in birthweights and centiles between the countries which could not be explained by adjusting for maternal characteristics. Also, the 10th centile for AC in IG was lower than in the WHO study, despite the fact that that study had a strict prescriptive purpose. When, in the WHO study, maternal and fetal complications were excluded, the results were unaltered, suggesting a limited effect of these strict criteria. The authors recommended that the performance of the references should be evaluated in specific populations to determine if adjustments would be needed.

Other relevant references for fetal growth

To ensure a good fit, with adequate distributions over the centiles, population-based references are widely used. High-quality references are, however, lacking in many parts of the world, hence the initiatives to create international universal references. Swedish biometry guidelines[25] recommend the growth reference by Maršál et al, from 1996.[10] The study was conducted on only 86 women, of whom 24% were smokers, and used the formula by Persson and Weldner to calculate EFW from BPD, MAD, and FL. Apart from the fact that the reference does not represent a low-risk group, there were no measurements earlier than 25 weeks, making estimations in the early viable span unreliable. Further, the underestimation of EFW in fetuses with abnormal head shape mentioned earlier is a limitation. Lastly, the SDs for EFW were calculated in a cross-sectional fashion despite repeated measurements. They are also not readily available and were generalized to 12% throughout pregnancy, leading to a clinical practice in Sweden to express fetal weight deviation in percent rather than in z-scores or centiles. There was thereby, for many reasons, a need to reconsider the choice of reference.

In 2006, updated fetal growth references were published in Norway.[38, 39] The setup was descriptive and while including only healthy women, no exclusions were made due to complications. While the Norwegian population is expected to be similar to the Swedish, birthweights are higher in Norway. The techniques to measure BPD are also different between the countries, making

it difficult to adopt these references into clinical use in Sweden. In Sweden, callipers are placed on the proximal faces of both parietal bones, while in Norway, the outer margins are used. However, the formula by Combs for EFW, used in Norway, includes HC, AC, and FL, but not BPD.[40] In comparison with the Maršál reference, there were subtle differences for girls, but boys were smaller in the Norwegian reference.[38] In addition to describing the population, the influence of different maternal and fetal factors was examined, enabling further customisation of the expected weight curve for the individual fetus. The value of such individualisation is another debated subject, again reflecting different views on the underlying causes of differences in growth. If customisation is done by individual factors, the detection rate for growth restriction could theoretically be higher and the false positive rate lower.

Ethnic-specific growth references for an American multi-ethnic population were created in the National Institute of Child Health and Human Development (NICHD) Fetal Growth studies.[41] Despite strict inclusion and exclusion criteria, with exclusions of neonatal complications, ethnic-specific differences in EFW were shown from 16 weeks of gestation. A substantial proportion of non-white fetuses would have been classified as $<5^{th}$ centile if a standard derived from only the white group had been applied to the other groups. Another study evaluated distributions of EFW and AC between NICHD, IG and WHO, concluding that all of the references showed different distributions across centiles between subgroups, arguing for ethnic-specific references.[42] A study including over 100 000 term births showed that an ethnic-specific birthweight reference was superior to a population-based reference in identifying adverse neonatal outcomes.[43]

For many years, Gardosi has advocated customised growth references, where Term Optimal Weight is calculated in early pregnancy by computerised adjustments for maternal, non-pathological conditions. In comparison with universal standards or population-based references, adjusted SGA boundaries have shown higher agreement with the risks for stillbirth.[44] In women with high Body Mass Index (BMI), seemingly having lower risks for SGA, a customised cut-off placed on a higher centile was more accurate in identifying fetuses at risk. Arguments against customised size charts focus on the lack of apparent advantages and challenge the notion of normal smallness in populations where perinatal risks are significantly higher than in others. Also, the majority of stillbirths at term occur in AGA fetuses, suffering demise due to hypoxia before showing decreased growth,[34] or having a slow growth rate without reaching the cut-off for SGA.[45] The detection of fetuses at risk for adverse outcomes, rather than size, is highlighted. A study on pregnancies in Spanish and Indo-Pakistani couples showed that birthweight was associated with maternal characteristics, and Cerebro-Placental Ratio (CPR), rather than ethnicity. The conclusion was that placental dysfunction, indicating FGR, instead of physiology, explained the differences in birthweight between the groups.[46]

Universal versus population-based references

Several studies have since the publication of international references evaluated these in specific populations. While constructing a population-based reference for a Southern Chinese population, the performances of both the IG standards and the WHO references were evaluated. Compared with the population-based reference, the 10th, 50th and 90th centiles were all higher for WHO, resulting in 16% of fetuses below the 10th centile in week 34, and only 0.8% above the 90th centile. Contrasting, although this cohort was recruited from the general population, the IG 10th centile was lower, while the 90th centile was higher. In conclusion, both references would need customisation to fit the population.[47] The performance of the IG standards in a French setting was evaluated in comparison with the routinely used population-based reference.[48] The mean z-scores for AC and FL were higher using the IG standards. The proportion of measurements below the 10th centile was lower, and the proportion above the 90th centile was considerably higher compared with the French references, resulting in fewer fetuses classified as SGA, and more as LGA. As in the WHO study there was a very small effect of applying the strict IG criteria, questioning the ability of a standard derived from carefully selected individuals to describe universal normal growth.

In a study on a Swedish population of term births, the distribution across centiles related to the risk for adverse outcomes was evaluated for a reference based on the study population, a customised reference, and IG. When applying IG, only 3% were below the 10th birthweight centile, and the risk for perinatal mortality was increased already below the 35th centile. Either reference was considered possible to use, provided that thresholds were adapted to the specific chart.[49]

Infertility

Infertility is defined by the WHO as failure to achieve a pregnancy after at least one year of regular unprotected sexual intercourse,[50] and affects 8-12% of couples globally.[51] It may occur due to female, male or unexplained factors. As the ability to influence one's family planning is considered a human right, infertility is a prioritised health issue by the WHO.

Female infertility causes include hormonal, ovarian, tubal, and uterine factors. In many cases multiple risk factors may be present, such as metabolic, endocrine or inflammatory conditions, contributing to increased risks for adverse pregnancy outcomes.[52] Advanced maternal age is an increasingly contributing cause of infertility.[51] Polycystic ovarian syndrome (PCOS) is another common cause, characterised by hyperandrogenism and oligo- or anovulation,[53] often coexisting with obesity and reduced insulin sensitivity. It has been associated with risks for various pregnancy complications.[54, 55] Endometriosis

is related to reduced fertilisation rates and pregnancy complications including preterm birth. However, its association with abnormal fetal growth is unclear.[56, 57]

Male factor infertility primarily involves testicular dysfunction, characterised by abnormal sperm production or a complete absence of sperm, as well as post-testicular dysfunction, such as ejaculation disorders. Pre-testicular disturbances, such as hypogonadotropic hypogonadism or hyperprolactinemia, may also affect sperm quality.[51] The association between these conditions and pregnancy complications is less well understood.[58]

Unexplained infertility, which accounts for 10-20% of all infertility causes, is associated with elevated risks of pregnancy complications such as hypertensive disorders and preterm birth, regardless of the type of fertility treatment.[52]

Assisted Reproductive Technologies

Assisted Reproductive Technologies (ART) include various medical procedures to address infertility and achieve pregnancy, mainly conventional IVF or intracytoplasmic sperm injection (ICSI), with own or donated gametes, including controlled ovarian stimulation (COH), oocyte pick-up and transfer of fresh or frozen-thawed embryos.

Since the birth of the first child through IVF in 1978, the number of annual treatments has been steadily increasing. In Sweden, nearly 5% of children today are born from ART pregnancies. However, access to fertility treatments varies significantly between countries.[59] Over the years, ART has advanced and become available for new groups. In Sweden, oocyte donation was legalised in 2003, and ART became available to same-sex couples in 2005. Since 2016, ART treatments have been available to single women. Most recently, in 2019, the requirement for a genetic connection between the child and at least one of the parents was removed, enabling double donation of both oocyte and sperm, as well as embryo donation.[60] These changes have resulted in a more heterogeneous group, now including women with social causes of infertility in addition to those with medical infertility.

In earlier years, to increase live birth rates, multiple embryo transfer was not uncommon. The marked increase in multiple gestation pregnancies that followed led to a single embryo transfer policy, established by the Swedish National Board of Health and Welfare in Sweden in 2003. Despite the shift, high birth rates have been sustained, partly due to advances in laboratory techniques and extended embryo culture, which allows for the natural selection of high-quality embryos, resulting in higher live birth rates per transfer. Today, the incidence of multiple gestation after ART in Sweden is approximately 2%.[60] In cases with few embryos, extended culture increases the risk that no embryos will be available for transfer. Therefore, decisions regarding the

number of embryos to transfer, and at which embryo stage, are based on clinical evaluation.[61]

The development of new techniques has led to a shift from primarily fresh embryo transfer of cleavage-stage embryos (2-3 days) to longer embryo cultures to blastocyst stage (5-6 days) for fresh as well as frozen transfers. There has also been a shift in freezing techniques, from slow freezing to vitrification, leading to higher embryo survival rates.[62] An advantage of frozen ET is the reduced risk for ovarian hyperstimulation syndrome compared with fresh ET.[63] As a result, a total freeze approach is often preferred for patients at increased risk for hyperstimulation. Some centres even advocate elective freezing, a "freeze all" policy; however, evidence is sparse that this approach is superior in terms of live birth rates compared to standard treatment.[63, 64]

In women with regular menstrual cycles, transfer of a freeze-thawed embryo is often performed in a natural cycle, or in a modified natural cycle where ovulation is induced. When ovulation cannot be achieved, or sometimes for logistic reasons, ET is performed in an artificial cycle with administration of exogenous hormones. This results in a pregnancy where there is no corpus luteum present and risks for adverse outcomes have been described, further discussed in the sections below.[65]

Fetal growth after ART

Associations between ART and elevated risks for preterm birth, SGA and low birthweight have been reported.[66, 67] When assessing fresh and frozen ET separately, several studies have shown that frozen ET is associated with increased risks for offspring being born LGA and macrosomic, as well as higher risks for hypertensive disorders during pregnancy, compared with fresh ET and natural conception (NC).[68-72]

However, intrauterine growth has been studied to a much lesser extent, with most studies focusing on early fetal growth. Two large retrospective cohort studies showed larger CRL after frozen ET compared with fresh ET.[73, 74] A prospective study also showed larger CRL after frozen than after fresh ET at 6-14 weeks. In weeks 6-10 both groups had negative z-scores, indicating lower values than the published reference, but due to more rapid growth, z-scores were positive after 65 days for frozen ET and after 85 days for fresh ET.[75]

A retrospective cohort study that evaluated growth throughout pregnancy noted that ART pregnancies in general had positive z-scores for CRL in the first trimester, and positive z-scores for EFW in the second trimester, while in the third trimester only frozen ET had positive z-scores for EFW.[76] A prospective study on growth from week 19 to 41, reported that both fresh and frozen ET had positive z-scores for EFW in the second trimester, and thereafter non-

divergent growth with negative z-scores from gestational week 32 for fresh ET and 35 for frozen ET.[77]

A large study showed differences in birthweights at different gestational ages with higher weight for frozen compared with fresh ET from 33 weeks of gestation,[78] while another showed differences only after 37 weeks.[79] However, as previously discussed, making assumptions of preterm fetal weights based on preterm deliveries inevitably introduces a source of bias.

Causes of growth differences

Despite many studies showing differences in growth outcomes between different ART methods and NC, a causal relationship cannot be readily established. Apart from the need to address the higher prevalence of multiple gestations, accounting for increased risks for adverse outcomes, different background factors could influence the choice of treatment as well as fetal growth. Infertility is the most apparent factor, why a reasonable approach is to compare outcomes from ART with those from subfertile couples achieving pregnancies without the use of ART. The majority of treatments are performed due to medical infertility, but the indications have widened and the treated group is becoming increasingly heterogenous.[60] The different aetiologies of infertility, as well as associations with other risk factors further complicate comparisons. Moreover, the time aspect is of importance, since treatment methods are evolving, and practice routines are continuously changing.

ART involves multiple interventions affecting the woman, oocytes, sperm and embryos, including hormonal therapy, embryo culture in artificial media, and exposure to fluctuations in temperature and oxygen pressure. The effects of these factors on fetal growth and health, as well as the underlying mechanisms and interactions, are still not sufficiently understood.[80]

A possible pathophysiological mechanism behind divergent growth patterns after ART is impaired endometrial receptivity and placental development due to COH, performed in most treatments.[81, 82] Supraphysiologic oestradiol levels have been identified as an independent risk factor for low birthweight,[83] and fresh ET is performed shortly after hormonal stimulation. Fresh ET in natural cycles, without exogenous ovarian stimulation, has been associated with lower rates of both preterm delivery and low birthweight.[84] Oocytes used for frozen ET are also exposed to COH, and a negative correlation between high oestradiol levels and birthweight has been shown, also for frozen ET.[85, 86]

To separate the effect of embryo freezing from that of COH, outcomes after fresh and frozen ET of donated oocytes were compared. No differences in birthweights or preterm birth rates were noted, suggesting that the negative effects on the endometrium in fresh ET, rather than embryo freezing, may account for differences between fresh and frozen ET.[81]

Conflicting results have been reported regarding the possible effects of embryo culture media. Whereas some studies have shown differences in birthweights and fetal size in the second trimester,[87, 88] others did not show any differences between different media.[89]

Likewise, the effect of the duration of embryo culture on fetal growth is uncertain. A large retrospective study of birthweights after frozen ET found an association between culture duration and LGA,[90] and similar results were shown in a register study restricted to fresh ET.[91] Contrasting results were shown in another large register-based study, where neither embryo stage, nor freezing technique or vitrification media affected birthweight. Frozen ET in artificial cycles, however, resulted in significantly higher birthweight compared with natural cycles,[92] a finding that is supported also by other studies.[93]

The use of ICSI has increased dramatically and is now frequently performed even in cases without male infertility, often lacking a clear medical indication. However, its usage shows significant regional variation.[59] The possible effects of the ICSI technique, as opposed to conventional IVF, on fetal growth have been relatively scarcely studied, but no apparent impact has been shown on birthweight or gestational age in singletons of couples with non-male factor infertility.[94]

When exploring the effects of embryo freezing, one question is to what extent the freezing technique affects fetal growth. Since the shift from slow freezing to vitrification coincides with the transfer of blastocysts rather than cleavage-stage embryos, the effect of the freezing technique is difficult to isolate.[95] A study comparing outcomes of blastocyst transfers showed higher birth rates after vitrification compared with slow freezing, but no differences in neonatal outcomes, such as proportions of SGA or LGA.[96]

Sibling studies can overcome many confounding factors regarding background characteristics, including the role of infertility. One study showed that fresh ET had a lower mean birthweight and higher risk of SGA while frozen ET had a higher mean birthweight and higher risk for LGA, with consistent results when restricting to single ET and blastocyst stage.[97] Another study comparing birthweights of siblings from the same embryo cohort, either after fresh-frozen or frozen-frozen transfers. There was a statistically significant higher birthweight in the younger sibling only when the first was fresh ET and the subsequent frozen ET.[98] Another sibling study showed substantially higher risks for hypertensive disorders of pregnancy after frozen ET, compared with fresh ET and NC, but was not able to distinguish between natural and artificial cycles. Other studies reported higher risks for both preeclampsia and macrosomia after artificial cycles compared with stimulated or true natural cycles.[65, 99]

The association between macrosomia and preeclampsia could seem contra-intuitive, but where early onset preeclampsia is strongly associated with fetal growth restriction and abnormal placentation, only a minority of cases of late preeclampsia share these features. There are higher incidences of both SGA and LGA in this group, and for the latter, the pathophysiology is rather thought

to be related to high placental demands and cardiac dysfunction.[100, 101] In studies on ART, the risk for bias should be noted, since artificial cycles more often are used in anovulatory patients, for instance in women with PCOS, having independent risk factors for preeclampsia.[102] The corpus luteum, however, plays additional roles beyond producing oestrogen and progesterone, particularly in cardiovascular regulation and angiogenesis. These functions are supported by the secretion of relaxin and vascular endothelial growth factor (VEGF).[103] Uterine artery pulsatility index has been shown to be lower in pregnancies after frozen ET in artificial cycles, compared with fresh ET. Whether this is a cause or a consequence of increased growth rate, and thereby increased risk for late preeclampsia is unclear.[104]

Much remains unknown about the extent to which the previously mentioned factors contribute to varying outcomes after ART and NC, and their exact mechanisms of action are not yet fully understood. Epigenetic alterations due to one or more of these factors have been proposed as a possible explanation. Manipulations of the oocytes and embryos are performed during stages where demethylation and remethylation of DNA normally occur.[80] Imprinting disorders, such as Beckwith-Wiedemann and Angelman syndrome, are more common after ART, but whether these conditions are effects of ART or the underlying infertility is not sufficiently understood.[105] Many studies have explored the relation between ART and epigenetic alterations, but variations regarding both methods for analyses and tissues analysed complicate the interpretation of the relevance of the findings, as methylation varies in different tissues and over time. A recent meta-analysis did not find consistent alterations between studies, suggesting a minimal effect of ART on DNA methylation.[106]

While many studies have shown evidence of differences in birthweights and proportions of SGA and LGA between fresh and frozen ET, intrauterine fetal growth is still scarcely studied.

Further studies of intrauterine growth are therefore needed, to separate the individual contributions of infertility and ART.

Birthweight and postnatal growth

While the risk for adverse obstetric outcomes is high in FGR, and low in SGA without placental insufficiency, both groups have elevated risks for long-term consequences. Being born SGA is associated with negative neurocognitive outcome as well as impaired glucose tolerance, obesity, type 2 diabetes, and cardiovascular disease.[15, 107] The developmental origin of health and disease (DoHaD) suggests that adaptation to different environmental influences in early development results in programming for future disease. Either pathological conditions or physiological adaptations suitable for the current situation can result in a predisposition, the development of disease depending on

subsequent factors.[5] Analogous with fetal weight, the risks are not necessarily caused by low birthweight, which instead could be viewed as an indicator of increased risk. The catch-up growth that the majority of SGA children show during their first years is associated with lower risks for cognitive disabilities but with increased risks for metabolic disease.[108, 109] There is also evidence that being born LGA or macrosomic is associated with increased risks for obesity, cardiovascular disease and diabetes mellitus.[110] These risks are more closely related to the lack of catch-down growth and obesity,[111] whereas children born SGA can develop visceral adiposity and thereby increased risks despite normal weight.[15]

Postnatal growth and health after ART

Most studies on postnatal growth after ART show reassuring results. Where differences between fresh and frozen ET and NC are evident at birth, the differences in size are often attenuated in early childhood.[112] There are, however, a limited number of studies, and they have often not distinguished between fresh and frozen ET. Results are also somewhat conflicting. One study showed no difference in size from birth to five years of age,[113] while another found that, despite those after fresh ET being smaller than NC at birth and frozen being similar to NC, both ART groups exhibited higher postnatal growth rates.[114] Results are also conflicting regarding the sex-specific influence of ART. Whereas one study showed diminishing differences between fresh and frozen ET at 7-10 years of age, except for girls after frozen ET being taller and heavier than NC,[115] another showed that girls after fresh ET, despite lower birthweights, were taller than frozen ET and NC during childhood.[116] A meta-analysis of a large number of offspring from ART and NC pregnancies, showed that children from fresh ET were shorter and lighter than NC while no differences were seen when comparing frozen ET with NC. In late adolescence, the differences were attenuated, but no sex-specific analyses were performed.[117] A recent register-based study of ART offspring aged seven to 18 years, showed that boys after frozen ET had a higher risk of being overweight compared with fresh ET and NC, while girls after frozen ET had a slightly lower risk of being overweight compared with NC.[118]

There is evidence of increased cardiometabolic risk after ART, including premature vascular ageing and increased blood pressure, also in the absence of other risk factors.[119-121] These studies, however, did not distinguish between fresh and frozen ET. A recent study of children aged seven to ten years, showed that girls after frozen ET had higher blood pressure than girls after fresh ET, which was not found in boys, where instead a more favourable lipid profile was found.[122] Another recent study showed a probable mediating effect of LGA in children after ART, increasing the risk for obesity, higher blood pressure and fasting glucose, and thereby the risk for cardiovascular

disease.[123] There is also a moderately increased risk for type 1 diabetes mellitus after frozen ET, compared with fresh ET and NC, possibly also associated with the increased risk of LGA.[124]

The evaluation of long-term health in offspring from ART is complicated by the usually late onset of metabolic disease, requiring long follow-up times, and by the rapidly evolving treatment methods.[125]

Postnatal growth in children conceived with ART is still relatively scarcely studied, many studies being rather small and with various sources of bias. Therefore, further population-based studies are warranted.

Research aims

The overall aim of the thesis was to investigate normal fetal growth as well as fetal and postnatal growth trajectories after NC and ART treatments, to describe differences and identify underlying mechanisms.

The specific aims of the studies were:

I. To create updated national reference ranges for fetal size and growth from gestational week 12 to 42, by applying updated statistical methods to longitudinally collected data on ultrasonically derived intrauterine biometric measurements in a large Swedish cohort of low-risk pregnancies.

II. To compare fetal growth trajectories between pregnancies after fresh and frozen embryo transfer and natural conception, using population-based Swedish data on ultrasound measurements throughout pregnancy as well as birthweights.

III. To compare fetal growth from the first trimester until birth in pregnancies after fresh and frozen embryo transfer and natural conception, in a prospective Swedish multicentre study.

IV. To examine postnatal growth up to five years of age in children born after fresh and frozen embryo transfer and NC, in a population-based cohort in Uppsala County.

Materials and Methods

Study	Study population	Study design	Exposure	Outcome
I	583 pregnancies of low risk for growth aberration	Prospective observational longitudinal multicentre study		Normal fetal growth
II	10 970 fresh ET, 6520 frozen ET and 178 518 NC. Pregnancies with ultrasound data.	Retrospective population-based cohort study	Fresh or frozen ET or NC	Fetal growth and birthweights
III	82 fresh ET, 175 frozen ET and 583 NC.	Prospective observational longitudinal multicentre study	Fresh or frozen ET or NC	Fetal anthropometrics and fetal weight
IV	Children in Uppsala county from 517 fresh ET, 284 frozen ET and 17 214 NC	Retrospective population-based cohort study	Fresh or frozen ET or NC	Height, weight and BMI from birth to five years of age

Data sources

The Swedish Pregnancy Register (SPR)

This national quality register contains information on maternal health, prenatal diagnostics and maternal and neonatal outcomes for pregnancies leading to delivery from 22+0 gestational weeks. The national coverage is approximately 90%, except for ultrasound data where the coverage is lower.[126]

The Medical Birth Register (MBR)

The Swedish MBR contains prospectively collected data on all pregnancies leading to birth from 22 weeks of gestation, including medical history, antenatal care and complications during delivery or in the neonatal period. The register has a national coverage of 98%.[19]

The National Quality Register for Assisted Reproduction (Q-IVF)

The Q-IVF started in 2007 and contains data on infertility, fertility treatments, and related outcomes, with national coverage of close to 100%.[60]

The National Patient Register (NPR)

A national register including data on treatments from specialist care, classified by the International Classification of Diseases 10th revision (ICD-10) codes.

The Total Population Register

The Total Population Register provided data on country of birth.

Longitudinal Integrated Database for Health Insurance and Labour Market Studies (LISA)

The LISA database provided information on the highest achieved level of education.

The Prescribed Drug Register (PDR)

The PDR contains data on all prescribed drugs on a national level, organised by Anatomical Therapeutic Chemical (ATC) codes.

The Uppsala County Mother and Child (UCMC) Cohort 2000-2015

The UCMC is a local research cohort including data on all children born at Uppsala University Hospital between the years 2000 and 2015, with following child healthcare controls in Uppsala County. The database also contains data from the MBR, the NPR, the Total Population Register, and LISA. It comprises a total of 62 491 children, with prospectively collected measurements of height, weight and BMI at birth, 18 months, 3, 4, and 5 years of age (± 2 months).

Study population and exposures

Exposure groups

The main exposure in Studies II-IV was mode of conception, defined as fresh ET, frozen ET, or NC. In sensitivity analyses, the impact of further exposures was investigated.

Study I

In this prospective longitudinal multicentre study, healthy pregnant women with regular menstrual cycles and low risk of fetal growth aberrations were recruited from 18 antenatal care centres at five different study sites, Uppsala, Falun, Katrineholm, Västerås, and Örebro. At a first study visit in gestational weeks 12+3 to 13+6 evaluation of eligibility and pregnancy dating with ultrasound was performed, allowing ± 7 days compared with LPM-dating. To ensure an even distribution over gestation, each participant was randomly assigned to one of nine examination schedules for the additional four ultrasound scans, from gestational weeks 14 to 41. Data was collected from the medical records for antenatal care and delivery, including neonatal complications. Exclusions were made if pregnancy complications arose, including preterm birth (<37+0 weeks of gestation) and FGR (EFW<-2SD according to the Maršál reference,[10] in combination with either abnormal umbilical artery Doppler or oligohydramnios). At each examination, BPD, HC, MAD, AC and FL were measured thrice and EFW was calculated using Hadlock's third formula including BPD, HC, AC and FL.[26]

Study II

This was a longitudinal population-based retrospective cohort study based on the SPR, between 2013 and 2017, including pregnancies with records of a first trimester ultrasound. Data on ART and many potential confounders were made available through linkage with the MBR, Q-IVF, NPR, the Total Population Register, and the PDR. Multiple gestations were excluded. Three exposure groups were compared: fresh ET, frozen ET and NC. For ART-pregnancies, pregnancy dating was done using embryo culture duration and date of ET, conditioning on a maximum difference from the registered EDD date of ±7 days. NC pregnancies were dated by LMP, and pregnancies with a difference of 14 days or more compared with the first-trimester ultrasound dating were excluded. Infertility was defined as either a diagnosis of female infertility (from NPR) or self-reported involuntary time to pregnancy of more than one year. In frozen ET pregnancies with a prescription of oestrogen within 70 days before ET, the cycle was considered as programmed.

Study III

The setup for this prospective longitudinal multicentre study was similar to that of Study I. Women pregnant after either fresh or frozen ET were recruited at the fertility centres at Uppsala and Örebro University Hospitals in gestational weeks 8-10. Conventional IVF and ICSI cycles were eligible, whereas oocyte donations and multiple gestations were excluded. Inclusions were made in gestational weeks 12+3 to 13+6 and study participants were randomly allocated to one of nine examination protocols for the following four ultrasound scans. Detailed data on infertility and ART, as well as early CRL in weeks 8-10, were collected from the medical records at the fertility centres. Infertility causes were categorised into six groups: anovulation, endometriosis, tubal, male, social (same-sex couples and singles), unexplained, and other. The records also included information on endometrial preparation for frozen ET. However, since stimulated cycles could not be distinguished from programmed cycles, both were categorised as artificial cycles in this study. Further, data related to antenatal care and delivery, including neonatal outcomes, were collected from medical records. No exclusions were made for pregnancy complications except for severe fetal malformations and chromosomal abnormalities. EDD was calculated by adding 266 days to the date of ET and subtracting the embryo culture duration. Data on the 583 low-risk NC pregnancies from Study I acted as a reference group, and for this study pregnancy dating was calculated using LMP-dates. As in Study I, agreement with ultrasound dating of a maximum of seven days was a prerequisite. At each study visit, BPD, HC, MAD, AC, and FL were measured thrice. According to the power calculation, 112 participants for each fresh and frozen ET would be required to detect a difference of 100 g at term. Assuming smaller differences at lower gestations we aimed to include 175 participants in each group.

Study IV

A retrospective population-based cohort study based on the UCMC, restricted to year of birth from 2007, to ensure the availability of reliable ART data from Q-IVF, to 2014, to enable available measurements taken at 18 months of age. Three exposure groups were compared, children born after fresh ET, frozen ET, and NC. Data from Q-IVF, linked by each inhabitant's unique personal identification numbers, as well as available data from the MBR, the NPR, the Total Population Register, and the LISA database, provided detailed information on potential confounders. Subfertility was self-reported, as the inability to achieve pregnancy within one year of regular intercourse. Multiple gestations and preterm deliveries (<37+0 weeks) were excluded. To account for the remaining variation in gestational age, the postnatal age variable was adjusted according to gestational age at birth.

Outcomes

Study I

Prescriptive population-based references for EFW, presented as median and variance, expressed as centiles and standard deviations.

Study II

CRL, BPD, and a combined weight variable, constituted of EFW and birthweight. SGA, LGA and macrosomia at birth.

Study III

CRL, BPD, HC, MAD, AC, FL, and EFW. SGA and LGA at birth.

Study IV

Height, weight and BMI from birth to five years of age.

Directed Acyclic Graph (DAG)

Concluding causality from observed associations in observational studies is challenging. Assumptions of the causal pathways and relations between covariates can be visualised in a DAG.[127] This can aid in identifying a minimal sufficient adjustment set, and thereby to control for confounding. DAGs were created for Studies II to IV using the DAGitty software.

Statistical methods

Study I

With a one-way ANOVA, differences in mean birthweight between sites and different countries of birth were assessed. The discrepancy between EFW and birthweight for deliveries within two days of fetal weight estimation was evaluated using a paired samples t-test. To generate reference curves, a fractional polynomial model was first fitted to the log-transformed EFW to obtain an optimal transformation of gestational age. The transformed gestational age was entered into a random coefficient model to account for the repeated measurements and to obtain the estimated variance between and within fetuses. The regression coefficients and the variance components from the random coefficient model were used to estimate the expected mean EFW, standard deviation and centiles for a given gestational age. Sex-specific references were also

constructed using the same method. A sensitivity analysis was performed, excluding women with abnormal BMI at enrolment to antenatal care (<18.5 or ≥30.0). Comparisons with other references were made, for the Maršál reference,[10] of the median and ±2SD, and for Johnsen,[38] IG,[35] and WHO,[37] of the median and 2.5th and 97.5th centiles.

Study II

The effect of fresh or frozen ET on CRL and fetal weight was estimated by generalised least squares (GLS) models. Since repeated measurements are non-independent, an AR1 correlation structure was applied, assuming the correlation to be higher for values closer in time compared with values further apart. As the distribution of the residuals increased with gestational age, all values were log-transformed before modelling. Gestational age (GA) was modelled using restricted cubic splines with 5 knots, the number of knots chosen by the Akaike information criterion. The model also included a GA by group interaction to allow the associations between GA and outcome to differ by group. An appropriate minimal adjustment set was decided using a DAG. When comparing fresh and frozen ET with NC, adjustments were made for maternal age, BMI, parity, level of education (as a proxy for socioeconomic status), smoking, PCOS and infertility, and when comparing fresh ET with frozen ET, adjustments were also made for oocyte donation and embryo stage. Several sensitivity analyses were performed. To account for the possible effect on fetal growth of infertility per se, fresh and frozen ET were compared with subfertile NC without ART treatment. In an attempt to eliminate the effect of differences in embryo culture duration further sensitivity analyses were performed, restricted to cleavage stage or blastocyst stage ET respectively. Finally, a comparison was made within the frozen ET group, between programmed and non-programmed cycle ET.

Adjusted OR for SGA, LGA and macrosomia were calculated using logistic regressions with the same adjustment set as the longitudinal analysis. For the within-group analysis of frozen ET, aOR was also calculated for gestational hypertension.

Study III

We applied GLS models to investigate the effect of the mode of conception on fetal growth, evaluating each anthropometric parameter separately, as well as the calculated EFW. The minimal sufficient adjustment set for the modelling was decided using a DAG. This set included maternal age, BMI, smoking, country of birth, parity, maternal disease, embryo stage, infertility diagnosis, and for within frozen ET comparisons, also the method of endometrial preparation. In the main analysis, we chose not to adjust for infertility diagnoses, maternal disease or smoking, the impact of which was instead studied in

sensitivity analyses. The outcomes were log-transformed before modelling, using a similar approach as in Study II. In the main analysis, comparisons were made between fresh ET, frozen ET and NC with adjustments for maternal age, BMI, smoking, country of birth and parity, and for the within-analysis of ART, also embryo stage. Fetal sex was not considered a confounder, but in a sensitivity analysis, sex-specific differences between the exposure groups were evaluated. Further, the effect of embryo stage was explored in a sensitivity analysis restricted to blastocyst stage transfers. Separate sensitivity analyses were also performed where maternal chronic disease and smoking were excluded, as well as a comparison between fresh and frozen ET in a subgroup where anovulation as the cause of infertility was excluded. Finally, a subgroup analysis of frozen ET was performed, comparing artificial with natural cycles.

Adjusted OR for SGA and LGA at birth were calculated with logistic regressions, applying the same adjustment set as in the main analysis.

Study IV

Similar to Studies II and III, GLS models were applied to estimate the effect of fresh or frozen ET or NC on postnatal growth, according to a DAG. Adjustments were made for maternal age, BMI, country of birth, parity, smoking, level of education (acting as a proxy for socioeconomic status), and embryo culture duration, the latter only relevant for comparisons of fresh and frozen ET. All outcomes were log-transformed. In a sensitivity analysis, sex-specific growth patterns were explored, and in another sensitivity analysis, fresh and frozen ET were compared to subfertile NC.

Ethical considerations

Study I

The study was approved by the Regional Ethics Board in Uppsala, reference numbers 2014/209 and 2014/209/2.

Study II

The study was approved by the Regional Ethical Review Board in Stockholm, reference numbers 2013/1849-31/2 and 2018/386-32.

Study III

For this study, an amendment to the Study I ethical application was approved by the Regional Ethics Board in Uppsala, reference number 2014/209/3, and

a second amendment was approved by the Swedish Ethical Review Authority, reference number 2023-07676-02.

Study IV

To the main application with reference number 2012/410, an amendment for this study, for the inclusion of Q-IVF-data, was approved by the Swedish Ethical Review Authority, reference number 2021-05752.

In the two prospective studies involving exposing fetuses to ultrasound, participants have given informed written consent. All examinations were performed by experienced sonographers with awareness of safety indicators. The time of exposure was as short as possible and only grayscale ultrasound was used. Any suspected pregnancy complications detected within the studies were reported to, and managed by routine obstetric care. All data was stored in dedicated databases where participants were pseudonymised, and the code keys were stored securely at a separate location.

The two register-based studies did not involve personal contact with study subjects. Personal identity numbers were not included in the delivered register data, and the code keys for the identification of study subjects were kept at the National Board of Health and Welfare.

Results

Study I

Of 684 included participants, 583 completed the study. For details on exclusions, see Figure 1 below.

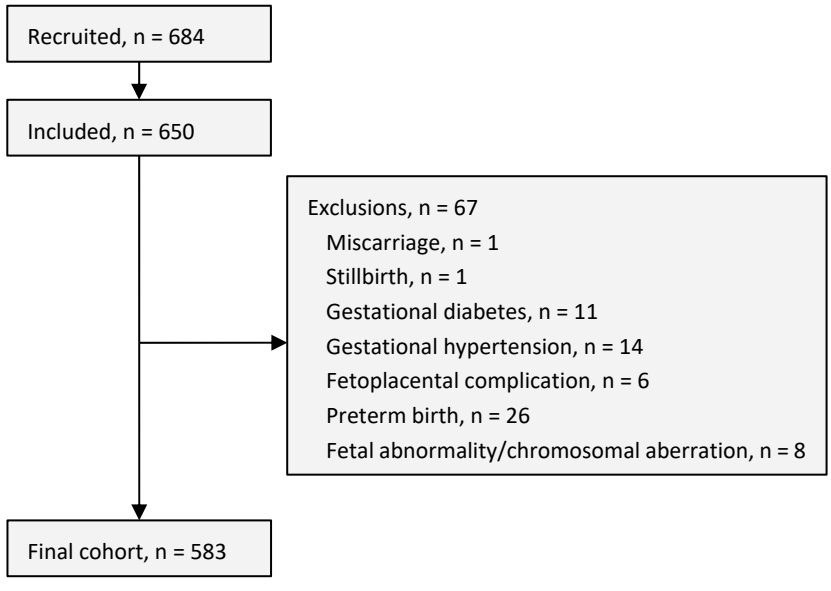

Figure 1. Flow chart with exclusions for pregnancy complications, resulting in the final cohort.

Of the total 2590 scans performed, 2551 had complete data for estimation of fetal weight. The dating discrepancy between BPD and LMP was -0.1 days. Eleven percent of the women were obese (BMI >30) and 92% were born in a Nordic country. There was no statistically significant difference in birthweight between women with Nordic versus non-Nordic origin, or between study sites.

The mean difference between EFW within two days of birth, and birthweight was 13 g. General and sex-specific medians for EFW were calculated, and the variances were expressed as centiles and SDs. Female fetuses had constantly lower centiles than male fetuses, with the exception that the 97.5th centile was higher than for males after 38 weeks, Figure 2.

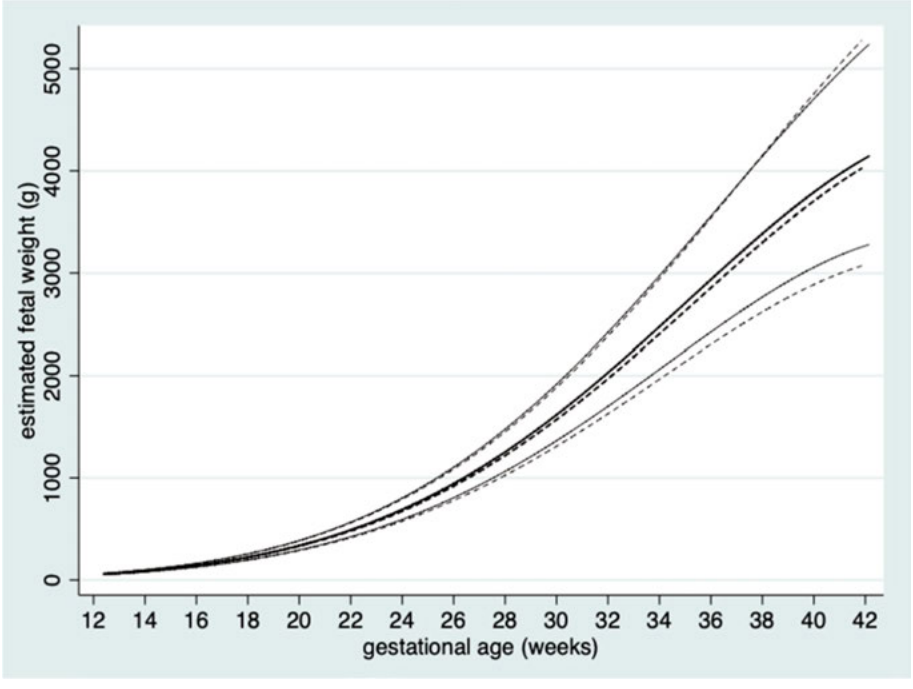

Figure 2. Sex-specific references for EFW, median, 2.5th and 97.5th centile for male (——) and female (- - -) fetuses.

In the sensitivity analysis excluding women with abnormal BMI, there were no statistically significant differences from the main analysis.

The comparisons with both the reference by Maršál et al.,[10] and the Norwegian reference by Johnsen et al.,[38] yielded similar results. The median and higher centiles were slightly lower until approximately 33 weeks, after which the new reference was increasingly higher. The lower centiles were constantly higher in the new reference. The weight differences at term between the new reference and the reference from Maršál were over 150 g for both the median and -2 SD, and 390 g for +2 SD.

Compared with IG,[36] the median, 2.5th and 97.5th centiles were similar from the beginning at 22 weeks, until 24 weeks, after which all centiles were increasingly higher in the new reference. Compared with the WHO reference,[37] the median was rather similar in the second trimester; however, the centiles were wider for the WHO reference. In the third trimester, the median, as well as the 2,5th and 97.5th centiles, were higher in the new reference, as shown in Figure 3.

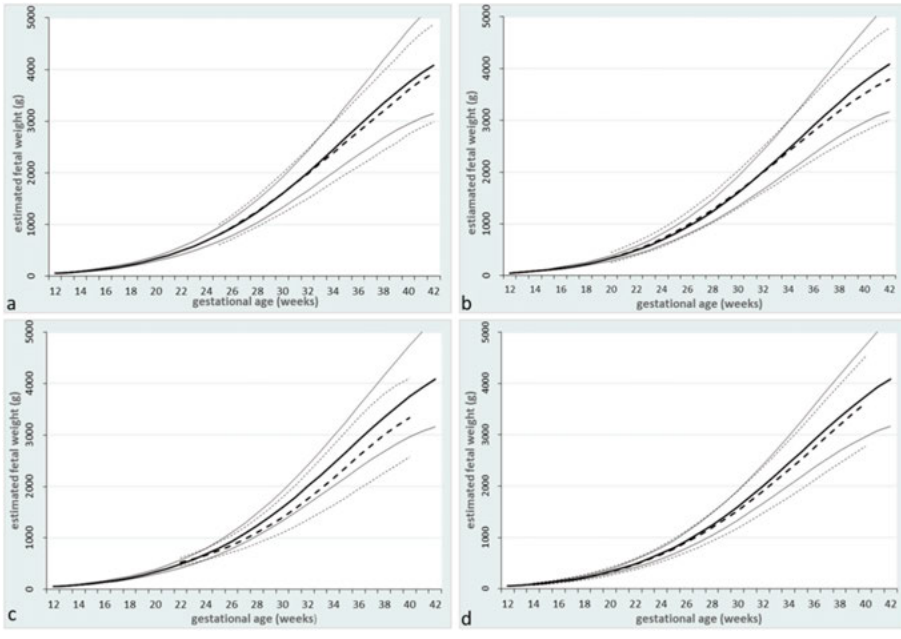

Figure 3. Comparisons of the median and ±2SD (——) with the reference by Maršál et al.(a), and median and 2.5th and 97.5th centile, with the references by Johnsen et al.(b), IG(c) and WHO(d) (- - -).

Study II

After exclusions, mainly due to lacking data on ultrasound altogether or a first-trimester scan, the analytic sample for the longitudinal analyses was constituted of 9875 fresh ET, 5857 frozen ET, and 158 697 NC, Figure 4.

There was no difference in CRL between fresh and frozen ET, but both had larger CRL compared with NC. Hence, there was a tendency towards overestimation of GA with CRL dating in ART-pregnancies (0.9 days for fresh and 1.1 days for frozen) compared with ET dating. In NC pregnancies, gestational age according to CRL was 1.4 days longer, and according to first trimester BPD 0,8 days shorter, than when calculated from LMP.

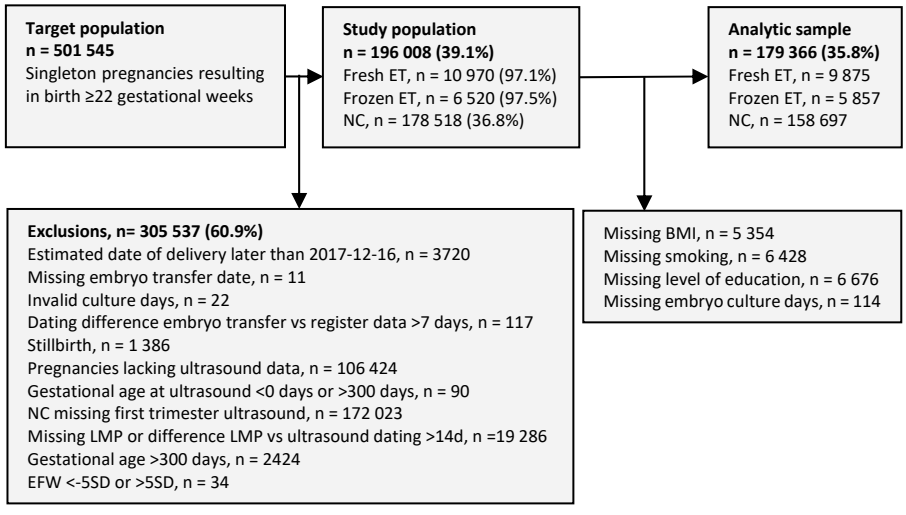

Figure 4. Flowchart illustrating the path to the final study cohort, with the analytic sample being a complete case population.

EFW was similar for fresh and frozen ET in the second trimester, fresh ET being slightly lower than NC at the start at 17 weeks, and thereafter both becoming higher. Fresh ET showed a lower growth rate than both frozen ET and NC from the second trimester, having lower EFW than frozen ET from 30 weeks, and lower than NC from 35 weeks of gestation, as shown in Figure 5. Frozen ET also had a lower growth rate than NC, but the difference was smaller and EFW remained higher than NC at term. Fresh ET had higher aOR for SGA (1.65, 95% CI 1.33-2.04), and lower aOR for LGA (0.66, 95% CI 0.56-0.79) and macrosomia (0.59, 95% CI 0.48-0.73) compared with frozen ET.

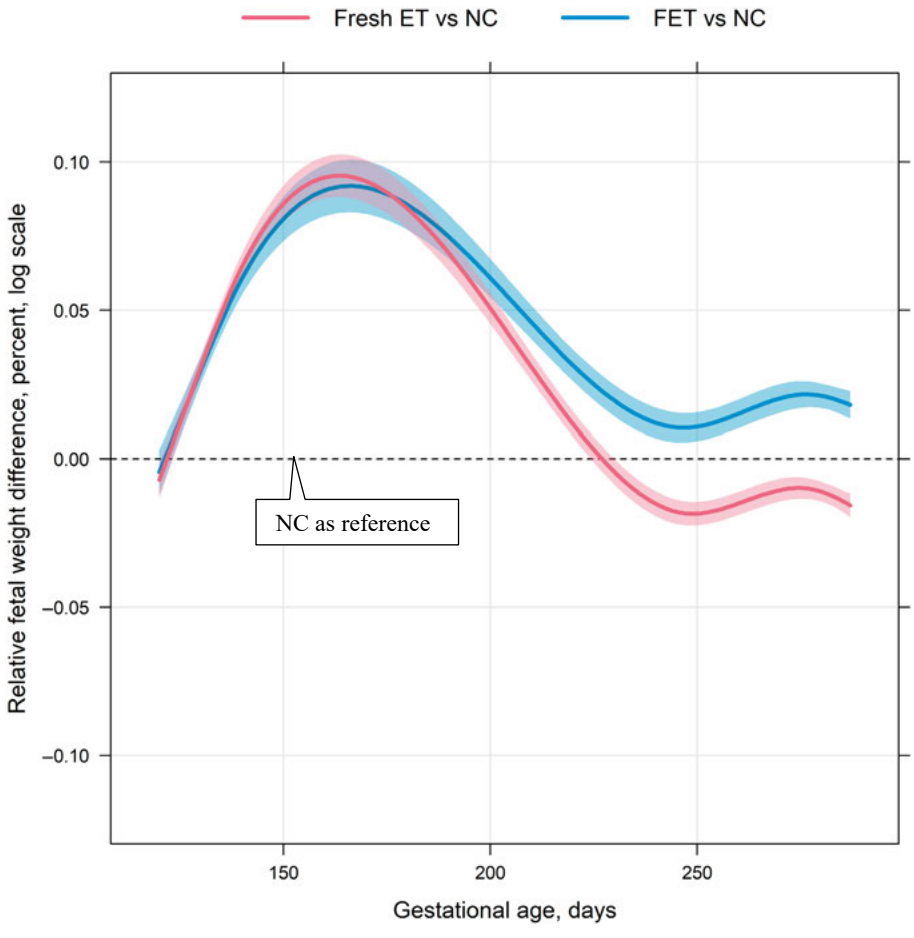

Figure 5. Difference in weight between fresh and frozen ET with NC as reference.

The results from the main analysis were consistent in the subgroup analyses. No statistically significant differences in EFW were seen between programmed and non-programmed frozen ET, but the risk for gestational hypertension was higher after programmed cycle transfer.

Study III

Due to a slower-than-expected inclusion rate, primarily for fresh ET, we decided to halt further inclusions on September 30, 2024, when 87 fresh and 175 frozen ET were included. Exclusions are shown in Figure 6.

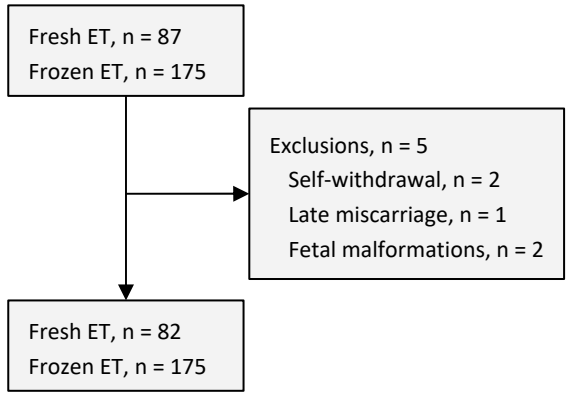

Figure 6. Flowchart of exclusions resulting in the final cohort.

In early pregnancy, there was a tendency towards larger biometric measurements and EFW for both ART groups, but the differences were more pronounced for frozen ET. No differences were seen between fresh and frozen ET in the first trimester. Both ART groups showed a decreased growth rate compared with NC, more apparent for fresh ET, having lower EFW than frozen ET from week 25, and lower than NC from week 35, as seen in Figure 7. At term, EFW was 4% lower for fresh ET compared with NC. There was no statistically significant difference between frozen ET and NC, but EFW was nearly 8% higher for frozen ET than fresh ET.

The sex-specific analyses showed very marginal differences between the groups for boys. For girls the influence of fresh ET was more pronounced, EFW at term was 8% lower than for NC, whereas EFW after frozen ET was 12% higher than after fresh ET.

When restricting the cohort to blastocyst stage transfers the differences were less obvious than in the main analysis, and at term, no statistically significant differences were seen between the groups. There were no differences between any of the outcomes in the comparison of artificial versus natural cycle frozen ET. The incidence of SGA at birth was higher after fresh ET compared with NC (aOR 2.83, 95% CI 1.164-6.890), but there were no statistically significant differences in the incidences of LGA.

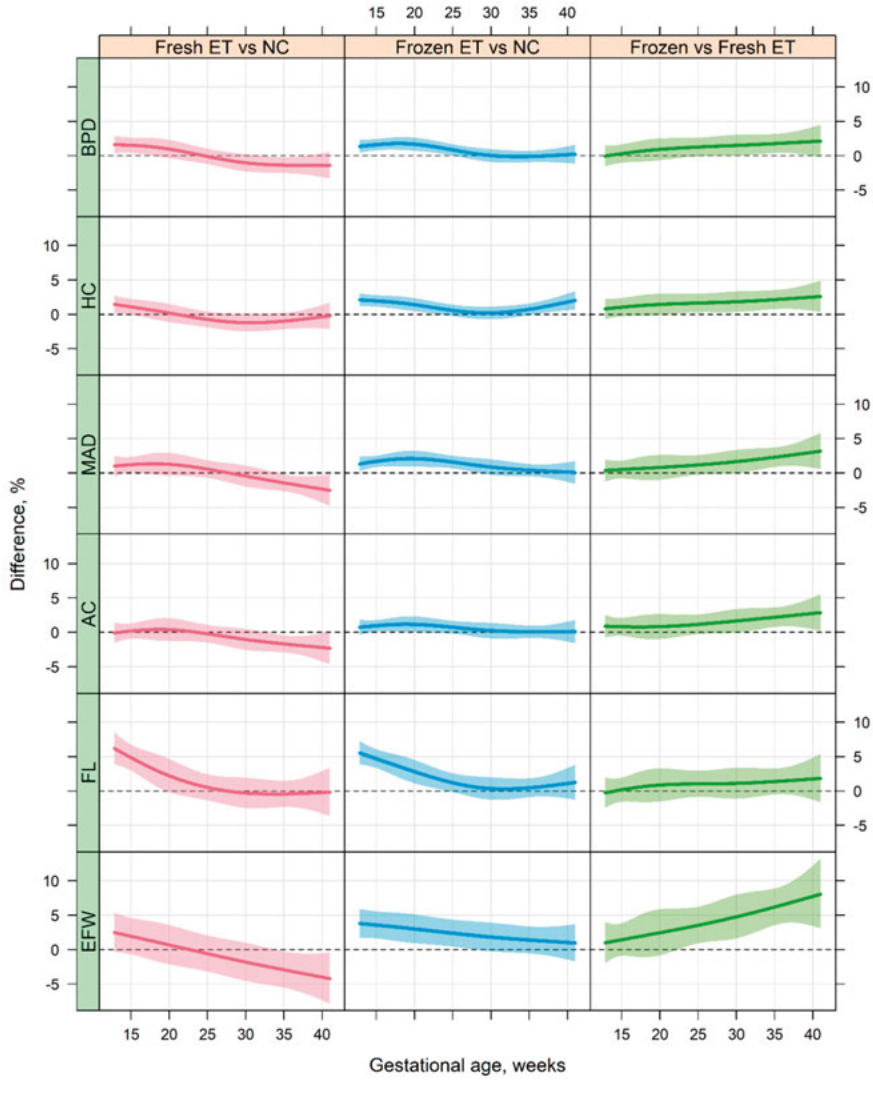

Figure 7. Contrasts of the outcomes between fresh ET - NC (red), frozen ET - NC (blue), and frozen - fresh ET (green).

Study IV

From the source population of 26 979 singleton births, 18 015 remained after exclusions, 517 from fresh ET, 284 frozen ET, and 17 214 NC, as shown in Figure 8.

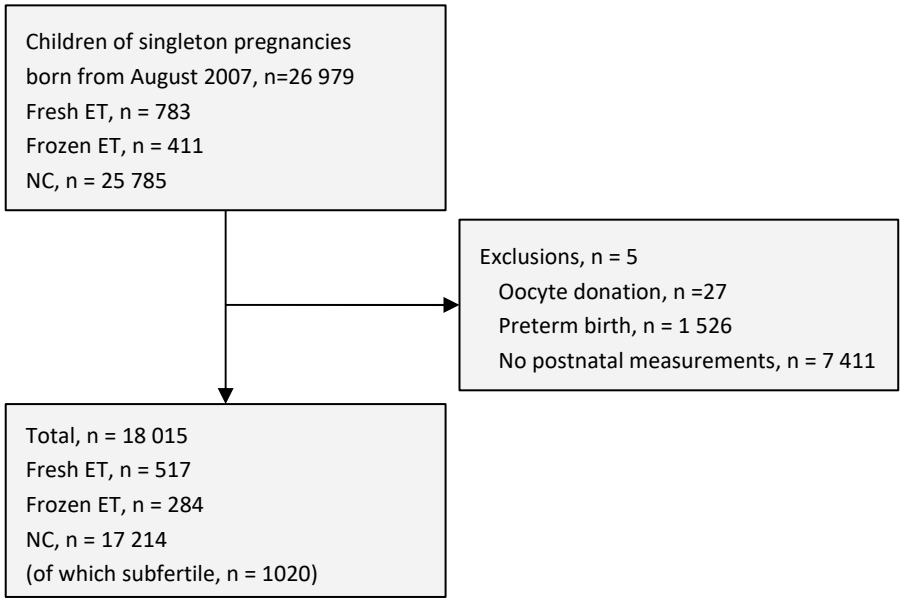

Figure 8. Diagram showing exclusions and the final cohort.

At birth, offspring from fresh ET were shorter and had lower weight and BMI, whereas they were all higher in offspring from frozen ET, compared with NC. Postnatal growth trajectories were different, where fresh ET showed an early catch-up growth, and frozen ET showed an early catch-down growth. The only statistically significant difference at 18 months was that children after fresh ET had lower BMI than NC, and at later time points, no differences were seen, results shown in Figure 9.

At birth, boys and girls after fresh ET were lighter than both frozen ET and NC. Girls after frozen ET were taller and heavier than girls after NC. Differences were largely attenuated by 18 months, but girls after frozen ET were taller without being heavier than NC from three to five years of age.

In the sensitivity analysis where ART was compared with subfertile NC the differences at birth between fresh ET and NC were no longer apparent. Frozen ET remained larger than NC in all aspects at birth, but later no differences were seen.

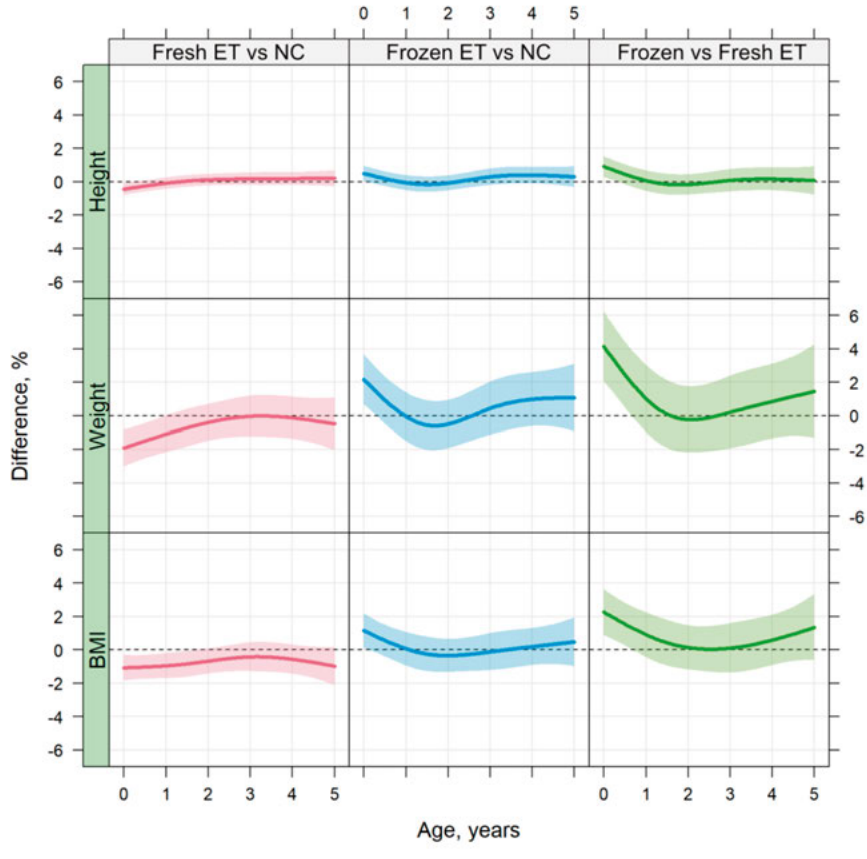

Figure 1. Differences in the outcomes between fresh ET - NC (red), frozen ET - NC (blue), and frozen - fresh ET (green).

Discussion

The general aim of the thesis was to study different aspects of growth. Study I aimed at creating updated references for fetal weight for the Swedish population, Studies II and III to describe and compare intrauterine growth of fetuses in pregnancies achieved by fresh or frozen ET, and Study IV the continuation of growth during the first five years of life for children from ART pregnancies, in comparison with those after NC.

In summary, the results from Study I, based on a large and carefully selected low-risk cohort, were general as well as sex-specific references for EFW from gestational weeks 12 to 42 with variance expressed as SD and centiles. Study II based on population-based national data showed that fetuses both from fresh and frozen ET were larger than NC in the second trimester, but thereafter showed a slower growth rate than NC, resulting in lower EFW for fresh ET, while frozen ET remained larger than NC at term. Study III yielded similar results, with ART having a tendency toward larger fetuses in the second trimester, thereafter declining growth compared with NC, but no difference in EFW between frozen ET and NC was seen at term. Study IV showed that differences in height, weight and BMI seen at birth were largely attenuated already at 18 months, but different effects were noted between sexes.

Methodological considerations

Study design

Studies I and III were prospective multicentre studies with similar designs but distinct objectives, reflected by different inclusion and exclusion criteria and statistical methods. In Study I we made efforts to only include a healthy population with low risk for growth abnormalities and used statistical methods allowing us to present both medians and values for variance. In contrast, Study III aimed to reflect the overall ART population rather than to create specific references. Consequently, the inclusion criteria were more inclusive. Using longitudinal statistics, growth of fresh and frozen ET fetuses was compared with the low-risk population from Study I.

Studies II and IV were both register-based epidemiological cohort studies with similar statistical methods as in Study III.

Bias

All included studies are observational, why potential bias has to be considered. In Study I, despite efforts made to include participants representative of the population in Sweden, a larger proportion of participants was born in a Nordic country. The strict inclusion and exclusion criteria inevitably led to a cohort with less resemblance to the general population, but the decision was intentional as the aim was for the reference to reflect fetal growth under optimal circumstances. As for underweight and obesity, the sensitivity analysis did not show any significant differences when excluding those participants, why they were included in the main analysis. Further, the willingness to participate in clinical studies can be influenced by for instance educational level and socioeconomy, relevant for both Study I and Study III. Despite all sonographers being experienced, measurement bias could be a source of error, and measurements on the screen were not blinded, why expected-value bias cannot be excluded.[128] In Study II, inclusion bias could have been introduced when conditioning on data from a first-trimester ultrasound, which led to the exclusion of a substantial proportion of NC pregnancies. There were variations in the offering of first-trimester screening in different regions, in some cases dependent on maternal age. After exclusions, the cohort's background characteristics more closely resembled those of the ART groups compared to the pre-exclusion cohort. Whereas in Studies I and III the collection of data was predefined, and in Study IV collection was performed prospectively within the routine child healthcare program, in Study II ultrasound observations later than the mid-trimester scan were likely motivated by identified risk factors or suspected pregnancy complications. This results in a risk for bias, including a higher likelihood of follow-up examinations when results are abnormal. However, we have no reason to believe that this would be different between the exposure groups.

Both when relying on register data, and medical records, the possibility of misclassification has to be considered. For instance, smoking status and time to pregnancy are self-reported and may be subject to inaccuracies. In Study II, attempts were made to evaluate the effect of programmed endometrial preparation in frozen ET by the assumption that a prescription of oestrogen within 70 days before ET resulted in a programmed cycle transfer. However, we had no information on whether the medication was used for the actual ET.

Confounding

Confounders are factors associated with the exposure, with the potential to influence the outcome, and are important to address in observational studies. When aiming to explore the effect of different ART, infertility is the most apparent confounder, with the potential to impact both mode of conception and growth. For instance, PCOS is associated with increased risks for

abnormal growth, and these patients are also more likely to have frozen ET after total freeze, due to the higher risk for ovarian hyperstimulation. Of the three studies exploring the effects of ART, information on infertility diagnosis was only available for Study III, but the diversity of infertility causes and the significant proportion of unexplained infertility posed challenges to conducting further analyses. Sensitivity analyses, comparing ART with subfertile NC without the use of ART, are means to isolate the effect of fresh or frozen ET, and were used in Studies II and IV, but the increasing group of participants with social infertility, which would not be expected to have increased risks for abnormal growth, complicates this approach. Further, other methodological factors are associated with a specific type of ART. An example is the duration of embryo culture, where, at least until recently, cleavage stage transfers were more common in fresh ET, and blastocyst stage transfers were more common in frozen ET.

We have used DAGs to decide minimal sufficient adjustment sets for confounders, and either adjusted for all confounders suggested, or explored their effects in separate substudies. Despite this, some degree of residual confounding cannot be ruled out.

Study I

The results of Study I are population-based prescriptive references for EFW from gestational weeks 12 to 42 for a Swedish population. Strengths of this study are the prospective design, with a well-defined low-risk cohort, and readily available data on detailed background through medical records, as well as ultrasounds performed by experienced sonographers. Triplicate measurements of each anthropometric and distribution of scans over the whole second and third trimesters, with a sufficiently large cohort to produce reliability also in extreme centiles, and the use of updated statistical methods, add to the strength.

Both the median and centiles were different from the reference by Maršál et al.,[10] Johnsen et al.,[38] and two multinational references, IG[36] and WHO.[37] There are multiple possible reasons for differences compared with the reference from Maršál. The methods for fetal weight estimations used are different, and the formula by Persson and Weldner[24] used for the Maršál reference is known to underestimate fetal weight, this being most apparent in early gestations and the lower centiles.[28] The formula by Hadlock et al.,[26] on the other hand, tends to overestimate fetal weight.[27] The differences between EFW and actual birthweights were very small in participants giving birth within two days of ultrasound, although this included only 36 participants. All but one were term deliveries, and differences caused by different formulae could be greater in earlier gestations. The mean birthweight within the study was 150 g higher than in the general population, possibly an effect of the strict inclusion

criteria. However, the impact of a careful selection of low-risk pregnancies versus the general population in high-income settings has been questioned since comparisons have shown very discrete differences.[48] The 50th centile of the new reference was higher at term than the Norwegian reference by Johnsen et al.,[38] despite the fact that mean birthweights were higher in the Norwegian general population than in the Swedish. Future validation studies will give answers to the fit of the new references in the Swedish population, and which cut-off values most accurately identify fetuses at risk.

Statistical considerations

The statistical method used when constructing references for fetal size and growth has to be carefully decided. Cross-sectional data can be used to create size charts, but for growth charts, longitudinal data is needed. When, as in data based on repeated measurements, the assumption of independent observations is violated, the statistical methods used need to be adapted, or else the distribution of centiles will be too narrow.[129] Whereas the reference by Maršál et al. was based on longitudinal data, and the variance was calculated in a cross-sectional manner, later publications have used updated statistical methods. The method must allow the distribution of EFW to increase with age, and due to this fact, log transformation of EFW is often needed to achieve a fairly constant distribution of the residuals. Ensuring a good fit of the curves to the raw data is very important, with a correct proportion of data outside extreme centiles, where the uncertainty is greatest. This can be demonstrated by superimposing the curves on plots of raw data. For this study, we chose to use a fractional polynomial function in combination with a multilevel model to account for depending data, both between repeat measurements in each examination, and also between different examinations of the same fetus.

Study II

This population-based study added knowledge to the largely unexplored field of intrauterine growth after different methods of ART, where previous studies mainly examined early growth or were based on birthweights. The ability to link multiple national registers by each inhabitant's unique personal identification number enabled reliable data on many important covariates. This, in combination with the large number of pregnancies, are major strengths of this study. The results, showing different growth patterns already in early pregnancy that remain until term, align well with one of the few earlier studies,[76] and contribute to the understanding of the previously shown differences in birthweights. As mentioned, there are many difficulties and pitfalls when trying to isolate the effect of different ART methods on fetal growth. The results from the sensitivity analyses indicate that different growth trajectories in fresh

and frozen ET and NC pregnancies were not mainly driven by infertility or the duration of embryo culture.

In large studies, statistical significance can often be demonstrated even when differences are small and perhaps clinically insignificant. The mean differences between the groups in this study were rather small, and the statistical methods used did not allow analyses of the variation within groups. This was, however, examined with a separate logistic regression on birthweights, where the proportions of SGA and LGA were different between fresh and frozen ET. Although differences are small, a better understanding of how ART methods have implications on growth is important, as are further efforts to understand the exact underlying mechanisms.

Study III

Having similar aims as Study II, this study has the potential to overcome some important weaknesses associated with register-based studies.

The well-defined protocol and reliable data on maternal background and ART, with in total 3 872 visits and 69 696 measurements are major strengths. The results are to a large extent similar to Study II, with some exceptions.

Whereas in Study II there was an increasing positive difference in EFW between both ART groups and NC in the second trimester, in this study, EFW tended to be higher than NC in the first trimester, but the growth rate was already then lower than NC. At term, results were similar between the two studies, where EFW in fresh ET was lower than NC and frozen ET, but in this study, no difference between frozen ET and NC was noted. This could be an effect of the much smaller sample size and thereby wider confidence intervals, but the estimate of the difference was also smaller. A possible explanation is that the mean birthweight in Study I, used as reference in this study, was significantly higher than in the general population, the reference category in Study II. There is a possibility that the comparisons to the selected healthy population in Study I overestimated the negative effect of fresh ET, and underestimated the positive effect on fetal growth of frozen ET, also reflected in the markedly higher aOR for SGA after fresh ET compared with NC in this study, than in Study II and previous studies.[78]

The sex-specific effects, with non-significant differences between boys in all groups, and a more marked decrease in growth in girls after fresh ET, can however not be explained by the reference population, where boys have a constant slightly higher median than girls. Most earlier studies have reported a larger effect on fetal growth of frozen ET in boys than in girls,[130, 131] but there are also contradictory findings where no apparent sex-specific differences were found,[78] and studies where the effect of frozen ET was more apparent in female fetuses.[132, 133] The study by Cavoretto et al., which also had a

prospective design, and a larger cohort, did not examine sex-specific growth differences or individual biometrics. Their results were somewhat contradictory to ours, showing non-divergent growth for fresh and frozen ET, and that both groups had negative z-scores for EFW in the third trimester. However, z-scores for birthweights were, despite this, positive for frozen ET in term deliveries.[77]

Exclusion of maternal disease and smoking, as well as anovulation, respectively, did not change the results of the main analysis to any large extent. The prevalence of maternal disease was generally low in the ART groups and only one participant was smoking in gestational weeks 30-32; these were non-eligible in the NC group. The high proportion of undefined infertility causes (unexplained 39.4-42.6% and missing 19.5-22.3%) resulted in a limited value of that sensitivity analysis. The inability to show differences between programmed and non-programmed frozen ET can probably be attributed to suboptimal statistical power and the fact that the subgroup of artificial cycles was constituted of both programmed and stimulated cycles, whereas in earlier studies exaggerated fetal growth was only shown for the former.[92]

Study IV

The most apparent change in ART during recent years is the increased use of frozen ET. The noted higher risks for LGA and macrosomia, as well as the well-known association between fresh ET and lower birthweights, have raised questions about the future growth and health of these children.

By combining population-based data from prospectively collected routine measurements with linked data on many relevant covariates, we were able to explore adjusted growth differences between offspring from fresh and frozen ET compared with NC. Since the UCMC cohort includes all children born at Uppsala University Hospital and having following child healthcare controls in Uppsala County, with visits at predefined time points, the risk of biased results is low. However, there were many exclusions due to lacking measurement data, and also a decreasing number of observations with age. At five years, only 31-38% had complete data. As this was driven mainly by fewer children reaching that age before the end of the study period, we have no reason to assume that this reduction was biased or related to the mode of conception. We did not use sex-specific references, since the age variable was corrected according to gestational age. Therefore, differences were instead expressed in percent. Our results are consistent with the majority of earlier studies, with differences in size at birth diminishing due to early postnatal differences in growth. Girls after frozen ET being taller but not heavier than NC aligns well with a recent study on adolescents, where girls after frozen ET had a lower risk of obesity.[118] In that study, boys after frozen ET had a higher risk for obesity, whereas our results show a non-significant increase in BMI

for boys toward the end. We did not, however, specifically study the risks of obesity. Nor did we analyse separate growth trajectories for children born SGA and LGA, which prevented us from studying the proportions of catch-up and catch-down growth in these groups.

Clinical implications/future perspectives

If the new references for EFW are to be used in clinical practice, they have to be validated for the Swedish population. However, since data on HC and AC to a large extent is lacking in the SPR, this cannot be easily done based solely on register data.

A general question is whether population-based references are the most suited approach for a multi-ethnic population, like the Swedish, the answer for which depends on whether ethnic background has physiological effects on growth. The impact of applying the strict IG criteria on a Norwegian multi-ethnic population did not cancel out ethnic differences in fetal growth, and neither were the incidences of birth complications different between women fulfilling the criteria and those not fulfilling the criteria.[134] The question of whether the reference should primarily reflect the population, or if it should describe optimal growth of that same population might thereby not have such a high significance.

International references can be of value in settings where population-based references are lacking and can make comparisons between regions easier but do not seem to be suitable for universal use without controlling for goodness of fit and when needed customisation to the relevant population.

Further customisation into a more personalised reference seemingly has the potential to increase sensitivity and specificity for abnormal growth and adverse outcomes, but are not yet broadly accepted.

There are now many studies supporting that there is a direct effect of ART on fetal growth and birthweights, while not yet fully understood. Further studies exploring the mechanisms behind these differences are needed, in particular well-designed epigenetic studies. Non-communicable diseases typically manifest late in life, and the techniques involved in ART are constantly evolving. This means that outcomes of today do not necessarily reflect the risks of today's treatments. Therefore, the long-term health of offspring from ART has to be continuously monitored. The risks for disease are not necessarily mediated through weight, as cardiovascular risk factors have been identified in ART offspring also in the absence of other risk factors.

If possible, programmed endometrial preparation could be reconsidered, unless medically motivated, since risks are seemingly higher both for exaggerated fetal growth and hypertensive disease. As the impact of individual procedures related to ART on growth and health still is relatively unknown, it

might be sensible to avoid unnecessary manipulations, for instance, the use of ICSI when a medical indication is lacking.

The higher incidences for SGA after fresh ET, and for LGA after frozen ET, should be considered in the overall assessment of the risk for abnormal growth.

Conclusions

We have produced updated population-based references for EFW intended for the Swedish population using updated robust methods, from a large cohort of low-risk pregnancies. Future validation studies will help in deciding which reference is best suited for clinical use, also considering the formulae for calculating EFW. Further customisation and individual conditioning may also be considered.

Using two different approaches, both a retrospective study on a nationwide population-based cohort and a prospective well-defined cohort with predefined ultrasound examinations, the previously scarcely studied field of fetal growth after fresh and frozen ET was explored. The studies consistently showed that fresh and frozen ET fetuses tended to be larger than NC in the first and second trimesters, indicating a higher growth rate, and thereafter showing lower, but diverging, growth rates compared with NC. Fresh ET was associated with increased risks for SGA in both studies, while the risk for LGA was higher after frozen ET in one of the studies. The effect of ART method on fetal growth was more apparent for female fetuses.

At birth, offspring from fresh ET were shorter and lighter, while those from frozen ET were taller and heavier than NC. These differences were largely attenuated already at 18 months of age. Girls after frozen ET, however, were slightly taller than NC from three to five years of age.

The early postnatal equalisation of differences in size evident in fetal life and at birth is reassuring, but catch-up growth is associated with increased risks for obesity and metabolic disease. Although several theories exist, the underlying causes and mechanisms have yet to be elucidated.

Sammanfattning på svenska

Huvudsyftet med denna avhandling är kartlägga olika aspekter av tillväxt där både intrauterin och postnatal tillväxt kan ha konsekvenser för hälsa på kort och lång sikt. De ingående studierna berör i huvudsak tillväxt under fosterlivet, men en av studierna behandlar postnatal tillväxt. Syftet var att beskriva normal fostertillväxt i okomplicerad graviditet, och därutöver att utforska effekterna av infertilitetsbehandling på intrauterin och postnatal tillväxt.

Fostertillväxt regleras av ett flertal faktorer i samspel. En väl fungerande placenta är vital för normal tillväxt men också många yttre faktorer spelar in, såsom nutritionsstatus, hormonella faktorer, men också frånvaron av negativa yttre faktorer, som till exempel toxiner och infektioner. Man brukar tala om att fostret har en inbyggd tillväxtpotential, det vill säga den storlek fostret kan uppnå under optimala förutsättningar. Denna potential är till stor del beroende av nedärvda anlag från föräldrarna, men kan också påverkas av exempelvis kromosomavvikelser eller andra genetiska varianter. I varje graviditet sker, efter implantationen av embryot i endometriet, en successiv invasion av trofoblastceller som leder till att de maternella spiralartärerna omvandlas till vida lågresistenskärl. Vid en onormal placentation påverkas överföringen av näringsämnen och gasutbytet till fostret negativt. Avvikande placentation är nära kopplad till tidigt debuterande tillväxthämning och preeklampsi. Tillväxthämning innebär att fostret inte uppnår sin tillväxtpotential, och i de flesta fallen är detta relaterat till nedsatt placentafunktion. Tillväxthämning är en vanlig graviditetskomplikation och innebär ökad risk för både intrauterin fosterdöd och medför betydande morbiditet, ofta som en följd av för tidig förlossning, som inte sällan är iatrogen. Det finns ingen specifik behandling mot tillväxthämning, varför målsättningen istället är att utifrån en helhetsbedömning förlösa vid optimal tidpunkt, där riskerna förknippade med fortsatt graviditet vägs mot riskerna med en förtida förlossning. Dessa bedömningar påverkas därför i hög grad av graviditetslängden. Stora ansträngningar görs för att identifiera foster i riskzonen för negativa utfall, och otaliga studier har undersökt diagnostiska metoder och på vilka grunder beslut om förlossning lämpligen tas.

Diagnostiken av tillväxthämning är inte enkel. Ofta misstänks tillväxthämning när ett foster har lägre uppskattad vikt än förväntat vid graviditetslängden (Small for Gestational Age – SGA). Men begreppen är inte liktydiga och därför viktiga att skilja åt. Först och främst skiljer sig tillväxtpotentialen åt foster

emellan, och variationen är stor även bland friska foster utan förhöjd risk för fosterdöd. Ett foster som ligger under en arbiträr gräns för normal storlek behöver därför inte vara tillväxthämmat. På motsvarande sätt kan ett foster med större tillväxtpotential vara tillväxthämmat även om vikten ligger inom normalområdet. Internationellt används ofta den tionde percentilen som gräns för SGA, medan SGA i Sverige definieras som fostervikt under -2 standarddeviationer av förväntat vid en given tidpunkt. Ju större avvikelse från förväntad vikt, desto större är sannolikheten att fostret är tillväxthämmat. Andra parametrar kan också vägas in, exempelvis dopplerundersökningar och bedömning av fostervattenmängd. Merparten av tillväxthämningar är emellertid sent debuterande och i dessa fall uppvisar endast en mindre andel avvikande blodflöde i navelsträngen, vilket komplicerar diagnostiken av sen tillväxthämning ytterligare.

Det är viktigt att komma ihåg att bedömning av fostertillväxt bygger på en rad antaganden. För att kunna bedöma ett fosters storlek i förhållande till dess ålder behövs först och främst en tillförlitlig uppskattning av graviditetslängden, vilken kan göras baserat på dagen för senaste menstruation, eller utifrån ultraljudsmätning av fostret. Utifrån antagandet att alla foster har liknande tillväxt i tidig graviditet översätts storlek till ålder. Osäkerheten är mindre om ultraljudsdatering görs i första trimestern eftersom den biologiska variationen ökar med graviditetslängden. Exakt graviditetslängd kan dock inte fastställas eftersom tidpunkten för implantation är okänd, liksom den exakta durationen av en graviditet. Utöver en korrekt datering behövs också en metod för att beräkna fostervikten. Ofta baseras sådana ekvationer på ultraljudsmätningar av fostrets huvud, buk och lårben utförda kort tid före förlossningen. Slutligen behövs en referens för normal fostervikt med vilken den beräknade vikten kan jämföras. Att använda sig av en referens skapad utifrån födelsevikter är olämpligt eftersom förtidsbörd är nära förknippat med avvikande tillväxt. Referenser bör istället skapas utifrån mätningar av foster under pågående graviditet. Sammanfattningsvis finns ett flertal felkällor att ta hänsyn till i bedömningen av viktavvikelse. Med upprepade viktskattningar kan fostrets tillväxttakt utvärderas, vilket kan underlätta bedömningen huruvida fostret löper ökad risk för negativa utfall eller ej.

Trots att SGA och tillväxthämning inte är liktydiga, är identifiering av foster som är små för tiden en central del av modern obstetrisk vård. Dessvärre är detektionsgraden av SGA generellt låg. Detta är delvis beroende på i vilken utsträckning ultraljudsbaserade viktskattningar görs. I Sverige finns i nuläget ingen region där ultraljud i tredje trimestern är rutin. Det saknas också starkt stöd för att detta skulle medföra hälsofördelar i en lågriskpopulation. Merparten av de foster som dör intrauterint i slutet av graviditeten är normalviktiga för tiden och där kan foster antingen ha dött innan de hunnit uppvisa avvikande tillväxt, eller ha haft en onormal tillväxttakt men ändå ligga inom normalområdet. Icke desto mindre är det av yttersta vikt att använda pålitliga metoder för uppskattning av graviditetslängd, fostervikt och viktavvikelse. I en

referens som är väl anpassad för en given population bör kurvans percentiler vara samstämmiga med fördelningen av fostervikter. Till exempel bör 10% av friska foster ha en vikt under kurvans tionde percentil. En referens där percentilerna är för högt placerade kommer att klassa en större andel foster som SGA och därmed öka risken för onödiga interventioner. För låga percentiler å andra sidan, medför att detektionsgraden blir lägre och därmed att en förhöjd risk kan förbises. Därför anses det ofta vara lämpligt att använda sig av populationsbaserade referenser. Sådana saknas dock i stora delar av världen, varför stora internationella projekt tagit fram universella tillväxtkurvor. Långvariga diskussioner har förts om huruvida skillnader i fostervikt och födelsevikt mellan olika populationer beror på fysiologiska skillnader, eller om de är uttryck för suboptimala levnadsförhållanden som återspeglas i ökad risk för komplikationer. Oavsett referens har de gränsvärden som definierar SGA stor påverkan både på detektionsgraden och andelen falskt positiva.

Den i Sverige rekommenderade referensen från Maršál et al. publicerades 1996 utifrån mätningar av endast 86 foster. Inga fostermätningar gjordes före v 25 och 24% av studiedeltagarna var rökare. Underlaget kan inte betraktas vara en lågriskpopulation, och avsaknad av data från större delen av andra trimestern medför att bedömningar under denna tid är osäkra.

Infertilitet definieras som ofrivillig barnlöshet trots minst ett års försök att uppnå en graviditet, och berör uppskattningsvis 8-12% av alla par. Bland orsakerna till kvinnlig infertilitet finns exempelvis störningar i äggstockar eller äggledare. Manlig infertilitet beror ofta på testikeldysfunktion. I vissa fall kan ingen orsak identifieras. Assisted Reproductive Technology (ART) är ett samlingsnamn för medicinska åtgärder av spermier och ägg i syfte att åstadkomma en graviditet. Tillgängligheten till ART skiljer sig mycket åt i olika länder. I Sverige har behandlingar de senaste åren gjorts tillgängliga för nya grupper, exempelvis spermiedonation till ensamstående, eller samkönade par. Den senaste förändringen skedde 2019, då kravet på en genetisk koppling till någon av föräldrarna togs bort, vilket möjliggjorde för ART med både donerade ägg och spermier. Därav är gruppen som genomgår behandlingar idag mer heterogen än tidigare. Användningen av ART ökar ständigt och står i dagsläget för cirka 5% av alla födda barn i Sverige. Det sker en kontinuerlig metodutveckling vilket har lett till bättre behandlingsresultat. Den mest påtagliga förändringen på senare år är en ökning av andelen fryst embryotransfer (ET), med motsvarande minskning av andelen färsk ET. Detta har skett parallellt med längre odlingstider och modernare frysteknik. Äldre studier har visat ökade risker för förtidsbörd och SGA efter ART. Senare har man kunnat se skillnader mellan olika metoder, där dessa ökade risker framför allt gäller färsk ET. Fryst ET har å andra sidan ökad risk för överdriven fostertillväxt (LGA). De bakomliggande orsakerna till infertilitet kan också ha påverkan på fostertillväxt, varför det är utmanande att bedöma den direkta effekten på tillväxt av färsk eller fryst ET. ART innebär dessutom ett flertal olika procedurer, involverande såväl kvinnan, som ägg och spermier, varför det är svårt att avgöra vilka moment

i behandlingen som eventuellt orsakar tillväxtskillnader. Hormonella faktorer relaterade till stimulering för utmognad av ägg misstänks vara en bidragande orsak. Andra möjliga orsaker är odlingstid, odlingsmedier, frysmetod och endometriepreparering. Påverkad placentation eller epigenetiska förändringar är två ofta diskuterade föreslagna verkningsmekanismer.

Ett stort antal studier har visat skillnader i graviditetslängd och födelsevikt efter olika typer av ART men tillväxtmönstren under fosterlivet, som leder fram till dessa skillnader är sparsamt studerade.

Att födas SGA innebär ökade risker för neonatala komplikationer, men också ökade hälsorisker på längre sikt. Majoriteten uppvisar tidigt en ökad tillväxttakt och merparten når normal slutlängd. En sådan ”catch-up”-tillväxt medför minskad risk för neurokognitiv påverkan men har visat sig öka risken för övervikt, diabetes mellitus typ 2 och kardiovaskulär sjukdom. Anpassningar som kan ha varit ändamålsenliga under fosterlivet antas kunna medföra en predisposition för sjuklighet på lång sikt. LGA innebär också ökade risker, i perinatalperioden för till exempel asfyxi och förlossningsskador, och för maternella komplikationer. Om hög födelsevikt inte följs av en lägre tillväxttakt, finns ökad risk för bestående övervikt, hypertoni och kardiovaskulär sjukdom. Det finns också studier som visar högre riskprofil för kardiovaskulär sjukdom efter ART, även i frånvaro av andra riskfaktorer. Därmed finns goda skäl att fortsätta följda dessa barns utveckling också efter förlossningen. Ett mindre antal studier talar i huvudsak för en snabb utjämning av skillnader i storlek vid födseln, men färsk och fryst ET har sällan undersökts separat, alternativt har studierna varit små, eller haft risk för bias. Ett fåtal studier har också undersökt könsspecifik påverkan av ART, men resultaten har varit tvetydiga.

Syftet med Studie I var att skapa uppdaterade referenser för fostervikt och tillväxt för den svenska populationen från vecka 12 till 42, utifrån en betydligt större kohort och en mer renodlad lågriskpopulation än tidigare referens. Rekrytering skedde vid 18 mödravårdscentraler på fem olika orter. Inkludering skedde sedan i vecka 12-13 där endast friska kvinnor med regelbunden menstruation erbjöds att delta. För att få en jämn fördelning av mätningar över hela graviditetslängden fördelades studiedeltagarna genom lottning till olika undersökningsscheman för ytterligare fyra ultraljudsundersökningar. Datering gjordes utifrån fostrets biparietaldiameter (BPD), med krav på överensstämmelse med datering enligt sista menstruation på maximalt ± 7 dagar. Vid varje undersökningstillfälle gjordes tre mätningar vardera av olika fosterbiometrier, av huvud, buk och lårben (BPD, HC, MAD, AC och FL). Bakgrundsdata från mödravårdsjournaler och förlossningsutfall från förlossningsjournaler matades också in i en dedikerad studiedatabas. Studiedeltagare där graviditetskomplikationer tillstötte exkluderades, till exempel på grund av förtidsbörd eller graviditetshypertoni. Av 650 inkluderade deltagare fullföljde 583 studien. Utifrån fosterbiometrier beräknades uppskattad fostervikt med Hadlocks tredje formel inkluderande BPD, HC, AC och FL. Med en longitudinell statistisk

metod med hänsyn tagen till upprepade mätningar av samma foster, skapades generella och könsspecifika referenser för medianvärde och varians uttryckt som percentiler och standarddeviationer. Den nya referensen skilde sig från den i dagsläget i Sverige rekommenderade referensen och tre andra relevanta referenser, med varierande grad av skillnader i percentilernas placering vid olika graviditetslängder.

Syftet med Studie II var att undersöka påverkan av färsk och fryst ET på fostertillväxt i jämförelse med NC i en retrospektiv kohortstudie. Populationen baserades på Graviditetsregistret, innefattande bakgrundsdata, ultraljudsundersökningar, graviditetskomplikationer och förlossningsutfall för cirka 90% av Sveriges alla graviditeter som lett till förlossning från vecka 22. Till dessa data länkades uppgifter från ett flertal andra register vilket gav tillgång till information om ART, utbildningsnivå, födelseland och läkemedelsförskrivning. Flerbördsgraviditeter, som i sig innebär en stor risk för påverkad fostertillväxt, uteslöts. Registrerade data från ett ultraljud i första trimestern var en förutsättning för deltagande för NC-graviditeter, för att samstämmighet skulle kunna bekräftas mellan datering utifrån sista menstruation och ultraljudsdatering (± 14 dagar). ART-graviditeter daterades utifrån datum för ET och antal odlingsdagar. Det huvudsakliga utfallet var en kombinerad viktvariabel, baserad både på uppskattad fostervikt via ultraljud och födelsevikt.

Från den ursprungliga kohorten på 501 545 graviditeter återstod 196 008 efter exkluderingar, varav 10 970 färsk ET, 6520 fryst ET, och 178 518 NC. Med en longitudinell statistisk metod som tar hänsyn till upprepade, och därmed beroende, mätningar modellerades skillnader i fostervikt mellan de tre grupperna. Justeringar gjordes i modellen för relevanta störfaktorer. Vid startpunkten, 17 veckor, fanns en minimal skillnad mellan grupperna. Både färsk och fryst ET visade därefter en initialt högre tillväxttakt och var under resterande delen av andra trimestern större än NC. Tillväxttakten övergick dock till att vara lägre än för NC redan under andra trimestern, mest uttalat för färsk ET som i slutet av graviditeten hade lägre vikt än NC. Fryst ET var trots lägre tillväxttakt än NC fortfarande större än dessa i fullgången tid. Vid jämförelse av födelsevikter var risken för SGA högre för färsk jämfört med fryst ET, medan risken för LGA var lägre.

Syftet med Studie III, var liksom i Studie II att jämföra fostertillväxt mellan färsk och fryst ET och NC. Detta var en prospektiv multicenterstudie med ett studieupplägg mycket snarlikt studie I. Inklusionskriterierna var dock inte lika strikta, men flerbördsgraviditet, äggdonation och allvarliga fosteravvikelser uteslöts. Rekrytering gjordes vid fertilitetsenheterna vid Akademiska sjukhuset och Universitetssjukhuset i Örebro. Protokollet för undersökningar var likartat det i Studie I, vars deltagare agerade referenspopulation i denna studie. Enligt powerberäkningen planerades att 175 deltagare från vardera färsk och fryst ET skulle inkluderas. Inklusionstakten var dessvärre lägre än förväntat, framför allt för färsk ET, och 30/9 2024 beslutades att i förtid stoppa ytterligare inkluderingar. Då hade 87 färsk och 175 fryst ET inkluderats. Efter

exkluderingar återstod 82 respektive 175 graviditeter. Med en statistisk metod snarlik den i Studie II modellerades tillväxten av olika biometrier och uppskattad fostervikt, och skillnaderna mellan dessa beräknades. Detaljerade data från patientjournaler, inklusive journaler från fertilitetsenheterna möjliggjorde justering för relevanta störfaktorer.

I tidig graviditet tenderade ART-graviditeter generellt att vara något större än NC, men detta var tydligare för fryst ET. I motsats till Studie II sågs därefter ingen ökad tillväxttakt, snarare en successivt avtagande tillväxt. Även i denna studie var tillväxttakten lägre för färsk ET och i fullgången tid var uppskattad fostervikt lägre än hos både fryst ET och NC. Ingen skillnad sågs i fullgången tid mellan fryst ET och NC. Vid en jämförelse uppdelat på kön sågs en större påverkan på flickfoster, där färsk ET i fullgången tid var påtagligt mindre än fryst ET och NC.

Syftet med Studie IV var att undersöka skillnader i tillväxt från födseln fram till fem års ålder hos barn födda efter ART och NC. Det var en populationsbaserad retrospektiv kohortstudie utgående från Uppsala Mor-Barn-kohort där samtliga barn födda på Akademiska sjukhuset med efterföljande barnahälsovårdskontroller inom Region Uppsala mellan år 2000 och 2015 ingår. Rutinmässigt utförda mätningar av barn vid födseln, 18 månader samt 3, 4 och 5 års ålder ingår. Till databasen finns också länkad data från flertalet nationella register. För denna studie länkades också information från Nationellt kvalitetsregister för assisterad befruktning, med uppgifter om infertilitet och ART sedan 2007. Med anledning av detta begränsades kohorten till barn födda från 2007, för att möjliggöra detaljerad information om infertilitetsbehandlingar, till 2014 för att ha data från åtminstone mätningar vid 18 månaders ålder. Barn från flerbördsgraviditeter och barn födda före vecka 37+0 exkluderades. Utgående från 26 979 födda barn återstod efter exkluderingar 18 015, varav 517 efter färsk och 284 efter fryst ET, samt 17 214 efter NC. En statistisk metod, snarlik den för studie II och III, användes för denna studie och justeringar gjordes för identifierade tillgängliga störfaktorer. Som förväntat var barn efter färsk ET kortare och lättare än NC vid födseln, medan barn efter fryst ET var längre och tyngre. Tidiga skillnader i tillväxttakt resulterade i att i princip alla skillnader var utjämnade redan vid 18 månaders ålder, bortsett från lägre BMI hos barn efter färsk ET. Vid senare tidpunkter sågs inga statistiskt signifikanta skillnader. När uppdelning gjordes utifrån kön noterades att flickor efter fryst ET var längre, men inte tyngre, än flickor efter NC vid tre till fem års ålder.

Sammanfattningsvis har nya referenser för fostertillväxt och vikt skapats. Genom en tillräckligt stor studiepopulation och uppdaterade statistiska metoder finns förutsättningar för referensen att passa den svenska populationen. Den behöver dock utvärderas i framtida studier. Först därefter kan en bedömning göras av vilken referens som är bäst lämpad, och vilka gränsvärden som bör användas i klinisk praktik.

Tillväxtmönstren skiljer sig åt mellan färsk ET, fryst ET och NC redan från tidig graviditet. Mycket talar för en direkt effekt av typ av ART på fostertillväxt men det är utifrån denna typ av studier svårt att säkert identifiera de underliggande mekanismerna, liksom att helt isolera effekten av ART från bakgrundsfaktorer. Skillnaderna i medelvikt mellan grupperna är i många fall relativt liten men ökade risker för SGA respektive LGA noterades . Trots att skillnaderna i längd och vikt vid födseln till största delen utjämnades postnatalt kan risken för framtida hälsoproblem vara fortsatt förhöjd. Fortsatt övervakning av hälsoeffekter på kort och lång sikt för barn födda efter ART är angelägen.

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References

1. Gardosi J, Madurasinghe V, Williams M, Malik A, Francis A. Maternal and fetal risk factors for stillbirth: population based study. BMJ. 2013;346:f108-f.
2. Figueras F, Gratacos E. An integrated approach to fetal growth restriction. Best Practice & Research Clinical Obstetrics & Gynaecology. 2017;38:48-58.
3. Scifres CM. Short- and Long-Term Outcomes Associated with Large for Gestational Age Birth Weight. Obstet Gynecol Clin North Am. 2021;48(2):325-37.
4. Lunde A, Melve KK, Gjessing HK, Skjaerven R, Irgens LM. Genetic and environmental influences on birth weight, birth length, head circumference, and gestational age by use of population-based parent-offspring data. Am J Epidemiol. 2007;165(7):734-41.
5. Hanson MA, Gluckman PD. Early developmental conditioning of later health and disease: physiology or pathophysiology? Physiol Rev. 2014;94(4):1027-76.
6. Mullis PE, Tonella P. Regulation of fetal growth: consequences and impact of being born small. Best Pract Res Clin Endocrinol Metab. 2008;22(1):173-90.
7. Murphy VE, Smith R, Giles WB, Clifton VL. Endocrine regulation of human fetal growth: the role of the mother, placenta, and fetus. Endocr Rev. 2006;27(2):141-69.
8. Kaufmann P, Black S, Huppertz B. Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. Biol Reprod. 2003;69(1):1-7.
9. Burton GJ, Redman CW, Roberts JM, Moffett A. Pre-eclampsia: pathophysiology and clinical implications. BMJ. 2019;366:l2381.
10. Marsál K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. Acta Paediatr. 1996;85(7):843-8.
11. Wikland KA, Luo ZC, Niklasson A, Karlberg J. Swedish population-based longitudinal reference values from birth to 18 years of age for height, weight and head circumference. Acta Paediatr. 2002;91(7):739-54.
12. Lees C, Marlow N, Arabin B, Bilardo CM, Brezinka C, Derks JB, et al. Perinatal morbidity and mortality in early-onset fetal growth restriction: cohort outcomes of the trial of randomized umbilical and fetal flow in Europe (TRUFFLE). Ultrasound Obstet Gynecol. 2013;42(4):400-8.
13. Gordijn SJ, Beune IM, Thilaganathan B, Papageorghiou A, Baschat AA, Baker PN, et al. Consensus definition of fetal growth restriction: a Delphi procedure. Ultrasound Obstet Gynecol. 2016;48(3):333-9.
14. Figueras F, Caradeux J, Crispi F, Eixarch E, Peguero A, Gratacos E. Diagnosis and surveillance of late-onset fetal growth restriction. Am J Obstet Gynecol. 2018;218(2S):S790-S802 e1.

15. Nordman H, Jääskeläinen J, Voutilainen R. Birth Size as a Determinant of Cardiometabolic Risk Factors in Children. Horm Res Paediatr. 2020;93(3):144-53.
16. Hertting E, Herling L, Lindqvist PG, Wiberg-Itzel E. Importance of antenatal identification of small for gestational age fetuses on perinatal and childhood outcomes: A register-based cohort study. Acta Obstet Gynecol Scand. 2024;103(1):42-50.
17. Gabbay-Benziv R, Aviram A, Hadar E, Chen R, Bardin R, Wiznitzer A, et al. Pregnancy outcome after false diagnosis of fetal growth restriction. The Journal of Maternal-Fetal & Neonatal Medicine. 2017;30(16):1916-9.
18. Kieler H, Axelsson O, Nilsson S, Waldenström U. Comparison of ultrasonic measurement of biparietal diameter and last menstrual period as a predictor of day of delivery in women with regular 28 day-cycles. Acta Obstet Gynecol Scand. 1993;72(5):347-9.
19. Cnattingius S, Källén K, Sandström A, Rydberg H, Månsson H, Stephansson O, et al. The Swedish medical birth register during five decades: documentation of the content and quality of the register. Eur J Epidemiol. 2023;38(1):109-20.
20. Saltvedt S, Almstrom H, Kublickas M, Reilly M, Valentin L, Grunewald C. Ultrasound dating at 12-14 or 15-20 weeks of gestation? A prospective cross-validation of established dating formulae in a population of in-vitro fertilized pregnancies randomized to early or late dating scan. Ultrasound Obstet Gynecol. 2004;24(1):42-50.
21. Johnsen SL, Wilsgaard T, Rasmussen S, Hanson MA, Godfrey KM, Kiserud T. Fetal size in the second trimester is associated with the duration of pregnancy, small fetuses having longer pregnancies. BMC Pregnancy Childbirth. 2008;8:25.
22. Kullinger M, Wesström J, Kieler H, Skalkidou A. Maternal and fetal characteristics affect discrepancies between pregnancy-dating methods: a population-based cross-sectional register study. Acta Obstet Gynecol Scand. 2017;96(1):86-95.
23. Mathewlynn S, Kitmiridou D, Impey L, Ioannou C. The impact of late pregnancy dating on the detection of fetal growth restriction at term. Acta Obstet Gynecol Scand. 2024.
24. Persson PH, Weldner BM. Intra-Uterine Weight Curves Obtained by Ultrasound. Acta Obstetricia et Gynecologica Scandinavica. 1986;65(2):169-73.
25. SFOG Fetometry Guideline. 2019.
26. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements--a prospective study. Am J Obstet Gynecol. 1985;151(3):333-7.
27. Hammami A, Mazer Zumaeta A, Syngelaki A, Akolekar R, Nicolaides KH. Ultrasonographic estimation of fetal weight: development of new model and assessment of performance of previous models. Ultrasound in Obstetrics & Gynecology. 2018;52(1):35-43.
28. Lindström L, Cnattingius S, Axelsson O, Granfors M. Accuracy and precision of sonographic fetal weight estimation in Sweden. Acta Obstet Gynecol Scand. 2023;102(6):699-707.
29. Salomon LJ, Bernard JP, Ville Y. Estimation of fetal weight: reference range at 20–36 weeks' gestation and comparison with actual birth-weight reference range. Ultrasound in Obstetrics & Gynecology. 2007;29(5):550-5.

30. Ioannou C, Talbot K, Ohuma E, Sarris I, Villar J, Conde-Agudelo A, et al. Systematic review of methodology used in ultrasound studies aimed at creating charts of fetal size. Bjog. 2012;119(12):1425-39.
31. Ohuma EO, Altman DG. Design and other methodological considerations for the construction of human fetal and neonatal size and growth charts. Stat Med. 2019;38(19):3527-39.
32. Nicolaides KH, Wright D, Syngelaki A, Wright A, Akolekar R. Fetal Medicine Foundation fetal and neonatal population weight charts. Ultrasound in Obstetrics & Gynecology. 2018;52(1):44-51.
33. Kierans WJ, Joseph KS, Luo ZC, Platt R, Wilkins R, Kramer MS. Does one size fit all? The case for ethnic-specific standards of fetal growth. BMC Pregnancy Childbirth. 2008;8:1.
34. Thilaganathan B. Ultrasound fetal weight estimation at term may do more harm than good. Ultrasound in Obstetrics & Gynecology. 2018;52(1):5-8.
35. Papageorghiou AT, Ohuma EO, Altman DG, Todros T, Cheikh Ismail L, Lambert A, et al. International standards for fetal growth based on serial ultrasound measurements: the Fetal Growth Longitudinal Study of the INTERGROWTH-21st Project. Lancet. 2014;384(9946):869-79.
36. Stirnemann J, Villar J, Salomon LJ, Ohuma E, Ruyan P, Altman DG, et al. International estimated fetal weight standards of the INTERGROWTH-21(st) Project. Ultrasound Obstet Gynecol. 2017;49(4):478-86.
37. Kiserud T, Piaggio G, Carroli G, Widmer M, Carvalho J, Neerup Jensen L, et al. The World Health Organization Fetal Growth Charts: A Multinational Longitudinal Study of Ultrasound Biometric Measurements and Estimated Fetal Weight. PLoS Med. 2017;14(1):e1002220.
38. Johnsen SL, Rasmussen S, Wilsgaard T, Sollien R, Kiserud T. Longitudinal reference ranges for estimated fetal weight. Acta Obstet Gynecol Scand. 2006;85(3):286-97.
39. Johnsen SL, Wilsgaard T, Rasmussen S, Sollien R, Kiserud T. Longitudinal reference charts for growth of the fetal head, abdomen and femur. Eur J Obstet Gynecol Reprod Biol. 2006;127(2):172-85.
40. Combs CA, Jaekle RK, Rosenn B, Pope M, Miodovnik M, Siddiqi TA. Sonographic estimation of fetal weight based on a model of fetal volume. Obstet Gynecol. 1993;82(3):365-70.
41. Buck Louis GM, Grewal J, Albert PS, Sciscione A, Wing DA, Grobman WA, et al. Racial/ethnic standards for fetal growth: the NICHD Fetal Growth Studies. Am J Obstet Gynecol. 2015;213(4):449.e1-.e41.
42. Grantz KL, Hediger ML, Liu D, Buck Louis GM. Fetal growth standards: the NICHD fetal growth study approach in context with INTERGROWTH-21st and the World Health Organization Multicentre Growth Reference Study. American Journal of Obstetrics and Gynecology. 2018;218(2, Supplement):S641-S55.e28.
43. Hanley GE, Janssen PA. Ethnicity-specific birthweight distributions improve identification of term newborns at risk for short-term morbidity. Am J Obstet Gynecol. 2013;209(5):428.e1-6.
44. Gardosi J, Hugh O. Stillbirth risk and smallness for gestational age according to Hadlock, INTERGROWTH-21st, WHO, and GROW fetal weight standards: analysis by maternal ethnicity and body mass index. Am J Obstet Gynecol. 2023;229(5):547.e1-.e13.
45. Hugh O, Gardosi J. Fetal weight projection model to define growth velocity and validation against pregnancy outcome in a cohort of serially scanned pregnancies. Ultrasound Obstet Gynecol. 2022;60(1):86-95.

46. Morales-Roselló J, Buongiorno S, Loscalzo G, Scarinci E, Giménez Roca L, Cañada Martínez AJ, et al. Birth-weight differences at term are explained by placental dysfunction and not by maternal ethnicity. Study in newborns of first generation immigrants. J Matern Fetal Neonatal Med. 2022;35(8):1419-25.
47. Cheng YKY, Lu J, Leung TY, Chan YM, Sahota DS. Prospective assessment of INTERGROWTH-21(st) and World Health Organization estimated fetal weight reference curves. Ultrasound Obstet Gynecol. 2018;51(6):792-8.
48. Heude B, Le Guern M, Forhan A, Scherdel P, Kadawathagedara M, Dufourg MN, et al. Are selection criteria for healthy pregnancies responsible for the gap between fetal growth in the French national Elfe birth cohort and the Intergrowth-21st fetal growth standards? Paediatr Perinat Epidemiol. 2019;33(1):47-56.
49. Vieira MC, Relph S, Persson M, Seed PT, Pasupathy D. Determination of birth-weight centile thresholds associated with adverse perinatal outcomes using population, customised, and Intergrowth charts: A Swedish population-based cohort study. PLoS Med. 2019;16(9):e1002902.
50. World Health Organization (2019). International Statistical Classification of Diseases and Related Health Problems (11th ed.). https://icd.who.int/.
51. Vander Borght M, Wyns C. Fertility and infertility: Definition and epidemiology. Clin Biochem. 2018;62:2-10.
52. Vannuccini S, Clifton VL, Fraser IS, Taylor HS, Critchley H, Giudice LC, et al. Infertility and reproductive disorders: impact of hormonal and inflammatory mechanisms on pregnancy outcome. Hum Reprod Update. 2016;22(1):104-15.
53. Persson S, Elenis E, Turkmen S, Kramer MS, Yong E-L, Sundström-Poromaa I. Fecundity among women with polycystic ovary syndrome (PCOS)—a population-based study. Human Reproduction. 2019;34(10):2052-60.
54. Palomba S, de Wilde MA, Falbo A, Koster MP, La Sala GB, Fauser BC. Pregnancy complications in women with polycystic ovary syndrome. Hum Reprod Update. 2015;21(5):575-92.
55. Valgeirsdottir H, Sundström Poromaa I, Kunovac Kallak T, Vanky E, Akhter T, Roos N, et al. Polycystic ovary syndrome and extremely preterm birth: A nationwide register-based study. PLoS One. 2021;16(2):e0246743.
56. Stephansson O, Kieler H, Granath F, Falconer H. Endometriosis, assisted reproduction technology, and risk of adverse pregnancy outcome. Hum Reprod. 2009;24(9):2341-7.
57. Horton J, Sterrenburg M, Lane S, Maheshwari A, Li TC, Cheong Y. Reproductive, obstetric, and perinatal outcomes of women with adenomyosis and endometriosis: a systematic review and meta-analysis. Hum Reprod Update. 2019;25(5):592-632.
58. Hu S, Xu B, Huang B, Jin L. The impact of male infertility or intracytoplasmic sperm injection technique on perinatal outcomes. J Matern Fetal Neonatal Med. 2022;35(4):685-91.
59. Smeenk J, Wyns C, De Geyter C, Kupka M, Bergh C, Cuevas Saiz I, et al. ART in Europe, 2019: results generated from European registries by ESHRE†. Hum Reprod. 2023;38(12):2321-38.
60. Q-IVF. Annual Report 2024, National Quality Registry for Assisted Reproduction. 2024.
61. Glujovsky D, Farquhar C, Quinteiro Retamar AM, Alvarez Sedo CR, Blake D. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. Cochrane Database Syst Rev. 2016(6):Cd002118.

62. Rienzi L, Gracia C, Maggiulli R, LaBarbera AR, Kaser DJ, Ubaldi FM, et al. Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. Hum Reprod Update. 2017;23(2):139-55.
63. Zaat T, Zagers M, Mol F, Goddijn M, van Wely M, Mastenbroek S. Fresh versus frozen embryo transfers in assisted reproduction. Cochrane Database Syst Rev. 2021;2(2):Cd011184.
64. Maheshwari A, Bell JL, Bhide P, Brison D, Child T, Chong HY, et al. Elective freezing of embryos versus fresh embryo transfer in IVF: a multicentre randomized controlled trial in the UK (E-Freeze). Hum Reprod. 2022.
65. Asserhøj LL, Spangmose AL, Aaris Henningsen AK, Clausen TD, Ziebe S, Jensen RB, et al. Adverse obstetric and perinatal outcomes in 1,136 singleton pregnancies conceived after programmed frozen embryo transfer (FET) compared with natural cycle FET. Fertil Steril. 2021;115(4):947-56.
66. Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. Obstet Gynecol. 2004;103(3):551-63.
67. McGovern PG, Llorens AJ, Skurnick JH, Weiss G, Goldsmith LT. Increased risk of preterm birth in singleton pregnancies resulting from in vitro fertilization-embryo transfer or gamete intrafallopian transfer: a meta-analysis. Fertil Steril. 2004;82(6):1514-20.
68. Maheshwari A, Raja EA, Bhattacharya S. Obstetric and perinatal outcomes after either fresh or thawed frozen embryo transfer: an analysis of 112,432 singleton pregnancies recorded in the Human Fertilisation and Embryology Authority anonymized dataset. Fertil Steril. 2016;106(7):1703-8.
69. Sazonova A, Kallen K, Thurin-Kjellberg A, Wennerholm UB, Bergh C. Obstetric outcome in singletons after in vitro fertilization with cryopreserved/thawed embryos. Hum Reprod. 2012;27(5):1343-50.
70. Maheshwari A, Pandey S, Amalraj Raja E, Shetty A, Hamilton M, Bhattacharya S. Is frozen embryo transfer better for mothers and babies? Can cumulative meta-analysis provide a definitive answer? Hum Reprod Update. 2018;24(1):35-58.
71. Pelkonen S, Koivunen R, Gissler M, Nuojua-Huttunen S, Suikkari AM, Hyden-Granskog C, et al. Perinatal outcome of children born after frozen and fresh embryo transfer: the Finnish cohort study 1995-2006. Hum Reprod. 2010;25(4):914-23.
72. Pinborg A, Loft A, Aaris Henningsen AK, Rasmussen S, Andersen AN. Infant outcome of 957 singletons born after frozen embryo replacement: the Danish National Cohort Study 1995-2006. Fertil Steril. 2010;94(4):1320-7.
73. Yang H, Miao H, Yin M, Wang Y, Zhao D, Yang M, et al. The difference in early trimester fetal growth between singletons after frozen embryo transfer and fresh embryo transfer. AJOG Global Reports. 2024;4(2):100334.
74. Mitta K, Tsakiridis I, Giougi E, Mamopoulos A, Kalogiannidis I, Dagklis T, et al. Comparison of Fetal Crown-Rump Length Measurements between Thawed and Fresh Embryo Transfer. J Clin Med. 2024;13(9).
75. Cavoretto PI, Farina A, Girardelli S, Gaeta G, Spinillo S, Morano D, et al. Greater fetal crown-rump length growth with the use of in vitro fertilization or intracytoplasmic sperm injection conceptions after thawed versus fresh blastocyst transfers: secondary analysis of a prospective cohort study. Fertil Steril. 2021;116(1):147-56.

76. Ginod P, Choux C, Barberet J, Rousseau T, Bruno C, Khallouk B, et al. Singleton fetal growth kinetics depend on the mode of conception. Fertil Steril. 2018;110(6):1109-17 e2.
77. Cavoretto PI, Farina A, Gaeta G, Seidenari A, Pozzoni M, Spinillo S, et al. Greater estimated fetal weight and birth weight in IVF/ICSI pregnancies after thawed as compared with fresh blastocyst transfer: prospective cohort study with novel unified modeling methodology. Ultrasound Obstet Gynecol. 2021.
78. Terho AM, Pelkonen S, Opdahl S, Romundstad LB, Bergh C, Wennerholm UB, et al. High birth weight and large-for-gestational-age in singletons born after frozen compared to fresh embryo transfer, by gestational week: a Nordic register study from the CoNARTaS group. Hum Reprod. 2021;36(4):1083-92.
79. Ding Q, Wang Y, Suo L, Niu Y, Zhao D, Yu Y, et al. The gestational age-specific difference in birthweight between singletons born after fresh and frozen embryo transfer: A cohort study. Acta Obstet Gynecol Scand. 2023;102(3):323-33.
80. Mani S, Ghosh J, Coutifaris C, Sapienza C, Mainigi M. Epigenetic changes and assisted reproductive technologies. Epigenetics. 2020;15(1-2):12-25.
81. Rafael F, Robles GM, Navarro AT, Garrido N, Garcia-Velasco JA, Bosch E, et al. Perinatal outcomes in children born after fresh or frozen embryo transfer using donated oocytes. Human Reproduction. 2022;37(7):1642-51.
82. Vidal M, Vellvé K, González-Comadran M, Robles A, Prat M, Torné M, et al. Perinatal outcomes in children born after fresh or frozen embryo transfer: a Catalan cohort study based on 14,262 newborns. Fertil Steril. 2017;107(4):940-7.
83. Pereira N, Elias RT, Christos PJ, Petrini AC, Hancock K, Lekovich JP, et al. Supraphysiologic estradiol is an independent predictor of low birth weight in full-term singletons born after fresh embryo transfer. Human Reproduction. 2017;32(7):1410-7.
84. Mak W, Kondapalli LA, Celia G, Gordon J, DiMattina M, Payson M. Natural cycle IVF reduces the risk of low birthweight infants compared with conventional stimulated IVF. Hum Reprod. 2016;31(4):789-94.
85. Duan CC, Li C, He YC, Xu JJ, Shi CY, Hu HT, et al. Oocyte exposure to supraphysiological estradiol during ovarian stimulation increased the risk of adverse perinatal outcomes after frozen-thawed embryo transfer: a retrospective cohort study. J Dev Orig Health Dis. 2020;11(4):392-402.
86. Cai J, Liu L, Xu Y, Liu Z, Jiang X, Li P, et al. Supraphysiological estradiol level in ovarian stimulation cycles affects the birthweight of neonates conceived through subsequent frozen-thawed cycles: a retrospective study. Bjog. 2019;126(6):711-8.
87. Dumoulin JC, Land JA, Van Montfoort AP, Nelissen EC, Coonen E, Derhaag JG, et al. Effect of in vitro culture of human embryos on birthweight of newborns. Hum Reprod. 2010;25(3):605-12.
88. Nelissen EC, Van Montfoort AP, Smits LJ, Menheere PP, Evers JL, Coonen E, et al. IVF culture medium affects human intrauterine growth as early as the second trimester of pregnancy. Hum Reprod. 2013;28(8):2067-74.
89. Lin S, Li M, Lian Y, Chen L, Liu P. No effect of embryo culture media on birthweight and length of newborns. Hum Reprod. 2013;28(7):1762-7.
90. Pier BD, Roshong A, Santoro N, Sammel MD. Association of duration of embryo culture with risk of large for gestational age delivery in cryopreserved embryo transfer cycles. Fertil Steril. 2024;121(5):814-23.

91. Zhu J, Lin S, Li M, Chen L, Lian Y, Liu P, et al. Effect of in vitro culture period on birthweight of singleton newborns. Hum Reprod. 2014;29(3):448-54.
92. Hesters L, Sermondade N, Lambert C, Pouly JL, Pereira B, Lucas C, et al. Is large for gestational age in singletons born after frozen embryo transfer associated with freezing technique or endometrial preparation protocol? A longitudinal national French study. Hum Reprod. 2024;39(4):724-32.
93. Rosalik K, Carson S, Pilgrim J, Luizzi J, Levy G, Heitmann R, et al. Effects of different frozen embryo transfer regimens on abnormalities of fetal weight: a systematic review and meta-analysis. Hum Reprod Update. 2021.
94. Zhang Y, Yichun G. P-126 The impact of ICSI versus IVF on the reproductive outcomes of couples with non-male factor infertility and frozen-thawed embryo transfer cycles. Human Reproduction. 2024;39(Supplement_1).
95. Ginström Ernstad E, Spangmose AL, Opdahl S, Henningsen AA, Romundstad LB, Tiitinen A, et al. Perinatal and maternal outcome after vitrification of blastocysts: a Nordic study in singletons from the CoNARTaS group. Hum Reprod. 2019;34(11):2282-9.
96. Li Z, Wang YA, Ledger W, Edgar DH, Sullivan EA. Clinical outcomes following cryopreservation of blastocysts by vitrification or slow freezing: a population-based cohort study. Hum Reprod. 2014;29(12):2794-801.
97. Westvik-Johari K, Romundstad LB, Lawlor DA, Bergh C, Gissler M, Henningsen AA, et al. Separating parental and treatment contributions to perinatal health after fresh and frozen embryo transfer in assisted reproduction: A cohort study with within-sibship analysis. PLoS Med. 2021;18(6):e1003683.
98. Anav M, Phillips S, Ferrieres-Hoa A, Gala A, Fournier A, Vincens C, et al. Cryopreserved embryo replacement is associated with higher birthweight compared with fresh embryo: multicentric sibling embryo cohort study. Sci Rep. 2019;9(1):13402.
99. Ginström Ernstad E, Wennerholm U-B, Khatibi A, Petzold M, Bergh C. Neonatal and maternal outcome after frozen embryo transfer: Increased risks in programmed cycles. American Journal of Obstetrics and Gynecology. 2019;221(2):126.e1-.e18.
100. Verlohren S, Melchiorre K, Khalil A, Thilaganathan B. Uterine artery Doppler, birth weight and timing of onset of pre-eclampsia: providing insights into the dual etiology of late-onset pre-eclampsia. Ultrasound in Obstetrics & Gynecology. 2014;44(3):293-8.
101. Masini G, Foo LF, Tay J, Wilkinson IB, Valensise H, Gyselaers W, et al. Preeclampsia has two phenotypes which require different treatment strategies. Am J Obstet Gynecol. 2022;226(2s):S1006-s18.
102. Valdimarsdottir R, Vanky E, Elenis E, Lindström L, Junus K, Jonsson M, et al. Polycystic ovary syndrome and risk of pre-eclampsia: A national register-based cohort study. Bjog. 2024;131(7):985-95.
103. Kornfield MS, Gurley SB, Vrooman LA. Increased Risk of Preeclampsia with Assisted Reproductive Technologies. Curr Hypertens Rep. 2023;25(9):251-61.
104. Cavoretto PI, Farina A, Gaeta G, Sigismondi C, Spinillo S, Casiero D, et al. Uterine artery Doppler in singleton pregnancies conceived after in-vitro fertilization or intracytoplasmic sperm injection with fresh vs frozen blastocyst transfer: longitudinal cohort study. Ultrasound Obstet Gynecol. 2020;56(4):603-10.

105. Cortessis VK, Azadian M, Buxbaum J, Sanogo F, Song AY, Sriprasert I, et al. Comprehensive meta-analysis reveals association between multiple imprinting disorders and conception by assisted reproductive technology. J Assist Reprod Genet. 2018;35(6):943-52.
106. Schaub AM, Gonzalez TL, Dorfman AE, Novoa AG, Hussaini RA, Harakuni PM, et al. A systematic review of genome-wide analyses of methylation changes associated with assisted reproductive technologies in various tissues. Fertil Steril. 2024;121(1):80-94.
107. Saenger P, Czernichow P, Hughes I, Reiter EO. Small for gestational age: short stature and beyond. Endocr Rev. 2007;28(2):219-51.
108. Lindström L, Wikström AK, Bergman E, Lundgren M. Born Small for Gestational Age and Poor School Performance - How Small Is Too Small? Horm Res Paediatr. 2017;88(3-4):215-23.
109. Sacchi C, Marino C, Nosarti C, Vieno A, Visentin S, Simonelli A. Association of Intrauterine Growth Restriction and Small for Gestational Age Status With Childhood Cognitive Outcomes: A Systematic Review and Meta-analysis. JAMA Pediatr. 2020;174(8):772-81.
110. Johnsson IW, Haglund B, Ahlsson F, Gustafsson J. A high birth weight is associated with increased risk of type 2 diabetes and obesity. Pediatr Obes. 2015;10(2):77-83.
111. Lei X, Zhao D, Huang L, Luo Z, Zhang J, Yu X, et al. Childhood Health Outcomes in Term, Large-for-Gestational-Age Babies With Different Postnatal Growth Patterns. Am J Epidemiol. 2018;187(3):507-14.
112. Bay B, Lyngsø J, Hohwü L, Kesmodel US. Childhood growth of singletons conceived following in vitro fertilisation or intracytoplasmic sperm injection: a systematic review and meta-analysis. Bjog. 2019;126(2):158-66.
113. Terho AM, Pelkonen S, Toikkanen R, Koivurova S, Salo J, Nuojua-Huttunen S, et al. Childhood growth of term singletons born after frozen compared with fresh embryo transfer. Reprod Biomed Online. 2021;43(4):719-26.
114. Magnus MC, Wilcox AJ, Fadum EA, Gjessing HK, Opdahl S, Juliusson PB, et al. Growth in children conceived by ART. Hum Reprod. 2021;36(4):1074-82.
115. Asserhøj LL, Mizrak I, Heldarskard GF, Clausen TD, Hoffmann ER, Greisen G, et al. Childhood BMI after ART with frozen embryo transfer. Hum Reprod. 2023;38(8):1578-89.
116. Green MP, Mouat F, Miles HL, Hopkins SA, Derraik JG, Hofman PL, et al. Phenotypic differences in children conceived from fresh and thawed embryos in in vitro fertilization compared with naturally conceived children. Fertil Steril. 2013;99(7):1898-904.
117. Elhakeem A, Taylor AE, Inskip HM, Huang J, Tafflet M, Vinther JL, et al. Association of Assisted Reproductive Technology With Offspring Growth and Adiposity From Infancy to Early Adulthood. JAMA Netw Open. 2022;5(7):e2222106.
118. Terho AM, Tiitinen A, Salo J, Martikainen H, Gissler M, Pelkonen S. Growth of singletons born after frozen embryo transfer until early adulthood: a Finnish register study. Hum Reprod. 2024;39(3):604-11.
119. Cui L, Zhao M, Zhang Z, Zhou W, Lv J, Hu J, et al. Assessment of Cardiovascular Health of Children Ages 6 to 10 Years Conceived by Assisted Reproductive Technology. JAMA Netw Open. 2021;4(11):e2132602.

120. Guo XY, Liu XM, Jin L, Wang TT, Ullah K, Sheng JZ, et al. Cardiovascular and metabolic profiles of offspring conceived by assisted reproductive technologies: a systematic review and meta-analysis. Fertil Steril. 2017;107(3):622-31 e5.
121. Meister TA, Rimoldi SF, Soria R, von Arx R, Messerli FH, Sartori C, et al. Association of Assisted Reproductive Technologies With Arterial Hypertension During Adolescence. J Am Coll Cardiol. 2018;72(11):1267-74.
122. Asserhøj LL, Mizrak I, Lebech Kjaer AS, Clausen TD, Hoffmann ER, Greisen G, et al. Blood pressure and lipid profiles in children born after ART with frozen embryo transfer. Hum Reprod Open. 2024;2024(2):hoae016.
123. Zhang Y, Dai K, Chen X, Cui L, Chen ZJ. Association between being large for gestational age and cardiovascular metabolic health in children conceived from assisted reproductive technology: a prospective cohort study. BMC Med. 2024;22(1):203.
124. Norrman E, Petzold M, Clausen TD, Henningsen AK, Opdahl S, Pinborg A, et al. Type 1 diabetes in children born after assisted reproductive technology: a register-based national cohort study. Hum Reprod. 2020;35(1):221-31.
125. Pinborg A, Wennerholm UB, Bergh C. Long-term outcomes for children conceived by assisted reproductive technology. Fertil Steril. 2023;120(3 Pt 1):449-56.
126. Skogsdal Y CP, Elvander C, Hed C, Ageheim M, Algovik M, Petersson K, Bjelke M, Granfors M, Svanvik T on behalf of the Pregnancy Register. Pregnancy Register's Annual Report 2023 2.0. 2023.
127. Tennant PWG, Murray EJ, Arnold KF, Berrie L, Fox MP, Gadd SC, et al. Use of directed acyclic graphs (DAGs) to identify confounders in applied health research: review and recommendations. Int J Epidemiol. 2021;50(2):620-32.
128. Drukker L, Droste R, Chatelain P, Noble JA, Papageorghiou AT. Expected-value bias in routine third-trimester growth scans. Ultrasound in Obstetrics & Gynecology. 2020;55(3):375-82.
129. Ohuma EO, Altman DG. Statistical methodology for constructing gestational age-related charts using cross-sectional and longitudinal data: The INTERGROWTH-21(st) project as a case study. Stat Med. 2019;38(19):3507-26.
130. Litzky JF, Boulet SL, Esfandiari N, Zhang Y, Kissin DM, Theiler RN, et al. Effect of frozen/thawed embryo transfer on birthweight, macrosomia, and low birthweight rates in US singleton infants. American Journal of Obstetrics and Gynecology. 2018;218(4):433.e1-.e10.
131. Tang X, Yu Y, Ding Q, Liu H, Niu Y, Li Y, et al. The sex-specific difference in singleton birth weight after frozen embryo transfer compared with fresh embryo transfer: a secondary analysis of 3 randomized trials. Fertility and Sterility. 2022;117(5):1004-12.
132. Shinohara S, Hirata S, Suzuki K. Association between infertility treatment and intrauterine growth: a multilevel analysis in a retrospective cohort study. BMJ Open. 2020;10(4):e033675.
133. Keane KN, Mustafa KB, Hinchliffe P, Conceicao J, Yovich JL. Higher β-HCG concentrations and higher birthweights ensue from single vitrified embryo transfers. Reprod Biomed Online. 2016;33(2):149-60.
134. Sletner L, Kiserud T, Vangen S, Nakstad B, Jenum AK. Effects of applying universal fetal growth standards in a Scandinavian multi-ethnic population. Acta Obstet Gynecol Scand. 2018;97(2):168-79.

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