Sari Pelkonen

FROZEN EMBRYO TRANSFER
EARLY PREGNANCY, PERINATAL OUTCOMES,
AND HEALTH OF SINGLETON CHILDREN
SARI PELKONEN

FROZEN EMBRYO TRANSFER
Early pregnancy, perinatal outcomes, and health of singleton children

Academic dissertation to be presented with the assent of the Doctoral Training Committee of Health and Biosciences of the University of Oulu for public defence in Auditorium 4 of Oulu University Hospital, on 17 June 2016, at 12 noon
Abstract

The main goal of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) treatment is a healthy mother and a healthy child. The most important complication following IVF/ICSI arises from the increased risk of multiple pregnancies. An elective single embryo transfer (eSET) with the freezing of spare embryos and subsequent treatment with frozen embryo transfer (FET) is the only way to avoid this complication. For this reason, the number of children born after FET is steadily rising.

The aim of this study was to provide more detailed evidence on the safety of FET, particularly focusing on serum hormone profiles during the first trimester weeks of singleton pregnancies after IVF/ICSI fresh embryo transfer (ET), after FET during a natural menstrual cycle, and after spontaneous conception. Another part of this study compared the perinatal outcomes, congenital anomalies (CAs), and morbidity of singletons born after FET and IVF/ICSI fresh ET. The reference group was those born after spontaneously conceived (SC) pregnancies.

In the clinical prospective study, the maternal serum estradiol and progesterone levels in pregnancies after fresh ET (n=39) were higher during early pregnancy weeks than in FET (n=30) and SC pregnancies (n=41), while the hormonal profiles after FET did not differ from SC pregnancies.

In the large register study, FET children (n=1830) were found to have a reduced risk for adverse perinatal outcomes, such as preterm birth, a low birthweight, and being small for their gestational age compared with children born after fresh ET (n=2942). However, FET children have an increased risk for being large for their gestational age. The major CAs and morbidity until three years of age did not differ between groups. When compared with SC children (n =31 243), the perinatal outcome was worse and the rates of CAs and morbidity were higher in FET children.

The FET cycle seemed to provide a better physiological environment for early fetal development than fresh ET. Further, FET protects against some of the adverse perinatal outcomes of children when compared with fresh ET, but not when it comes to the major CAs and early somatic health.

This study provides further evidence of the safety of FET in comparison with fresh ET. This information should further encourage clinicians to implement eSET combined with cryopreservation in their IVF/ICSI program.

Keywords: childhood morbidity, congenital anomalies, controlled ovarian hyperstimulation, early pregnancy, frozen embryo transfer, in vitro fertilization
Tiivistelmä


Kliinisessa prospektiivisessa tutkimuksessa havaittiin naisilla, joilla oli tuorealkion siirrosta alkanut raskaus (n=39), merkittävästi koholla olevat seerumin estradioli- ja progesteronipitoisuudet 7-8 raskausviikolle asti verrattuna naisiin, joilla raskaudet olivat alkanet PAS:sta (n=30) tai luonnollisesti (n=41). Vastaavasti PAS-raskauksissa hormonipitoisuudet eivät eroonee merkittevästi luonnolliseen raskauteen verrattuna.

Laajassa rekisteritutkimuksessa havaittiin PAS-lapsilla (n=1830) olevan pienempi riski ennenaiakaisuuteen ja pienpainoisuuteen kuin tuorealkiolasilla (n=2942). Kuitenkin PAS-lapsilla oli lisääntynyt riski syntyä isokokoisinä raskausviikkoihin nähden. Synynnäisten epämuodostumien ja eri sairauksien esiintyvyysissä ei ollut eroja. Luonnollisesti alkunsa saaneisiin lapsiin (n=31 243) verrattua, PAS-lapsilla oli vastasyntyneisysyyskaudelta lähtien enemmän terveyteen liittyviä ongelmia. Kuitenkin PAS-lapsilla ollut vastasyntyneisyyskaudelta lähtien enemmän terveyteen liittyviä ongelmia.

To my family
Acknowledgements

The research for this thesis was carried out in collaboration with the Departments of Obstetrics and Gynecology, University Hospitals of Oulu and Helsinki, Väestöliitto Fertility Clinics in Helsinki and Oulu and the National Institute for Health and Welfare, during the years 2007–2015.

In particular, I owe my sincerest gratitude to my supervisor, Professor Aila Tiitinen, for all her support, advice, and encouragement throughout the years. It has been a long journey, and her thorough knowledge of this field of study has guided me through the difficulties. Our cooperation has been excellent in spite of the long geographical distance between us. It has been a true privilege to work with her.

I thank my other supervisor, Professor Hannu Martikainen, for his support, fruitful discussions, practical criticism, and guidance in the field of infertility.

With warm thoughts, I thank the former Department Head, Professor Juha Tapanainen, for his supportive attitude toward my research and Professor Emeritus Pentti Jouppila, for providing me the opportunity to specialize in the field of Obstetrics and Gynecology. I also express my warmest thanks to the current Department Head, Eila Suvanto, MD, Ph.D., for offering me the opportunity to perform this work on the side of clinical practice.

I owe my deepest thanks to my research group, who have also been assisting in the work of this project. I would like to give special thanks to Professor Emeritus Anna-Liisa Hartikainen for teaching me the secrets of epidemiology. I really appreciate her genuine interest in my project. I would also like to express my sincere gratitude to Riitta Koivunen, MD, Ph.D., who encouraged me to start this project and shared the very first and often challenging steps in the scientific world, and Professor Mika Gissler, who conducted all the statistical analysis in register-based studies and gave me his friendly support throughout the process. Without his professional contribution, this work would not have begun. The contribution of the National Institute for Health and Welfare has also been essential.

I warmly thank my other co-authors and collaborators: Docent Ilkka Järvelä for sharing the scientific world of early pregnancy after ART; Annukka Ritvanen MD for her expertise in the field of congenital anomalies; dear friends Satu Lehtinen, MD, Ph.D. and Sari Koivurova, MD, Ph.D. for their experience while studying the health of ART children; Docent Anne-Maria Suikkari and Sinikka Nuojua-Huttunen, MD, Ph.D. for their support in initiating this project.

I wish to express my gratitude to Docent Leena Anttila and Docent Olli Pitkänen for their expert work as reviewers. Their constructive comments and
suggestions had a major impact on the completion of this thesis. I also appreciate the official advisory group for this Ph.D. project, Docent Leila Unkila-Kallio and Docent Kaarin Mäkikallio-Anttila. I thank Elina Arokannas, Ph.M., for her skillful language review of the Finnish abstract. Further I warmly thank my colleague Eija Karjalainen, MD, who started this project prior to my involvement.

I would like to express my gratitude to the Department Head of the Reproductive Clinic, Docent Laure Morin-Papunen and the entire staff for their understanding and kind assistance whenever needed.

I own my warmest thanks to my colleagues and friends at the Department of Obstetrics and Gynecology for their empathy and interest in my work, and special thanks go to Hilkka Ijäs MD, Ph.D., Marianne Hinkula MD, Ph.D., Liisa Laatio MD, Ph.D., Maarit Niinimäki MD, Ph.D., and Anna Terho MD. Their friendship has provided me with the positive energy I needed to recharge my batteries.

All my dear friends are warmly thanked for their delightful company and support. Special thanks go to my childhood friends Arja Bertolli, Ella Korhonen and Anne Kolek and their families for their long-term friendship. I also wish to express my thanks to my dear friend Elina Takaluoma and her family, for sharing with my family all the sunny and rainy days in life.

I owe my deepest love and gratitude to my dearest parents, Eine and Mauri Holopainen, for their love and care throughout my life and for their kind helping hand in managing everyday life during these years. I also express my loving thanks to my brothers, Matti and his family and Jussi, for their support and understanding throughout my life. Further, I am thankful for my parents-in-law, Sylvie and Sakari Pelkonen, for all their support and help.

Finally, I thank my dearest husband Heikki. His love and continuous support have been crucial to navigate through this project. I am also grateful to our beloved children Hannes and Helmi. I love them.

This study was supported by research grants from the Finnish Foundation for Gynecology and Obstetrics, Finnish Medical Association, the Orion Research Foundation, the Emil Aaltonen Foundation, the Finnish Fertility Society, the Oulu and Helsinki University Hospital Research Funds, and University of Oulu, which are gratefully acknowledged.

Oulu, May 2016

Sari Pelkonen
Abbreviations

AOR  Adjusted odds ratio
ART  Assisted reproductive technology, includes IVF, ICSI, and FET
BMI  Body mass index
CA   Congenital anomaly
CI   Confidence interval
COH  Controlled ovarian hyperstimulation
DET  Double embryo transfer
eSET  Elective single embryo transfer
ESHRE European Society of Human Reproduction and Embryology
ET   Embryo transfer
FET  Frozen embryo transfer
FSH  Follicle-stimulating hormone
GnRH Gonadotrophin-releasing hormone
hCG  Human chorionic gonadotrophin
ICD  International Classification of Diseases
ICSI Intracytoplasmic sperm injection
IVF  In vitro fertilization
LBW  Low birthweight
LGA  Large for gestational age
LH   Luteinizing hormone
OHSS Ovarian hyperstimulation syndrome
OR   Odds ratio
PTB  Preterm birth
RR   Relative risk
SC   Spontaneously conceived
SGA  Small for gestational age
SET  Single embryo transfer
VLBW Very low birthweight
VPTB Very preterm birth
List of original articles

This thesis is based on the following publications, which are referenced throughout the text by their Roman numerals:


Some previously unpublished new results are also presented in this thesis.
# Contents

Abstract

Tiivistelmä

Acknowledgements

Abbreviations

List of original articles

Contents 15

1 Introduction 17

2 Review of the literature 19

2.1 Infertility ................................................................. 19

2.2 ART ......................................................................................... 19

2.2.1 Controlled ovarian hyperstimulation and oocyte retrieval ...... 21

2.2.2 Fertilization ................................................................. 22

2.2.3 Embryo culture and assessment........................................ 23

2.2.4 The luteal phase .......................................................... 24

2.2.5 Cryopreservation ......................................................... 25

2.2.6 Frozen embryo transfer................................................ 26

2.2.7 Treatment outcome after ART ........................................ 27

2.3 Singleton children born after ART ........................................ 28

2.3.1 Obstetric outcomes ....................................................... 28

2.3.2 Perinatal outcomes ....................................................... 30

2.3.3 Congenital anomalies and chromosomal disorders .......... 31

2.3.4 The other health outcomes .......................................... 32

2.4 Patient- and treatment-related factors and the risk of adverse pregnancy outcomes ......................................................... 34

2.4.1 Subfertility ................................................................. 34

2.4.2 IVF/ ICSI treatment .................................................... 36

2.4.3 Cryopreservation ......................................................... 38

2.4.4 The number of embryos transferred ................................. 39

2.5 Singleton children born after FET ......................................... 41

2.5.1 Obstetric outcomes ....................................................... 41

2.5.2 Perinatal outcomes ....................................................... 42

2.5.3 Congenital anomalies and chromosomal disorders .......... 47

2.5.4 The other health outcomes .......................................... 47

3 Aims of the present study 51
4 Materials and methods 53
4.1 Study population ................................................................. 53
4.1.1 Study I ........................................................................ 53
4.1.2 Studies II–IV ................................................................. 53
4.1.3 ART protocols ............................................................... 55
4.2 Study design ....................................................................... 56
4.2.1 Clinical data (I) ............................................................... 58
4.2.2 Register data (II–IV) ....................................................... 58
4.3 Statistical methods ............................................................. 63
4.3.1 Early pregnancy data (I) .................................................. 63
4.3.2 Mother’s and children’s data (II–IV) ............................... 63
4.3.3 Ethical aspects .............................................................. 64
5 Results 65
5.1 Early pregnancy hormone profiles (I) .................................... 65
5.1.1 Estradiol levels ............................................................... 65
5.1.2 Progesterone levels ........................................................ 65
5.2 Obstetric outcome (II) ........................................................ 66
5.3 Perinatal outcomes (II) ........................................................ 67
5.4 Major congenital anomalies (III) ......................................... 71
5.5 Physical health of children (IV) .......................................... 74
6 Discussion 79
6.1 Discussion of the results ...................................................... 79
6.1.1 The maternal serum hormonal profiles after FET pregnancies .............................................. 79
6.1.2 Pregnancy complications after FET .............................. 81
6.1.3 Perinatal outcomes of children born after FET ..... 81
6.1.4 Congenital anomalies of children born after FET 83
6.1.5 Somatic health of children born after FET ....................... 84
6.2 Clinical implications and future research ............................ 85
7 Conclusion 87
References 89
Original publications 103
1 Introduction

One in six couples worldwide experience some form of infertility problem at least once during their reproductive lifetime (Boivin et al. 2007). Infertility represents a complex and growing problem with varying causes and characteristics that affect the lives of both men and women across countries and socioeconomic boundaries (Petraglia et al. 2013b).

Today, assisted reproductive technology (ART) offers the most effective treatment for all types of infertility. The most customary ART procedures are in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), and frozen embryo transfer (FET) (European IVF-Monitoring Consortium (EIM) et al. 2016). The increasing use of ART treatments, due in part to the social trends of postponed childbearing and expanding availability, makes the safety aspects of ART an important public health concern (Opdahl et al. 2015). Over six million children have been conceived through ART (ESHRE 2015). In Finland, a total of 2007 children were born in 2013 as a result of ART treatments, representing 3.6% of all children born that year in Finland (THL 2015a).

The main goal of ART treatment is a healthy mother and a healthy child. The concerns regarding the safety of ART for the offspring have been expressed since the first pregnancy outcome reports in the 1980s. Today, multiple pregnancies still carry the most significant risks for complications of an ART treatment, i.e., preterm birth (PTB), low birthweight (LBW), and small for gestational age (SGA) (Tiitinen 2012).

The cryopreservation has expanded because single embryo transfer (SET) is the only way to avoid the complications associated with multiple pregnancies (Tiitinen et al. 2004). After the implementation of elective single embryo transfer (eSET) with embryo freezing, the twin rates after ART have decreased considerably in the Nordic countries concomitantly with improved outcomes for children conceived by ART (Henningsen et al. 2015). According to the latest report by the European Society of Human Reproduction and Embryology (ESHRE), the overall trend toward transferring fewer embryos seems to continue and FET cycles increase. The multiple delivery rates (19.2% in 2011 compared with 22.3% in 2007) are lower than ever in Europe, being the lowest in Sweden (4.8%) and Finland (6.2%) and the highest in Greece (41.6%) (European IVF-Monitoring Consortium (EIM) et al. 2016)

However, SET is not the final solution to reduce the adverse perinatal outcomes of ART children. Singletons born after ART treatment also exhibit an increased risk
of adverse perinatal outcomes compared with spontaneously conceived (SC) singletons, even after adjusting for maternal age and parity (Helmerhorst et al. 2004, Jackson et al. 2004, McDonald et al. 2009, Pandey et al. 2012). It is highly likely that the etiological factors behind these are diverse and parental characteristics play an important role (Henningsen & Pinborg 2014).

Finland is one of the pioneering countries regarding eSET with the freezing of spare embryos and subsequent treatment with FET (Martikainen et al. 2001, Tiitinen et al. 2001, Vilska et al. 1999). To date, the proportion of FET versus fresh embryo transfer (ET) cycles is higher than in most other countries (European IVF-Monitoring Consortium (EIM) et al. 2016), and a substantial proportion (37%) of live births after ET originate from FET cycles (THL 2015a).

The health of children born after FET is a fundamentally important issue when discussing the safety of eSET policy as well as the embryo cryopreservation technology. The present research was conducted to extend the data published on the children born after FET compared with fresh ET.
2 Review of the literature

2.1 Infertility

The failure to conceive after a year of unprotected timed intercourse is referred to as infertility in the medical literature (Evers 2002). Infertility is regarded as one of life’s major crises. It has social as well as psychological impacts on a couple’s life (Practice Committee of the American Society for Reproductive Medicine 2004). One in six couples worldwide experience some form of infertility problem at least once during their lifetime. In high-income countries, approximately 15% of the population is affected by infertility (Petraglia et al. 2013b). In Finland, 13–17% of women have reported difficulties in trying to conceive within 12 months at some point in their lives (Klemetti et al. 2010). In recent years, the prevalence of infertility has increased, which can be explained by lifestyle changes, such as delayed child bearing, obesity, not exercising or exercising excessively, inappropriate diet, smoking, psychological stress, alcohol and/or caffeine consumption, and exposure to environmental pollutants and chemicals (Petraglia et al. 2013a).

According to the ESHRE’s classification, 20–30% of infertility cases are linked to physiological causes in men, 20–35% to physiological causes in women, 25–40% of cases are due to a problem in both partners, and no cause is found in 10–20% of cases (ESHRE 2014).

The array of infertility services, and their availability, has increased dramatically over the last 30 years. Clinicians are now more aware of infertility and better trained to evaluate and treat its causes. The public has a greater awareness of infertility and modern treatments, largely due to the increased media attention surrounding the advances and controversies relating to ART.

2.2 ART

The most successful treatment for all types of infertility is ART, which refers to all treatments that include the in vitro handling of human gametes, both eggs and sperm, and embryos to establish a pregnancy. The most frequent procedures of ART are IVF, ICSI, FET, and oocyte and embryo donation; in contrast, intrauterine insemination is not considered a part of ART (Zegers-Hochschild et al. 2009).
ART has changed considerably since 38 years ago with the birth of the first baby by IVF in 1978 (Steptoe & Edwards 1978). A few years later, the cryopreservation of surplus embryos followed by the replacement of a thawed embryo became an option and the first child after FET was born in 1984 (Zeilmaker et al. 1984). In the same year, the first birth was achieved after ovum donation and IVF (Lutjen et al. 1984). In 1990, the first preimplantation genetic diagnose (PGD) was described (Handyside et al. 1990), and two years later, ICSI was introduced to treat male infertility (Palermo et al. 1992).

Today, IVF is a routine medical practice in the management of infertility. The availability of ART in terms of cycles per million populations is the highest in the Nordic countries, Belgium, Iceland, and Slovenia. In 2011, around 1.5 million ART cycles were performed each year worldwide, and infants born after ART have contributed to up to 5% of the national birth cohorts in some countries (European IVF-Monitoring Consortium (EIM) et al. 2016).

![Fig. 1. IVF, ICSI, and FET treatment cycles in Finland 1992–2014. Data for 2014 is preliminary.](image)

According to preliminary data (THL 2015a), in Finland 7875 ART treatments using the patients’ own gametes were started in 2014 (Figure 1). The number of ART
treatments has increased considerably in the 2000s (6770 treatment cycles were started in 2001), mainly as a result of growth in the use of FETs, accounting for 43% of all ART treatments. It is a common international trend that the increasing use of FETs is associated with a decrease in the number of IVF/ICSI treatments (European IVF-Monitoring Consortium (EIM) et al. 2016).

2.2.1 Controlled ovarian hyperstimulation and oocyte retrieval

The first IVF cycles were performed in a natural cycle resulting in SETs (Steptoe & Edwards 1978). The transfer of a single embryo yielded only moderate results; therefore, controlled ovarian hyperstimulation (COH) was developed to allow numerous oocytes to be fertilized and transferred in vitro.

Figure 2 illustrates the COH protocols used in the present study. In the conventional long-protocol, the pituitary-ovarian axis is first suppressed through the administration of a gonadotrophin-releasing hormone (GnRH) agonist, which is commonly started during the mid-luteal phase of the menstrual cycle. GnRH agonists first stimulate the pituitary to produce luteinizing hormone (LH) and follicle stimulation hormone (FSH), resulting in an increase in estrogen levels (a “flare-up”). With continued use, suppression occurs from exhaustion and desensitization of the gonadotrophic pituitary cells. As a result, the LH and FSH and estrogen levels decrease (“down-regulation”). After ovarian suppression, either recombinant FSH or human menopausal gonadotrophin with individualized doses is used to achieve a COH. In the short protocol, COH is started on cycle day 2 or 3 and GnRH antagonists are initiated on day 5th or 6th of the administration of gonadotrophins. GnRH antagonist binds to GnRH receptors on the pituitary gland. This directly inhibits the release of LH and, to a lesser degree FSH, and the inhibition is maintained if the administration of GnRH antagonist is continued (Fritz & Speroff 2011). The dose of stimulating hormones is dependent on the patient’s age, body mass index (BMI), the results of ovarian reserve testing, and the response observed in any previous stimulation cycles.

In both protocols, the ovarian response is monitored by transvaginal ultrasonography. Once the largest follicles have a diameter of 17 to 20 millimeters, human chorionic gonadotrophin (hCG), which works as an analogue of LH, is administered. From that moment on, the down-regulation and administration of gonadotrophins are discontinued. In GnRH antagonist cycles, ovulation can also be performed by injecting GnRH agonist, which stimulates the endogenous LH surge. Oocyte retrieval is performed 34–37 hours later under transvaginal ultrasound
guidance. One by one, the follicles are punctured and vacuum-aspirated into a test tube (Fritz & Speroff 2011).

During COH, the blood level of estradiol is 4– to 10–fold higher and progesterone 10–fold higher than during the normal menstrual cycle. The estradiol concentration at the time of oocyte retrieval correlates with the number of mature oocytes. Each mature follicle produces estradiol, which corresponds to the level of a serum estradiol 600–800 pmol/L (Orvieto et al. 2005).

### 2.2.2 Fertilization

Fertilization can be achieved by conventional IVF or by ICSI. In IVF, each oocyte is incubated with approximately 100,000–200,000 sperm. The semen for IVF is
prepared from ejaculated sperm by removing inactive cells and seminal fluid, allowing the sperm to penetrate and fertilize the egg. In ICSI, an individual sperm is injected directly into an oocyte (Palermo et al. 1992). ICSI is the treatment of choice in severe cases of male infertility or in surgically retrieved sperm, or in cases of previous fertilization failure in conventional IVF cycles. Currently, the indication of ICSI treatment is more liberal in cases with mixed infertility, unexplained infertility, mild male factor infertility, a low oocyte number, and fertilization failures. The ICSI treatment policies have changed in most countries in western and central Europe, where ICSI is used in two-thirds of cases; in contrast, in Finland, IVF has remained the dominant technology (European IVF-Monitoring Consortium (EIM) et al. 2016).

Oocytes are evaluated for evidence of fertilization at approximately 18 hours after insemination or ICSI. Normal fertilization has occurred when two polar bodies and two (female and male) pronuclei can be visualized under a light microscope. After fertilization, the pronuclear membranes break down, a diploid number of chromosomes are achieved, and the first cell division is possible (Fritz & Speroff 2011).

2.2.3 Embryo culture and assessment

An embryo culture is a component of IVF/ICSI where oocytes and sperms, and thereafter embryos, are allowed to fertilize and cleave in a medium under specified temperature and gas conditions. During the culture, either the same culture medium can be used throughout the period, or a sequential system, in which the embryo is sequentially placed in different media. Typically, embryo culture media are based on balanced salt-solutions or richer media, supplemented with energy-providing components like pyruvate, lactate, and glucose, as well as other components like amino acids, vitamins, and human serum albumin to improve the performance of embryonic growth and development. Furthermore, many other conditions in IVF laboratories such as the carbon dioxide concentration, incubation volume, embryo group size, and the type of protein supplementation of the culture are important. Of the external conditions, the maintenance of the correct pH and osmolality of the medium with temperature control are essential as well (Nagy et al. 2012).

The embryonic development, which is monitored on a daily basis in the IVF laboratory, follows a distinct pattern of events; approximately one day after insemination, the embryo is at a pronuclear stage as a zygote. Then, one cell zygote begins to divide into cleavage stage embryo (days 2–3), morula stage (day 4), and
a blastocyst stage (days 5–6). In vitro growth demonstrates the developmental competency of the embryo (Nagy et al. 2012).

The assessment of the embryo quality in IVF/ICSI is essential and determines the selection of embryos for fresh ET, cryopreservation, or destruction. The classification of early cleavage embryos is traditionally based on sequential screening on the cleavage rate, the degree of fragmentation and cytoplasm appearance as well as the number, size, symmetry, and the nuclear status of the blastomeres. The duration of culture of an embryo may vary from 1 to 6 days prior to its selection for fresh ET (Steer et al. 1992).

2.2.4 The luteal phase

The luteal phase in ART cycles is deficient, although steroid levels are higher in cycles after COH than in natural cycles. Co-treatment with GnRH analogues to prevent premature LH surges and luteinization effectively suppresses endogenous LH secretion, as intended. Unfortunately, although treatment with the agonists or antagonists ends abruptly (Figure 2) on the day of hCG administration, the residual suppression of endogenous LH does not. Abnormally low levels of LH during the luteal phase can be insufficient to stimulate and maintain the level of luteal function required to promote timely endometrial maturation in preparation for implantation or to support an early pregnancy once established (Fritz & Speroff 2011).

The luteal phase can be supported by several methods: vaginal/intra–mural progesterone supplementation or stimulation of the corpora lutea with hCG or GnRH-agonist (only in the antagonist cycle) to produce estradiol and progesterone. In clinical practice, there is considerable variation in the supplementation protocols without consensus of the golden standard treatment. In Finland, natural micronized progesterone is the most commonly used method, mainly administered vaginally. Progesterone can be started at the day of oocyte retrieval, the day before ET or the day of ET and continued until the day of a positive pregnancy test or the eighth gestation week (Nyboe Andersen et al. 2002). Nevertheless, prolonged use has not been proven to be beneficial because at 7–8 weeks of pregnancy, the placental trophoblastic tissue is mature enough to produce the steroids required to maintain the pregnancy and the corpus luteum is no longer crucial. In fact, corpus luteal function declines even before this, in spite of an increasing stimulus from the rising levels of hCG secreted from the growing placenta (Csapo & Pulkkinen 1978).
2.2.5 Cryopreservation

Human zygotes and embryos can be frozen and thawed from the pronuclear to the blastocyst stage and remain viable for at least several years, perhaps indefinitely (Michelmann & Nayudu 2006). At present, there are two basic methods for embryo cryopreservation, the “slow-freeze” technique and ultra–rapid “vitrification”. The objective of freezing is to avoid the ice crystallization of intracellular water, which can result in cellular damage. Freezing protocols vary with the stage of embryo development, which affects cellular permeability. In general, slow-freezing is mostly used in the cleavage stage and vitrification is used at all stages of preimplantation development. Further, vitrification is a current method of choice when cryopreserving oocytes (Edgar & Gook 2012).

In the slow-freezing method, intracellular water is gradually replaced by cryoprotectants (i.e., glycerol and 1,2 propanediol) via osmosis by passage through solutions with increasing concentrations of the cryopreservative to minimize intracellular ice-formation. The temperature of the final solution is then decreased below the freezing point in a programmed 2-step process, and embryos are then stored in liquid nitrogen at -196°C. The thawing process is reversed, and the embryo is gradually passing through decreasing concentrations of the cryoprotectant followed by incubation in a cell culture medium before ET (Nagy et al. 2012).

By vitrification, an embryo is flash frozen by direct immersion into liquid nitrogen, creating a solid glass-like state, which avoids ice crystal formation and thereby reduces associated cell injuries. However, to achieve these results, higher concentrations of cryoprotectants or cryoprotectant mixtures must be used. Rapid thawing and warming of the frozen embryo is achieved by immersing it directly into warm thawing solution. Up-to-date clinical IVF laboratories use several vitrification protocols for human embryo preservation (Sparks 2015).

In general, 70 to 80% of cleavage-stage embryos cryopreserved by the slow-freezing method survive after thawing and 50% of all thawed embryos will have 100% of the blastomeres intact. Successful blastocyst cryopreservation may be more consistently achieved with vitrification, but optimal slow freezing can produce similar results in terms of cumulative pregnancy rates per treatment (Edgar & Gook 2012). Multiple variables, such as the selection criteria for embryos to be cryopreserved, the method of freezing and thawing, the synchronization between embryo and endometrium development and the mode of hormone supplementation
during the FET cycle, and patient characteristics, such as the age of the women, determine the efficacy of embryo cryopreservation programs (Wong et al. 2014).

Cryopreservation increases the cumulative live birth rate per oocyte retrieval. It can also be used for all embryos when the risk of ovarian hyperstimulation syndrome (OHSS) is high or when there is some other reason to avoid refrain from fresh ET, i.e., there are complications after a follicle puncture, the endometrium is not favorable for fresh ET, in embryo donation programs, and for fertility preservation in women awaiting cytotoxic treatment (Wong et al. 2014).

### 2.2.6 Frozen embryo transfer

FET can be carried out through different cycle regimens, including spontaneous ovulatory cycles (natural cycle), cycles in which ovulation is induced by drugs (ovulation induction cycle), and cycles in which the endometrium is artificially prepared by exogenous estrogen and progesterone (artificial cycle).

For the timing of ET, synchronization of the stages of development of frozen-thawed embryos and the endometrium is important. In natural cycles, the patient is instructed to start urinary LH testing, which is based on the vaginal ultrasound examination. The ET is scheduled 3–5 days after the LH surge, depending on the cleavage stage of the embryo. Vaginal luteal phase support commences on the day of ET, using micronized progesterone or vaginal gel for two weeks (Fritz & Speroff 2011).

The artificial cycle is used in patients with anovulatory or irregular cycles, and the endometrial development is carefully controlled using transvaginal ultrasound during programmed sequential treatment with exogenous estrogen from the second cycle day of a natural or progestin-induced menstrual cycle. When the endometrium is developed sufficiently (>7 mm, trilaminar pattern), ET is scheduled. Substitution with micronized progesterone vaginally or vaginal gel starts 3–5 days before the day of the ET and treatment continues until 9–12 gestational weeks after a positive pregnancy test result (Hyden-Granskog et al. 2005).

Natural and artificial cycles seem to be similar in terms of their efficacy of clinical pregnancy outcomes. A 2010 systematic review including 22 randomized trials comparing different endometrial preparation regimens and 2013 published meta-analyses including 20 articles did not find evidence that one method of endometrial preparation was superior to others (Glujovsky et al. 2012, Groenewoud et al. 2013).
2.2.7 Treatment outcome after ART

Today in Europe 1–5% of all children born annually are born as a result of ART (European IVF-Monitoring Consortium (EIM) et al. 2016). There are now estimated to be more than six million ART children worldwide (ESHRE 2015). The proportion of ART children is not negligible and will influence the coming generations (Pinborg et al. 2013b). In Finland, almost 35,000 children were born after ART during the years 1992–2013. At present, nearly one child in every school class has been born after ART treatment (Raatikainen et al. 2012). Figure 3 shows the number of children born after IVF, ICSI, and FET treatments in Finland (THL 2015a).

Fig. 3. The number of children born after IVF, ICSI, and FET treatments in Finland 2001–2013 (THL 2015a).

According to the latest report of European registers by ESHRE, the clinical pregnancy rate was 33.2% per transfer and the delivery rate per transfer was 24.8% for IVF in 2011. The corresponding figures for ICSI were 31.8% and 22.7% and for FET 21.3% and 15.9%. Of all births, 18.6% were twins and 0.6% were triplets (European IVF-Monitoring Consortium (EIM) et al. 2016). In Finland, according
to the most recent report by THL, the clinical pregnancy rate per transfer for IVF was 32.8%, for ICSI 29.0%, and for FET 28.1% in 2013. Delivery rates per transfer were 24.6%, 21.4%, and 21.5%, respectively (THL 2015a).

A consensus regarding an objective way of assessing the success rate after ART is still lacking (Pinborg et al. 2004, Tiitinen et al. 2004). Traditionally, IVF/ICSI outcomes have been expressed in terms of clinical pregnancies and the live birth rate per embryo transfer. However, these parameters also include multiple pregnancies and births. The cumulative live birth rate per stimulated IVF/ICSI cycle after all embryo transfers, fresh and frozen, may be the most relevant end point of ART in the future due to a noticeable decline in the number of embryos transferred. This should allow better comparisons between infertility clinics and across countries (Pinborg 2012). For example, the live birth rate per IVF/ICSI fresh cycles and the cumulative live birth rate including IVF/ICSI fresh ET and FET cycles in the same calendar year were in Finland 20.4% and 33.8%, in Sweden 22.2% and 31.3%, and in Norway 20.9% and 26.9%, justifying the transfer and freezing policies performed in those countries in 2011 (European IVF-Monitoring Consortium (EIM) et al. 2016).

2.3 Singleton children born after ART

The first published studies on perinatal outcomes in the 1980s suggested that an excess of PTB and LBW were characteristic of singleton pregnancies after IVF (Australian in vitro fertilisation collaborative group 1985, Frydman et al. 1986, Steptoe et al. 1986). Although there were also early reports on congenital anomalies (CAs) following IVF, they were based on small numbers of affected children, as CAs are relatively rare. Hence, risk estimates were imprecise and often interpreted as showing no increase because the difference was not statistically significant. It took until the mid-2000s for the increased risk of major CAs in children born after ART to be generally acknowledged (Hansen & Bower 2014).

2.3.1 Obstetric outcomes

Preeclampsia, placenta previa, placental abruption, and antepartum hemorrhage are major obstetric complications that are associated with adverse acute and long-term consequences for both mothers and infants. Table 1 summarizes the major pregnancy complications in ART singleton pregnancies compared with SC pregnancies.
Table 1. The risk of major pregnancy complications in ART singleton pregnancies versus SC pregnancies expressed as adjusted ORs with 95% CIs. Results adopted from large cohort studies, systemic reviews, and meta-analyses where at least parity and maternal age were adjusted.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Children (n)</td>
<td>12,283</td>
<td>10,087</td>
<td>6730</td>
<td>13,544</td>
<td>20,807</td>
<td>501,766</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>1.6 (1.2–2.0)</td>
<td>1.2 (1.1–1.3)</td>
<td>1.0 (0.9–1.1)</td>
<td>1.5 (1.4–1.6)</td>
<td>1.3 (1.1–1.6)</td>
<td></td>
</tr>
<tr>
<td>Placental abruption</td>
<td>1.9 (1.4–2.5)</td>
<td>2.1 (1.4–2.9)</td>
<td>1.9 (1.4–2.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placenta previa</td>
<td>2.9 (1.5–5.4)</td>
<td>3.8 (3.3–4.5)</td>
<td>2.3 (1.9–2.9)</td>
<td>3.4 (2.7–4.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>APH†</td>
<td>2.5 (2.3–2.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

†APH ante partum hemorrhages including placental abruption, placenta previa, and third-trimester vaginal bleeding.

Two meta-analyses based on eight (Jackson et al. 2004) and 15 studies (Pandey et al. 2012) reported a 1.6 and 1.5 increased risk of preeclampsia in ART pregnancies compared with SC pregnancies, respectively. Similar findings were shown in two other population-based cohort studies (Kallen et al. 2005a, Tandberg et al. 2015). Further, the Swedish study showed increased risk for preeclampsia when adding BMI and smoking into the model (Sazonova et al. 2011).

The systemic review and meta-analyses reported a 1.5 times increased risk of hypertensive disorder in ART singleton pregnancies compared with SC singleton pregnancies with an absolute increased risk (95% CI) of 2% (1–2%) (Pandey et al. 2012). A very recently published large cohort study from Scandinavia including 47,088 ART pregnancies showed only a moderately increased risk of hypertensive disorders in ART pregnancies [Adjusted odds ratio (AOR) 1.16, 95% CI 1.10; 1.10–1.21] compared with SC pregnancies (Opdahl et al. 2015).

Significantly increased rates of placenta previa and placental abruption were found in four large cohort studies (Grady et al. 2012, Romundstad et al. 2006, Healy et al. 2010, Sazonova et al. 2011) and in two meta-analyses (Kallen et al. 2005d, Pandey et al. 2012) in ART pregnancies when compared with SC pregnancies. Romundstad and colleges (2006) reported that the risk of placenta previa was 3-fold higher in pregnancies achieved with ART than with natural conception in the same individual (Romundstad et al. 2006).
### 2.3.2 Perinatal outcomes

Perinatal outcomes for ART singletons have been studied extensively. In 2004–2009, five systematic reviews were published independently (Helmerhorst et al. 2004, Jackson et al. 2004, McDonald et al. 2005, McDonald et al. 2009, McGovern et al. 2004). In addition, two meta-analyses have been published recently (Pandey et al. 2012, Pinborg et al. 2013b) where the reasons for adverse outcomes of ART singletons were discussed as well. Table 2 shows the perinatal risks for the adverse outcome of ART singletons compared with SC singletons.

**Table 2. The risk of adverse perinatal outcome in singletons born after ART compared with SC singletons expressed as adjusted ORs with 95% CIs. The results adopted from systemic reviews and meta-analyses were adjusted at least for maternal age and parity.**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Children (n)</td>
<td>5361</td>
<td>12,283</td>
<td>31,032</td>
<td>28,352</td>
</tr>
<tr>
<td>No of studies</td>
<td>12</td>
<td>14</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td>LBW, &lt; 2500 g</td>
<td>1.7 (1.5–1.9)</td>
<td>1.8 (1.4–2.2)</td>
<td>1.6 (1.3–2.0)</td>
<td>1.7 (1.6–1.8)</td>
</tr>
<tr>
<td>VLBW, &lt; 1500 g</td>
<td>3.0 (2.1–4.4)</td>
<td>2.7 (2.3–3.1)</td>
<td>2.7 (1.8–3.8)</td>
<td>1.9 (1.7–2.2)</td>
</tr>
<tr>
<td>PTB, &lt; 37 weeks</td>
<td>2.0 (1.8–2.3)</td>
<td>2.0 (1.7–2.2)</td>
<td>1.8 (1.5–2.2)</td>
<td>1.5 (1.5–1.6)</td>
</tr>
<tr>
<td>VPTB &lt; 32 weeks</td>
<td>3.3 (2.0–5.3)</td>
<td>3.1 (2.0–4.8)</td>
<td>2.3 (1.7–3.0)</td>
<td>1.7 (1.5–1.9)</td>
</tr>
<tr>
<td>SGA</td>
<td>1.5 (1.4–1.7)</td>
<td>1.6 (1.3–2.0)</td>
<td>1.4 (1.0–2.0)</td>
<td>1.4 (1.3–1.5)</td>
</tr>
<tr>
<td>Perinatal mortality</td>
<td>1.7 (1.1–2.6)</td>
<td>2.2 (1.6–3.0)</td>
<td>1.9 (1.5–2.8)</td>
<td></td>
</tr>
</tbody>
</table>

LBW = low birthweight, VLBW = very low birthweight, PTB = preterm birth, VTPB = very preterm birth; SGA = small for gestational age.

The evidence from these systemic reviews and meta-analyses clearly points to the increased risk of adverse perinatal outcomes (LBW, VLBW, PTB, VPTB, SGA, and perinatal mortality) in ART singletons compared with SC singletons even after adjusting for maternal age and parity (Helmerhorst et al. 2004, Jackson et al. 2004, McDonald et al. 2009, Pandey et al. 2012).

However, in the meta-analyses (Pandey et al. 2012) the perinatal mortality risk did not persist when the analysis was done with random effects to control marked heterogeneity ($I^2 = 73\%$) amongst the studies included (Pandey et al. 2012). This can most likely be explained by the increased incidence of PTBs among ART children, but the immaturity of the preterm newborns is not the only explanation (Henningsen & Pinborg 2014). A recently published large population-based study including more than 62,000 ART singletons ($\geq 22$ weeks) showed that the risk of
a stillbirth was increased in ART pregnancies before 28+0 gestational weeks compared with pregnancies after spontaneous conception (RR 2.08, 95% CI 1.55–2.08). Even after stratification for SGA in the analyses on the risk of early neonatal, perinatal, and infant deaths, the singletons’ risk was higher in ART than in SC despite normal growth (Henningsen et al. 2014).

### 2.3.3 Congenital anomalies and chromosomal disorders

Four meta-analyses (Rimm et al. 2004, Hansen et al. 2005, McDonald et al. 2005, Hansen et al. 2013) have demonstrated an increased prevalence of CAs with an excess risk of 30-40% in singletons born after ART compared with the general population. Based on the latest meta-analyses, the risk further increased by 42% when data were restricted to major CAs (Hansen et al. 2013). Whether ICSI children have an added risk compared with IVF children is still not clear (Pinborg et al. 2012). In a large Swedish registry study (Kallen et al. 2010b), more than 32,000 ART children showed similar risks of CAs both after IVF and ICSI. However, these results are in contrast to a recently published population-based study from Australia finding a significantly increased risk of CAs in ICSI infants compared with IVF even after adjusting for maternal confounders (AOR 1.55, 95% CI 1.24–1.94). A history of infertility, either with or without ART, was also significantly associated with CAs (Davies et al. 2012).

Two study groups (Hansen et al. 2012, Kallen et al. 2010b) have shown that the younger or more recent ART populations may have a lower risk for CAs. Källen et al. (2010) revealed that the risk ratio decreased from 1.6 (1986–2001) to 1.3 (2001–2006). A decreasing risk has been seen especially for neural tube defects, cardiac septal defects, and esophageal atresia. However, hypospadias, which had previously been associated with ICSI, decreased and disappeared toward the end of the study period (Kallen et al. 2010b). Similarly, in an Australian study (Hansen et al. 2012), the OR decreased from 1.9 (1994–1998) to 1.3 (1999–2002). Further, a study from Finland using a more recent birth cohort (2006–2010) showed an even lower risk of CAs in ART singletons (OR 1.1; 96% CI 1.0–1.3) (Raisanen et al. 2013).

Since the introduction of the ICSI technique, men with azoospermia can now become genetic fathers of their offspring. The overall frequency of chromosomal aberrations in children born by the use of non-ejaculated sperm seems reassuringly low in comparison to the outcome of children born after ejaculated sperm (Belva...
et al. 2011, Woldringh et al. 2010). However, these studies are limited, and the sample sizes have been small.

Imprinting is an epigenetic modification of the genome by which only genes in one of two parental alleles are expressed. Epigenetic modification controls gene activity without changing the DNA sequence (Pinborg et al. 2016). Imprinting diseases, associated with inadequate epigenetic modification of the genome (impairment in DNA methylation) are rare (1 in 14,000–15,000 newborns), but have severe consequences for children (Bergh & Wennerholm 2012). The incidence of imprinting diseases shows a tendency toward an increased risk after ART. According to a comprehensive review (Vermeiden & Bernardus 2013), only Beckwith-Wiedemann syndrome was suitable for calculating the weighted relative risk (RR) for the birth of a child following IVF or ICSI compared with the normal population; RR was 5.2 (95% CI 1.6–7.4). There were insufficient data on Silver-Russell syndrome to draw a final conclusion, but the authors stated that a positive association with ART was likely, whereas this was not the case for Angelman’s syndrome. However, Prader–Willi syndrome may be associated with couples having fertility problems. The conflicting results most likely derive from the relatively small study populations (Vermeiden & Bernardus 2013). A recent systematic review with a meta-analysis demonstrated an association between children born after ART and imprinting disorders when compared with SC children. The combined OR on any imprinting disorder was 3.67 (95% CI 1.39–9.74), but the weighted mean differences of selected imprinted genes showed no differences in methylation levels between children born after ART and SC children (Lazaraviciute et al. 2014, Pinborg et al. 2016).

2.3.4 The other health outcomes

The long-term consequences of ART are quite poorly understood due to the lack of longitudinal data. According to Barker’s hypothesis on the developmental origins of adult disease, prenatal conditions may influence organ development and have a persistent impact on the physiological, endocrine, and metabolic function of the resulting organism, which may predispose infants to disease in adult life (Barker 1995).
Growth, cognitive development, and somatic health

Several small studies have evaluated the long-term growth and development of ART children compared with children in the general populations. No differences have been found between the two groups (Basatemur et al. 2010, Ceelen et al. 2008, Hart & Norman 2013). However, a study of ART children between 8 and 18 years old demonstrated higher blood pressure and blood glucose levels compared to age-matched controls (Ceelen et al. 2008, Ceelen et al. 2009). Further, a Belgium cohort study of children born after ICSI found a tendency toward a higher percentage of body fat in boys (Belva et al. 2012). In addition, ART infants may be at an increased risk of developing unfavorable fat distribution with a potential for an adverse metabolic profile and increased blood pressure during adolescence (Hart & Norman 2013).

Current evidence suggests that singleton ART children born at term have the same general neurodevelopmental outcomes as those born after natural conception (Hyrapetian et al. 2014, Yeung et al. 2016). According to a systematic review (Middelburg et al. 2008), PTB was associated with a certain risk of cognitive dysfunction, but no difference was found between ART and SC children. Further, ART has not been found to be associated with increased problems in child mental health or cognitive and social development (Bay et al. 2013, Hart & Norman 2013, Punamaki et al. 2016).

The main results of two recent systematic reviews regarding somatic health are controversial. In one, Hart et al. (2013) studied ART children and asthma, allergies, and atopy and found no an association between ART and these diseases (Hart & Norman 2013). On the other hand, Kettner et al. (2015) showed an increased risk of unspecified infectious and parasitic diseases, asthma, genitourinary diseases, and epilepsy or convulsions in ART children compared with SC children. According to this latter study, the disparity between the two reviews could be attributed to different study selection criteria, together with a limited number of studies on ART and childhood morbidity (Kettner et al. 2015).

Divergent results on cancer risk in children born after ART have been reported in the literature. The risk of cancer can only be assessed in large population-based registries, owing to the low frequency of this condition (Shankaran 2014). In a systematic review from Denmark (Hargrave et al. 2013), including 25 studies of varying quality, a significantly higher risk of cancer and several cancer subtypes was found among children born after ART. However, according to a later systemic review (Hart & Norman 2013) and some other large cohort studies (Kallen et al. 2016),
Källén et al. (2010) in a large population-based study including 26,692 ART children born between 1982 and 2005 reported a significantly increased total cancer risk (standardized incidence ratio SIR 1.42, CI 95% 1.09–1.87). An unexpectedly high incidence of histiocytosis was reported (SIR 5.6, CI 95% 1.8–13). Maternal age, parity, subfertility, BMI, and multiple births did not impact risk while a higher birthweight, PTB, respiratory distress, and a low Apgar score among infants were related to the cancer risk (Kallen et al. 2010a). A more recently published large population-based cohort study from the United Kingdom (Williams et al. 2013) covering 17 years (1992–2008) detected no increase in the overall risk of cancer among children born after ART. A total of 106,013 children aged less than 15 years conceived by ART were identified for inclusion in the study. One hundred and eight children were identified as having developed cancer compared with 109.7 children who were expected (SIR 0.98, 95% CI 0.81–1.1.9) (Williams et al. 2013). In a large population-based cohort study from Scandinavian countries including almost 92,000 children born after ART in four Scandinavian countries between 1982–2007, the overall incidence of cancer was insignificantly different among ART children when compared with children born after SC. This study detected 181 cancer cases in ART children (2.0/1000 children) and 638 cases born after SC (1.8/1000) corresponding to an adjusted hazard ratio of 1.08 (95% CI 0.91–1.27). For 2 of the 12 studied cancer types, CNS tumors and malignant epithelial neoplasms, a significantly increased risk was found among ART children (Sundh et al. 2014).

2.4 Patient- and treatment-related factors and the risk of adverse pregnancy outcomes

2.4.1 Subfertility

Infertility is a heterogeneous condition, caused by various underlying pathologies. It is possible that some of the mechanisms leading to infertility also compromise the pregnancy (Messerlian et al. 2013). It is well-known that women undergoing ART are on average older, and therefore predisposed to more comorbidities before conception than the younger general population of women who become pregnant naturally (Henningsen & Pinborg 2014).

In systemic reviews and meta-analyses (Messerlian et al. 2013, Pinborg et al. 2013b) and very recently published large population-based studies from the United
States (Declercq et al. 2015) and Australia (Marino et al. 2014), researchers have found that subfertility (time to pregnancy more than one year) plays an important role in the poorer perinatal outcome (PTB, LBW, stillbirth) of subfertility births without ART. This negative effect of subfertility on perinatal outcomes in ART has also been shown for CAs (Kallen et al. 2005b, Nygren et al. 2007). However, the impact of the duration of infertility on obstetric and neonatal outcomes in couples achieving pregnancy after ART has not been studied in detail.

It has not been possible to disentangle whether the increased risk of adverse perinatal outcomes after ART is attributable to reproductive technology per se or to factors related to inherent infertility (Pinborg et al. 2013b). In two Nordic studies, researchers have attempted to overcome this by using consecutive singleton sibling pairs, one conceived by ART and the other by SC, hence keeping the maternal and paternal factors the same. Romundstad and colleagues (2008) showed data on 2546 ART and non-ART sibling pairs and concluded that the increased perinatal risk observed after ART compared with the general population was related to the factors that led to infertility rather than to the parameters of reproductive technology, as the differences in perinatal outcomes were absent in the adjusted analyses compared with siblings after ART and non-ART (Romundstad et al. 2008). In contrast, Henningsen et al. (2011) included 13,692 pairs of singleton siblings after IVF, ICSI, FET, and SC and analyzed them according to the mode of conception. This sibling analysis demonstrated declining but persisting significantly increased perinatal risk in ART versus non-ART singleton siblings, indicating that factors related to the treatment per se adversely affect ART singleton outcomes. The authors suggested that the etiology behind the adverse outcomes in ART conception is multifactorial and is related to both the ART technology and the parental characteristics (Henningsen et al. 2011). Interestingly, a recent study showed similar risks for placenta previa, PTB, and LBW regardless of the type of infertility treatment used among singletons who were born after ovulation induction (n=4 111), intrauterine insemination (n= 2 351), or IVF (n=4 570). The authors concluded that the maternal factors associated with infertility may contribute to the adverse outcomes rather than ART procedures themselves (Hayashi et al. 2012).

Subfertility is a non-iatrogenic effect and cannot be prevented directly (Pinborg et al. 2013b). However, a changing patient mix toward a more reproductively healthy subfertile population over time as well as an improved lifestyle in couples seeking fertility treatments may diminish offspring’s perinatal risk (Henningsen et al. 2015, Kallen et al. 2010b, Pinborg et al. 2013b).
2.4.2 IVF/ICSI treatment

Controlled ovarian hyperstimulation

During COH and after the oocyte retrieval, the supraphysiological levels of estrogens may exhibit a potentially negative impact on endometrial angiogenesis and implantation (Kalra & Barnhart 2011, Maheshwari & Bhattacharya 2013). However, there is no consensus regarding at what threshold a cycle becomes supraphysiological and may lead to changes in the endometrium (Barnhart 2014). Weinerman and Mainigi (2014) suggested that a more natural uterine environment that occurs in a FET cycle is favorable for early placentation and embryogenesis, whereas in a fresh ET cycle the COH affects fetal growth through the effect on the implanting embryo that results in impaired trophoblast differentiation, and therefore abnormal placental development (Weinerman & Mainigi 2014), which might lead to ischemia, placental disease, and adverse obstetric outcomes including preeclampsia, intrauterine growth restriction, and placental abruption (Kroener et al. 2016).

Hu and colleagues (2014) have analyzed serum estradiol and progesterone levels before pregnancy and during the early pregnancy weeks after fresh ET (n= 190), after FET during natural menstrual cycle (n= 86), and after SC (n= 192) pregnancies. They found higher mean serum estradiol levels in pregnancies after fresh ET at 4 and 8 weeks of gestation than those of the pregnancies after FET or SC (p<0.01). The serum estradiol levels of pregnancies after fresh ET at 4 and 8 weeks of gestation were positively correlated to those on the day of hCG administration (p<0.01). Thus, the high maternal estradiol serum level was associated with an increased risk of LBW and SGA (Hu et al. 2014). Similar findings were published by Imudia et al. (2012) and Pereira et al. (2015), who showed that high estradiol levels on the day of hCG administration during COH were associated with an increased risk for LBW after fresh ET (Imudia et al. 2012, Pereira et al. 2015).

Although the number of oocytes retrieved has been considered to reflect the estradiol milieu and to be a sign of luteal activity, the first reports could not find any association between the number of oocytes and the incidence of LBW or PTB (Griesinger et al. 2008, Pinborg et al. 2013b, Sazonova et al. 2011, Shih et al. 2008). However, a very recently published large study from the UK including over 65,000 singleton live births after IVF/ICSI showed that women with more than 20 oocytes retrieved exhibited an increased risk of having singletons born preterm and having
LBW (Sunkara et al. 2015). As mentioned by the authors, an excessive response has its own underlying pathogenesis based on a high ovarian reserve, and without doubt a high prevalence of women with polycystic ovary syndrome (PCOS) can be found in this category. Women with PCOS have an increased prevalence of adverse obstetric outcomes, including PTB and LBW (Boomsma et al. 2008, Qin et al. 2013). A pilot study by Imudia et al. (2013) found that among patients at high risk of OHSS who chose elective cryopreservation of all embryos, the rates of preeclampsia and SGA, defined as 10% for gestational age, were lower than in patients who chose to proceed with fresh ET (Imudia et al. 2013).

**IVF laboratory conditions and procedures**

During the embryo culture, the embryo is exposed to an artificial milieu, which aims to mimic natural conditions. The current data, primarily based on animal models in ART technologies, suggest that in vitro culture conditions—i.e., the culture media itself, variations in temperature or oxygen part pressure of the incubator and the duration of the culture—may affect embryonal gene expression or cause epigenetic modifications of the embryonic genome (Kleijkers et al. 2014). However, it is not known whether these changes have significant long-term effects and if the results from other species are applicable to human embryos (Pinborg et al. 2013b). Besides that, other IVF-laboratory interventions, such as ICSI, assisted hatching, and blastomer biopsy, are able to cause damage.

The studies on the significance of the culture medium to the well-being of the embryo and pregnancy outcome in ART have yielded contradictory results. In a very recently published meta-analysis, including 11 human studies, only five reported a significant difference in birthweight that may have depended on the culture medium (Zandstra et al. 2015). Although the latter studies had high sample sizes and rigorous study designs, there were clearly methodological limitations since the composition of the culture media varied considerably, making comparison somewhat ambiguous (Zandstra et al. 2015).

Prolonged in vitro cultures of blastocysts are increasingly being adopted by ART clinics over shorter-term in vitro cultures. The data on the consequences of blastocyst transfers on perinatal outcomes have now started to emerge, albeit with conflicting results. According to a meta-analysis (Dar et al. 2014), the risk was increased for PTB (four studies, 54,792 cleavage-stage and 20,724 blastocyst-stage births; AOR 1.32, 95% CI 1.19–1.46) and for CAs (two studies, 22,068 cleavage stage and 4517 blastocyst stage births; AOR 1.29, 95% CI 1.03–1.62) in singletons.
born after blastocyst transfers compared with singletons born after early-cleaved transfers. Another meta-analysis showed only an increased risk for PTB (RR 1.27, 95% CI 1.22–1.31) in blastocyst cycles (Maheshwari et al. 2013). However, the researchers in these two meta-analyses were not able to control for such potential confounding factors as maternal age and parity, as the number of published studies were small (Dar et al. 2014, Maheshwari et al. 2013). Further, blastocyst cultures have been shown to affect the incidence of monozygotic twinning (Chang et al. 2009) and placenta previa (Sazonova et al. 2011), but this was not found in a large Japanese cohort study (Ishihara et al. 2014). Differences in the male-to-female ratio have also been reported after blastocyst cultures (Chang et al. 2009). Additionally, the male gender is associated with increased birthweight (Kaartinen et al. 2015), and a longer embryo culture period significantly increased the incidence of large for gestational age (LGA) newborn (Makinen et al. 2013). A possible explanation for adverse perinatal outcomes after extended culture may trigger genetic and epigenetic changes in trophodermal cells that can lead to abnormal placentation and implantation (Maheshwari et al. 2016). Results from animal studies support this hypothesis (Rizos et al. 2002).

A meta-analysis including five studies showed a lower risk of PTB in ICSI versus IVF singletons (OR 0.80, 95% CI 0.69–0.93). Three studies found a significantly lower risk of PTB or LBW in ICSI versus IVF, while the rest showed similar outcomes and none showed adverse outcomes with regard to ICSI (Pinborg et al. 2013b). A recent Australian study (Marino et al. 2014) showed LBW, PTB, and neonatal deaths more common in singleton births from IVF and to a lesser degree in births from ICSI compared with SC singletons. Further, the use of only frozen-embryos eliminated all significant adverse pregnancy outcomes associated with ICSI, but not IVF (Marino et al. 2014).

2.4.3 Cryopreservation

The extensive use of FET has increased the awareness of the safety aspects of embryo freezing. Large population-based register studies from Scandinavian countries have revealed a significantly higher risk of being LGA in FET singletons than fresh ET singletons (Pinborg et al. 2010, Sazonova et al. 2012, Wennerholm et al. 2013). Furthermore, the Danish sibling cohort study with one singleton sibling born after fresh ET and the other after FET showed that the higher mean birthweight in FET singletons remained even in the same mother after adjusting for maternal age and birth order (Henningsen et al. 2011). This indicates that factors
apart from maternal characteristics also influence the higher birthweight in FET singletons.

The oocyte donation cycle is an excellent comparator for assessing the outcome of ART pregnancies. Galliano and colleagues (2015) studied the effect of embryo freezing on children’s size (n = 731), including women who underwent oocyte donation pregnancy leading to singleton births (>28 weeks) originating at least one birth from a fresh ET and one birth from an FET cycle. In that study, FET had no positive or negative effect on the duration of pregnancy or the birthweight of children, even after adjusting for maternal and laboratory characteristics (Galliano et al. 2015).

The duration of embryo cultures can vary before cryopreservation. In a large retrospective study in Japan (Ishihara et al. 2014) on the neonatal outcomes of 53,023 singletons born after frozen-thawed early cleaved (day 3) and frozen-thawed blastocyst (day 5) transfers, the results showed that FET-blastocyst transfers are associated with improved perinatal outcomes in terms of PTB, LBW, and SGA being less frequent, although the rates of LGA were increased. Further, frozen-thawed blastocyst-transfer pregnancies have increased risks of placenta accrete and pregnancy-induced hypertension (Ishihara et al. 2014).

There is limited information available on the health of children born following different freezing methods. Most studies have been carried out on cleavage-stage embryos after slow freezing, and data comparing children’s outcomes after newer cryopreservation techniques, such as the vitrification of embryos, are limited. The literature on vitrification has mainly focused on the cryopreservation of blastocyst-stage embryos. Reported data on children born after the transfer of day 3 vitrified embryos compared with those born after slow-freezing embryos are relatively rare. According to some of these studies (Kato et al. 2012, Liu et al. 2013, Rama Raju et al. 2009, Shi et al. 2012), children born following the transfer of vitrified embryos seem to have a significantly higher birthweight than those of fresh embryos and slow freezing.

### 2.4.4 The number of embryos transferred

The strategy of eSET and additional cycles with the transfer of FET has, to a large extent, overcome the problems associated with multiple pregnancies. Today, eSET with a cryopreservation program is an essential part of a cost-effective ART program (Veleva et al. 2009) and eSET is the recommended strategy in Europe, Australia, Japan, Canada, and the United States, but clinical practice shows little
adherence to the guidelines outside the Nordic countries. As stated in the report of ESHRE in 2011 three countries reported an SET rate of over 50% (Belgium 50.4%, Finland 67.5%, and Sweden 73.3%) (European IVF-Monitoring Consortium (EIM) et al. 2016). Currently, in Finland the proportion of SET was even higher (79.3% of IVF fresh ET cycles and 74.9% of ICSI fresh ET cycles) (THL 2015a) and the number of multiple births has decreased, as shown in Figure 4.

Fig. 4. The rate (%) of multiple births after IVF, ICSI, and FET in Finland 1992–2013 (THL 2015a).

Despite extensive knowledge about the risks of multiple pregnancies, many women still have more than one embryo replaced per treatment cycle and the rate of multiples conceived after ART is still high. From 2000 to 2011, the multiple delivery rates in Europe declined steadily from 26.9% to 19.4% compared to a multiple delivery rate of 30% in the United States (27.5% twin, 2.5% triplet or more deliveries) (European IVF-Monitoring Consortium (EIM) et al. 2016). The reason for that is a consequence of the heterogeneity of national clinical guidelines, national law, and a government ART reimbursement strategy (Henningsen & Pinborg 2014).

Vanishing twin pregnancies, which involve about 10–18% of pregnancies resulting from a double embryo transfer (DET) strategy (Poikkeus & Tiitinen 2008), have also contributed to the increase, particularly the adverse neonatal outcomes.
such as PTB and LBW in children born after IVF (Pinborg et al. 2005, Pinborg et al. 2007, Sunkara et al. 2015). According to meta-analyses, the occurrence of PTB in eSET or SET versus DET singletons showed no significant difference (Pinborg 2013b).

2.5 Singleton children born after FET

The first live birth after FET was in 1984 (Zeilmaker et al. 1984). Since then, concern has arisen regarding the safety of embryo freezing/thawing techniques. Both the procedures themselves and the cryoprotectants were suspected to cause an increased risk of fetal malformations or other obstetric outcomes (Pinborg 2012).

2.5.1 Obstetric outcomes

Large population-based cohort studies have shown that women pregnant after FET were older and more likely to be multiparous compared with women who had conceived after fresh ET (Sazonova et al. 2012, Wennerholm et al. 2013). The incidence of Caesarean section was higher in FET singletons, but after adjusting for background factors, the differences disappeared (Sazonova et al. 2012, Wennerholm et al. 2013).

Major pregnancy complications after FET pregnancies have been demonstrated in a few studies (Healy et al. 2010, Ishihara et al. 2014, Kaser et al. 2015, Opdahl et al. 2015, Sazonova et al. 2012, Wennerholm et al. 2013). The data from Sweden (Sazonova et al. 2012) including 2348 FET pregnancies noted an increased risk of preeclampsia in FET pregnancies compared with fresh ET pregnancies (AOR 1.32, 95% CI 1.07–1.63). A recently published large Nordic study (Opdahl et al. 2015) including 6444 FET cycles from Norway, Sweden, and Denmark showed a higher risk of hypertensive disorders such as pre-eclampsia in FET pregnancies (OR 1.24, 95% CI 1.11–1.37), and the risk was increased even comparing FET and fresh ET pregnancies within the same mother (AOR 2.39, 95% CI 1.48–3.86). No differences were found between the IVF or ICSI treatment.

Placental disturbances such as placenta accrete have been associated more with FET pregnancies than fresh ET (Ishihara et al. 2014, Kaser et al. 2015). However, two other studies (Healy et al. 2010, Sazonova et al. 2012) have found significantly lower rates for placenta previa in FET pregnancies compared with fresh ET pregnancies. Further FET pregnancies in artificial cycles have an increased risk for
post-partum hemorrhage compared with FET pregnancies in natural cycles [OR 2.2 (95% CI 1.6–2.9), AOR 1.8 (1.3–2.6)] (Healy et al. 2010).

When comparing pregnancies from FET cycles with pregnancies from SC pregnancies, the results of major obstetric complications are rare. The Swedish study (Sazonova et al. 2012) found a significantly higher rate of preeclampsia in FET pregnancies (AOR 1.25, 95% CI 1.03–1.51). Further, a large Nordic study group showed an increased risk for hypertensive disorders in singleton FET pregnancies than SC singleton pregnancies (Opdahl et al. 2015). For placenta previa, a significantly increased crude OR was noted but disappeared after adjusting for confounders like the year of birth, maternal age, parity, smoking, BMI, and years of involuntary childlessness. However, when comparing singleton pregnancies from FET-SET cycles with pregnancies after SC, significantly increased crude ORs were noted for preeclampsia and placenta previa, but disappeared after adjusting for the confounders (Sazonova et al. 2012).

### 2.5.2 Perinatal outcomes

Until 2009, only a few studies (Sutcliffe et al. 1995, Wennerholm et al. 1997, Westergaard et al. 1999) had been done including an appropriate control group examining perinatal outcomes after FET. These were all included in a systematic review with birthweight data on about 15,000 children (Wennerholm et al. 2009), and PTBs reported for 11,000 (Pinborg 2012, Wennerholm et al. 2009). Since then, three large population-based studies from Nordic countries including 9952 singletons’ perinatal data (Pinborg et al. 2010, Sazonova et al. 2012, Wennerholm et al. 2013) and two meta-analyses (Maheshwari et al. 2013, Pinborg et al. 2014) have been published. Table 3 shows the perinatal risks for adverse outcomes of singletons born after FET using slow freezing compared with fresh ET and SC singletons.
Table 3. The perinatal risks for the adverse outcome of singletons born after FET versus fresh ET and FET versus SC singletons. The results from large register-based studies where at least maternal age, parity, and child year of birth were matched or adjusted.

<table>
<thead>
<tr>
<th>Study details</th>
<th>Pinborg et al. 2010</th>
<th>Sazonova et al. 2012</th>
<th>Wennerholm et al. 2013</th>
<th>Pinborg et al. 2014¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Country</td>
<td>Denmark</td>
<td>Sweden</td>
<td>Denmark, Norway, Sweden</td>
<td>Denmark</td>
</tr>
<tr>
<td>FET, n</td>
<td>957</td>
<td>2348</td>
<td>6647</td>
<td>896</td>
</tr>
<tr>
<td>Fresh ET, n</td>
<td>10 399</td>
<td>8944</td>
<td>42 242</td>
<td>9840</td>
</tr>
<tr>
<td>SC, n</td>
<td>4800</td>
<td>571 914</td>
<td>288 542</td>
<td>4510</td>
</tr>
<tr>
<td>Matching or adjustments</td>
<td>Maternal age, parity, year of birth, sex</td>
<td>Maternal age, parity, year of birth, smoking, BMI, years of involuntary childlessness</td>
<td>Maternal age, parity, year of birth, sex, country</td>
<td>Maternal age, primiparity, year of birth</td>
</tr>
<tr>
<td>PTB, &lt; 37 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FET vs Fresh ET</td>
<td>0.70 (0.53–0.92)</td>
<td>0.93 (0.77–1.11)</td>
<td>0.84 (0.76–0.92)</td>
<td></td>
</tr>
<tr>
<td>FET vs SC</td>
<td>1.12 (0.96–1.32)</td>
<td>1.05 (0.88–1.25)</td>
<td>1.49 (1.35–1.63)</td>
<td></td>
</tr>
<tr>
<td>VPTB, &lt; 32 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FET vs Fresh ET</td>
<td>0.81 (0.52–1.27)</td>
<td>1.14 (0.77–1.69)</td>
<td>0.79 (0.66–0.95)</td>
<td></td>
</tr>
<tr>
<td>FET vs SC</td>
<td>1.35 (1.02–1.77)</td>
<td>1.20 (0.83–1.74)</td>
<td>2.68 (2.24–3.22)</td>
<td></td>
</tr>
<tr>
<td>LBW, &lt; 2500 g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FET vs Fresh ET</td>
<td>0.63 (0.45–0.87)</td>
<td>0.76 (0.60–0.95)</td>
<td>0.81 (0.71–0.91)</td>
<td></td>
</tr>
<tr>
<td>FET vs SC</td>
<td>1.06 (0.89–1.28)</td>
<td>0.88 (0.71–1.10)</td>
<td>1.27 (1.13–1.43)</td>
<td></td>
</tr>
<tr>
<td>VLBW, &lt; 1500 g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FET vs Fresh ET</td>
<td>0.66 (0.33–1.29)</td>
<td>1.07 (0.70–1.63)</td>
<td>0.87 (0.68–1.12)</td>
<td></td>
</tr>
<tr>
<td>FET vs SC</td>
<td>1.19 (0.79–1.80)</td>
<td>1.28 (0.87–1.88)</td>
<td>1.69 (1.33–2.15)</td>
<td></td>
</tr>
<tr>
<td>SGA, – 2 SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FET vs Fresh ET</td>
<td>0.78 (0.58–1.04)</td>
<td>0.72 (0.62–0.83)</td>
<td>0.56 (0.36–0.86)</td>
<td></td>
</tr>
<tr>
<td>FET vs SC</td>
<td>0.80 (0.60–1.05)</td>
<td>1.18 (1.03–1.35)</td>
<td>0.69 (0.42–1.15)</td>
<td></td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>---------------------</td>
<td>----------------------</td>
<td>------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>LGA, +2 SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FET vs Fresh ET</td>
<td>1.59 (1.26–1.99)</td>
<td>1.45 (1.27–1.64)</td>
<td>1.34 (0.98–1.80)</td>
<td></td>
</tr>
<tr>
<td>FET vs SC</td>
<td>1.48 (1.22–1.81)</td>
<td>1.29 (1.15–1.45)</td>
<td>1.41 (1.01–1.98)</td>
<td></td>
</tr>
<tr>
<td>Macrosomia, &gt; 4500g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FET vs Fresh ET</td>
<td>1.46 (1.15–1.85)</td>
<td>1.58 (1.39–1.80)</td>
<td>1.91 (1.40–2.62)</td>
<td></td>
</tr>
<tr>
<td>FET vs SC</td>
<td>1.29 (1.04–1.59)</td>
<td>1.29 (1.15–1.45)</td>
<td>1.67 (1.18–2.37)</td>
<td></td>
</tr>
<tr>
<td>Perinatal mortality ≥ 22 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FET vs Fresh ET</td>
<td>0.83 (0.36–1.90)</td>
<td>1.90 (1.03–3.54)</td>
<td>1.38 (1.02–1.88)</td>
<td></td>
</tr>
<tr>
<td>FET vs SC</td>
<td>1.69 (0.73–1.31)</td>
<td>1.13 (0.66–1.91)</td>
<td>1.41 (1.06–1.86)</td>
<td></td>
</tr>
<tr>
<td>Stillbirth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FET vs Fresh ET</td>
<td></td>
<td></td>
<td>1.24 (0.82–1.91)</td>
<td></td>
</tr>
<tr>
<td>FET vs SC</td>
<td></td>
<td></td>
<td>1.09 (0.75–1.58)</td>
<td></td>
</tr>
</tbody>
</table>

1Population is a subcohort of FET children, where data on perinatal outcomes have been published earlier. PTB = preterm birth, VTPB = very preterm birth, LBW = low birthweight, VLBW = very low birthweight, SGA = small for gestational age, LGA = large for gestational age.
Preterm birth

In three large cohort studies (Pinborg et al. 2010, Sazonova et al. 2012, Wennerholm et al. 2013), the PTB rate for FET and fresh ET singletons varied between 6.2–7.9% and 7.5–9.8, respectively. With the exception of the Swedish study (Sazonova et al. 2012), all studies, including two large register-based studies (Pinborg et al. 2010, Wennerholm et al. 2013) and two meta-analyses (Maheshwari et al. 2012, Pinborg et al. 2013b), have shown a lower risk of PTB in FET singletons compared with fresh ET singletons. Further, the large Nordic population-based cohort study including 6647 singletons born after FET in Denmark, Norway, and Sweden (Wennerholm et al. 2013) showed a reduced risk for VPTB in FET singletons compared with fresh ET singletons.

When comparing singletons born after FET with singletons after spontaneous conception, the results from population-based cohort studies are more conflicting. Sazonova et al. (2012) showed the PTB and VPTB crude OR was increased in FET singletons, and significances disappeared after adjusting for confounders (Sazonova et al. 2012). Only the Swedish study (Sazonova et al. 2012) reported that the rate of extreme PTB (< 28 weeks) was increased for singletons from FET (AOR 1.99; 95% CI 1.04–3.81). In a Danish study (Pinborg 2010), a significantly increased risk was found for VPTB (<32 weeks) in FET singletons while other significances disappeared after adjustments. In a recently published Nordic population-based study (Wennerholm et al. 2013), significantly increased risks for PTB and VPTB were detected for singletons from the FET singleton compared to the SC group. The differences in outcomes between these three large studies might be attributable to different adjustments for confounders and/or selection of controls. In the Swedish study, the adjustment was performed for years of infertility, which was not done in the Danish and Nordic cohort studies. Further, in a Swedish study, the control group of singletons from the SC group was not matched to the original FET population instead of using the complete Swedish births as controls.

Fetal growth

Since 2002, it has been known that the rate of SGA singletons originating from frozen-thawed embryos was half that of singletons from fresh ET (Olivennes et al. 2002). Correspondingly, an Australian group reported a lower mean z-score in fresh ET singletons than in those born after FET (Shih et al. 2008). A lower z-score means that there are more children with LBW at a given gestational age. A large
database from the Society of Assisted Reproductive Technology from United States, including 21,083 fresh ET and 10,982 FET singletons were born in 2004–2006, showed an increased risk for LBW in fresh ET children compared with FET children even when LBW was stratified by term and preterm deliveries (term LBW AOR 1.73, 95% CI 1.31–2.29, p<0.001; preterm LBW; AOR 1.49, 95% CI 1.24–1.78, p<0.001). In addition, a higher odds of SGA in singletons conceived after fresh ET compared with FET was demonstrated (6.73% vs 3.5%, respectively; AOR 1.71, 95% CI 1.46–2.00, p<0.001) (Kalra et al. 2011). Consistent with these early observations, the latest two large population-based cohort studies showed a lower risk of being SGA in FET singletons versus fresh ET singletons (Pinborg et al. 2014, Wennerholm et al. 2013) while a Swedish study showed no significantly lower risk of SGA in FET singletons (Sazonova et al. 2012). The large Nordic study found the increased risk for SGA in FET singletons when compared with SC singletons (Wennerholm et al. 2013). However, no risk for SGA was found in other large population-based studies (Pinborg et al. 2014, Sazonova et al. 2012).

Two population-based large reports and one meta-analysis revealed a significantly higher adjusted risk of being LGA in FET singletons than singletons from fresh ET (Pinborg et al. 2014, Sazonova et al. 2012, Wennerholm et al. 2013). Further, the Swedish and Nordic cohort studies showed the risk of macrosomia (>4500 g) in FET children (Sazonova et al. 2012, Wennerholm et al. 2013). In the sibling cohort with the first child fresh and the second child FET, the risk of LGA in the FET sibling was significantly increased compared with the sibling conceived after fresh ET (AOR 3.45 95% CI 1.33–8.33) (Pinborg et al. 2014). As mentioned by the authors, this could be related to the birth order, as the FET sibling was the second born. However, in the complete sibling cohort where the first child FET/second child fresh ET sibling pairs were included, the risk of being born LGA in the FET sibling was increased compared with the fresh ET sibling (AOR 2.50; 95% CI 1.04–5.88) (Pinborg et al. 2014).

Perinatal mortality and stillbirth

In the Swedish (Sazonova et al. 2012) and Nordic registry studies (Wennerholm et al. 2013), a higher perinatal mortality (gestation age ≥ 22 weeks) was found for singletons born after FET compared with the fresh ET cycles, while no significant differences were found in the Danish study, either compared with singletons born after fresh ET or compared with singletons in SC (Pinborg et al. 2010).
this finding must be interpreted with caution. It might be a random finding due to several statistical comparisons being performed. Furthermore, the absolute rate of perinatal mortality was low (0.3%), particularly in the Swedish study (Sazonova et al. 2012). In the Nordic cohort dataset (Wennerholm et al. 2013), the risk of perinatal mortality in FET singletons even exceeds that of the SC singletons (AOR 1.27; CI 95% 1.13–1.43), which was not the case in the other Scandinavian datasets (Pinborg et al. 2014, Sazonova et al. 2012).

2.5.3 Congenital anomalies and chromosomal disorders

On the basis of existing literature (Davies et al. 2012, Kallen et al. 2010b, Maheshwari et al. 2012, Pinborg et al. 2013a, Shih et al. 2008, Wennerholm et al. 2009), children born after FET using slow freezing have not shown any significant risk for CAs compared with children born after fresh ET. Only the Belgian hospital-based study showed a higher CA rate in FET-ICSI singletons than the fresh-ICSI group, 6.4% versus 3.1% (OR 2.15, 95% CI 1.10–4.20) (Belva et al. 2008). However, this finding has not been verified by other larger population-based cohort studies (Davies et al. 2012, Kallen et al. 2010b). Belva et al. (2008) reported the rate of chromosome disorders, and no significant differences in rates were reported for FET versus fresh ET children (Belva et al. 2008).

To provide a clearer understanding of the possible mechanisms associated with the noted increase of CAs in ART groups and to address the ascertainment issue as much as possible, researchers from Australia (Halliday et al. 2010) effectively demonstrated that blastogenesis defects were increased more for fresh ET than for FET singleton births, with the risk of fresh ET relative to controls being more than three-fold.

2.5.4 The other health outcomes

Controlled studies focusing on the early growth, mental development, and morbidity of children born after FET are sparse (Table 4).
Table 4. Comparison of controlled studies focusing on the growth, mental development, and morbidity of children born after FET using slow freezing.

<table>
<thead>
<tr>
<th>Study group, country</th>
<th>Study design</th>
<th>Participants</th>
<th>Study period</th>
<th>Matching criteria</th>
<th>Child outcome</th>
<th>Follow up period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sutcliffe et al. (1995), England</td>
<td>Prospective</td>
<td>91 FET-IVF (68 singletons, 20 twins, 3 triplets) 83 SC</td>
<td>1989–1994</td>
<td>Age, sex, social class, geographical region</td>
<td>Mental development similar between the study groups.</td>
<td>From birth to 2 years of age</td>
</tr>
<tr>
<td>Wennerholm et al. (1998), Sweden</td>
<td>Retrospective hospital-based</td>
<td>255 FET (158 singletons, 97 twins, 9 triplets) 255 IVF, 252 SC</td>
<td>1990–1995</td>
<td>Maternal age, parity, plurality, date of delivery</td>
<td>Growth, mental development and chronic illness similar between ART groups.</td>
<td>From birth to 18 months of age</td>
</tr>
<tr>
<td>Nakajo et al. (2004), Japan</td>
<td>Retrospective questionnaire-based</td>
<td>74 FET singletons, 64 IVF, 217 ICSI</td>
<td>1995–2003</td>
<td></td>
<td>Growth of ART children was normal at 2 years of age.</td>
<td>From birth to 2 years of age</td>
</tr>
<tr>
<td>Källen et al. (2005), Sweden</td>
<td>Retrospective population-based</td>
<td>1427 FET (singletons and twins) 16,280 IVF</td>
<td>1982–2001</td>
<td>Year of birth, maternal age, parity, smoking, years of unwanted childlessness</td>
<td>Cancer; 3 FET children had a cancer diagnoses, while 1.94 were expected.</td>
<td>5.5 years (median)</td>
</tr>
<tr>
<td>Pinborg et al. (2010), Denmark</td>
<td>Retrospective, population-based</td>
<td>957 FET (singletons), 10 329 fresh ET, 4800 SC</td>
<td>1995–2006</td>
<td>Maternal age, parity, gender, infant year of birth</td>
<td>No increased risk for neurological (mental retardation + cerebral palsy), cancer and imprinting-related diseases.</td>
<td>From births to 12 months–13 years (age distribution)</td>
</tr>
</tbody>
</table>
Wennerholm et al. (1998) performed the largest study of somatic health to date and found that the postnatal growth and health of FET children was normal and similar between fresh ET and SC groups (Wennerholm et al. 1998). Moreover, they analyzed the prevalence of chronic diseases, which did not differ between the FET, fresh ET, and SC groups (18.0%, 15.3%, and 16.7%, respectively). In a Danish register study (Pinborg et al. 2010), no differences were found for cerebral palsy, intellectual disability, imprinting diseases, or malignancies in FET singletons compared with both fresh ET and SC children. However, due to the size of the study group (957 FET children), the low prevalence rate of these rare diseases does not allow detailed analysis. The systematic review (Wennerholm et al. 2009) concludes that long-term child follow-up studies with sufficient sample sizes for all cryopreservation techniques are essential, although almost absent.
3 Aims of the present study

During the last decades, the role of cryopreservation in ART has increased as a result of more widespread use of the eSET policy to avoid multiple pregnancies. Effective cryopreservation also increases the cost-effectiveness of the ART programs (Veleva et al. 2009).

At the time when the present study was initiated, large population-based controlled studies of children born after FET compared with fresh ET were sparse (Wennerholm et al. 2009): only three small studies including SC children as controls were available (Sutcliffe et al. 1995, Wennerholm et al. 1997, Westergaard et al. 1999). This research was conducted to extend the previous studies and provide more detailed evidence on the safety of FET.

The specific aims of the present study were as follows:

1. To analyze the maternal serum estradiol and progesterone profiles in pregnancies after fresh ET, after FET during a natural menstrual cycle, and after SC during the first trimester, when trophoblastic invasion and early placentation occurs (study I).
2. To compare the perinatal outcomes, major CAs, and the early health of children born after FET and fresh ET using data from SC children as a reference group (studies II–IV).
4 Materials and methods

4.1 Study population

Detailed characteristics of the subjects according to each study are given in Table 5.

4.1.1 Study I

The whole study population consisted of 110 women with singleton pregnancies. The ART group included 30 pregnancies after FET (FET group) and 39 pregnancies after fresh ET (COH+ET group). The control group included 41 women after SC pregnancies (SC group). The participants for the ART groups were recruited from the Reproductive Unit of the Department of Obstetrics and Gynecology, Oulu University Hospital, and women with SC pregnancies were offered participation in the study when they attended prenatal clinics provided by the local authorities in 2005–2012. The criteria for exclusion were women with a history of gynecological pathology (e.g., endometriosis, fibroids, and pelvic surgery) or currently smoking, as well as mothers with multiple or vanishing twin pregnancies.

4.1.2 Studies II–IV

Women who underwent ART treatment with ET leading to birth (n= 5692) at the infertility clinics of Väestöliitto Fertility Clinics in Oulu and Helsinki and at the University Hospitals of Oulu and Helsinki in 1995–2006 were included in the study. Using the women’s personal identification numbers, the corresponding births were matched with data from the Finnish Medical Birth Register. A random sample of 10% of mothers with SC pregnancies (n= 29,885) from the Medical Birth Register served as a reference group, which was matched to the study groups regarding their area of residence and the year of birth of the child. Births from the frozen and fresh ET groups were excluded if women had received donated eggs, or sperm, or needed preimplantation genetic examinations. The other exclusions are presented in the flow chart (Figure 5). In original study II, the data included singleton and multiple births. In this thesis, the final data presented in studies II and III were restricted to singleton live and stillbirths and consisted of 1830 children born after FET, 2942 children born after fresh ET, and 31,243 children born after SC pregnancies. In
study IV the final data were restricted to singleton live births and consist 1825 children born after FET, 2933 children born after fresh ET, and 31,137 children born after SC pregnancies.

1ET (embryo transfer) includes women who underwent in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), or frozen embryo transfer after in vitro fertilization (FET–IVF), or frozen embryo transfer after intracytoplasmic sperm injection (FET–ICSI).

2The same woman may have had several deliveries.

3Fresh ET group includes fresh–IVF (n= 1978) and fresh–ICSI (n= 964) singletons.

4FET (frozen embryo transfer) group includes FET–IVF (n=1296) and FET–ICSI (n=534) singletons.

5A random sample of 10% of mothers with spontaneous pregnancies served as a reference group which was matched to the study groups as regards area of residence and year of birth of the child.

Fig. 5. Flow chart of studies II–IV.
Table 5. Maternal characteristics of singleton pregnancies after FET, fresh ET, and SC in studies I–IV.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study I</th>
<th>Studies II–IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FET n (%)</td>
<td>Fresh ET n (%)</td>
</tr>
<tr>
<td>Women, n</td>
<td>30</td>
<td>39</td>
</tr>
<tr>
<td>Mean age, years (±SD)</td>
<td>32.0 (0.8)</td>
<td>31.2 (0.7)</td>
</tr>
<tr>
<td>Nulliparous</td>
<td>20 (66.7)</td>
<td>31 (79.5)</td>
</tr>
<tr>
<td>Smoking during pregnancy</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Socio-economic status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper white-collar</td>
<td>582 (31.8)</td>
<td>914 (31.1)</td>
</tr>
<tr>
<td>Lower white collar</td>
<td>801 (43.8)</td>
<td>1258 (42.8)</td>
</tr>
<tr>
<td>Blue-collar</td>
<td>166 (9.1)</td>
<td>286 (9.7)</td>
</tr>
<tr>
<td>Other</td>
<td>281 (15.4)</td>
<td>484 (16.5)</td>
</tr>
</tbody>
</table>

4.1.3 ART protocols

Fresh ET cycles

Fresh ET cycles were performed using either the long GnRH agonist (studies I–IV) and/or the short GnRH antagonist protocol (Studies II–IV). The FSH stimulation was followed by oocyte retrieval 34–36 h after the hCG injection (5000–10,000 IU). Oocytes were fertilized using IVF or ICSI methods. Embryos were cultured as previously described (Hyden-Granskog et al. 2005, Martikainen et al. 2001). ETs were carried out on days 2 or 3 after oocyte retrieval, depending on the day of the week—on Monday if oocytes were collected on Friday and 2 days after ovum pickup in all other cases. Such a strategy has been adopted to minimize work during the weekends. Luteal support with vaginal micronized progesterone (600 mg/ day) or vaginal gel (8%, 1.125 g / twice per day) was started on the day of ET and used for two weeks.

FET cycles

Spare good-quality embryos were frozen on the day of fresh ET, using a slow-freezing protocol with 1, 2-propanediol as the cryoprotectant (Hyden-Granskog et al. 2005).
In study I, FET was performed during a natural menstrual cycle. The women having spontaneous ovulation measured their urinary LH surge with a home test kit. Depending on the day the embryo was frozen, ET was carried out 2–5 days after a positive ovulation test. Luteal support with vaginal micronized progesterone (400–600 mg / day) or vaginal gel (8%, 1.125 g / day) was started on the day of ET and used for two weeks.

In studies II–IV, FET was performed during a natural or artificial cycle. In artificial FET cycles, estradiol valerate or 17β-estradiol was administered orally at a daily dose of 4–6 mg. Vaginal micronized progesterone was started when the endometrial thickness was $\geq 6$ mm on cycle days 11–20 and treatment was continued until 9–12 gestational weeks after a positive pregnancy test result. In cases of a thinner endometrium, the estradiol dose was increased to 6–8 mg/day and progesterone administration was delayed until a thickness of $\geq 6$ mm was reached.

4.2 Study design

The study designs are summarized in Table 6. The data were collected from patient records (I) and from national health registers (II–IV). Studies III and IV were a continuation of the previous study (II). A detailed description of the data sources and assessed variables is given below.
Table 6. Study design according to the original publications.

<table>
<thead>
<tr>
<th>Study details</th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aim</td>
<td>Hormonal profiles in early pregnancies after fresh ET and FET</td>
<td>Perinatal outcomes of children born after fresh ET and FET</td>
<td>Major congenital anomalies of children born after fresh ET and FET</td>
<td>Physical health of children born after fresh ET and FET</td>
</tr>
<tr>
<td>Study design</td>
<td>Prospective</td>
<td>Register-based cohort</td>
<td>Register-based cohort</td>
<td>Register-based cohort</td>
</tr>
<tr>
<td>Data source</td>
<td>Women with IVF, ICSI, and FET pregnancies in the University Hospital of Oulu. Women with SC pregnancies in a local prenatal clinic</td>
<td>Women who underwent ART treatments leading to births in four infertility clinics – linked with MBR and Cause-of-Death Registry</td>
<td>Women who underwent ART treatments leading to births in four infertility clinics – linked with MBR, RCM and Register of Induced Abortions</td>
<td>Women who underwent ART treatments leading to live births in four infertility clinics – linked with MBR, HDR and Cause-of-Death Register</td>
</tr>
<tr>
<td>Number of subjects</td>
<td>FET n=30, fresh ET n=39, SC n=41</td>
<td>FET n=1830, fresh ET n=2942, SC n=31,243</td>
<td>FET n=1830, fresh ET n=2942, SC n=31,243</td>
<td>FET n = 1825, fresh ET n=2933, SC n=31,137</td>
</tr>
<tr>
<td>Time of follow up</td>
<td>First trimester of pregnancy</td>
<td>From the 22nd gestational week until one year of age</td>
<td>From birth until one year of age</td>
<td>From live birth until three years of age</td>
</tr>
</tbody>
</table>

MBR = Medical Birth Register, RCM = Register of Congenital Malformations, HDR = Hospital Discharge Register.
4.2.1 Clinical data (I)

The follow-up visits in pregnancy weeks 4–11 included blood sampling for hormonal assays (estradiol and progesterone).

Estradiol and progesterone assays (I)

After blood sampling, the serum was collected. Samples were stored at −80°C for subsequent analyses. A radioimmunoassay was used for the estradiol (Orion Diagnostica, Oulunsalo, Finland) according to the manufacturers’ instructions. Progesterone levels were analyzed using an automated chemiluminescence system (Advia Centaur; Siemens Healthcare Diagnostics, Tarrytown, NY, USA). The respective intra-assay and inter-assay coefficients of variation were 4.5% and 4.0% for estradiol and 4.7% and 8.4% for progesterone. The external quality control of the hormone assays was organized by national (Labquality Ltd, Helsinki) and international (Bio-Rad Laboratories EQAS, Irvine, CA, USA) companies.

4.2.2 Register data (II–IV)

Studies II, III, and IV were based on five national registries: the Medical Birth Registry, the Cause-of-Death Register at Statistics Finland, the Register of Congenital Malformations, the Register of Induced Abortions, and the Hospital Discharge Register. The different outcome measures and corresponding data sources for studies II, III, and IV are shown in Table 7.

The Medical Birth Register (Studies II–IV)

The Medical Birth Register is currently run by the National Institute for Health and Welfare (THL 2015c). Information for the Medical Birth Register is recorded by midwives at delivery hospitals and submitted to the THL. The Medical Birth Register identifies the mother and child by their unique personal identification numbers (IDs) and contains information on the maternal background and on the live and stillbirths until the age of 7 days, including all live and stillbirths after the 22nd gestational week and birthweight of 500 g or more. From 2004 onward, new variables were introduced in the Medical Birth Register, e.g., the complete set of all diagnoses during pregnancy and birth and the mother’s weight and length.
The Register of Congenital Malformations (THL 2015d) collects information on all live births, stillbirths, and selective terminations of pregnancies or spontaneous abortions with CAs. The data are collected through several data sources from hospitals, pathology departments, and cytogenetic laboratories and by linkages to several other nationwide health registers. CAs have been defined as major congenital structural anomalies, chromosomal defects, and as a few other birth defects like congenital hypothyroidism and teratomas.

CAs are classified and coded according to an extended version of the International Classification of Diseases, ICD-9. The CAs reported to the Register of Congenital Malformations, which do not qualify the criteria of major CAs, are not accepted to the register (rejected, not registered cases). The physician responsible for the Register of Congenital Malformations routinely classifies CAs into major, other, and rejected. Other anomalies reported to the register can, for example, be minor anomalies related to major CAs. Rejected anomalies include some minor CAs, as defined by the European Surveillance of Congenital Anomalies EUROCAT exclusion list. The Register of Congenital Malformations records all notified births/fetuses, but only those with at least one major CA are considered malformation cases. There is no upper age limit for data collection, but most cases are registered before the end of the second calendar year after birth or the termination of pregnancy (THL 2012d).

For study III, only cases with major CAs, as defined in the Register of Congenital Malformations, were included in the analysis using organ system classification (ICD-9). A case was counted only once in each organ system, but a case with multiple major anomalies may appear in several different groups according to the organ systems affected. To obtain the total prevalence for CAs, the medical geneticist responsible for the Register of Congenital Malformations reviewed all diagnosis and inclusion criteria, without information on the mode of conception of the births and fetuses from selective pregnancy terminations.

The Register of Induced Abortions (THL 2015e) collects information on induced abortions, and data are collected from all hospitals in Finland that perform induced abortions. The current legislation permits pregnancies to be terminated up to 20 weeks of gestation for social, medical, or ethical reasons and up to 24 weeks of
gestation in cases of a severe fetal disease or a structural anomaly that has been detected by a reliable prenatal diagnostic method. The healthcare unit performing the procedure is required to report the case to the National Institute for Health and Welfare (THL) within 1 month using a specific data collection form approved by the Ministry of Social Affairs and Health.

**Hospital Discharge Register (Study IV)**

The Hospital Discharge Register (THL 2015b) collects information on inpatient care as well as on visits to outpatient clinics. It gathers information of diagnoses, given as ICD–10 (since January 1, 1996) codes and dates of hospital admissions and discharges. The diagnoses included the main diagnoses (which can consist of separate diagnoses of the reasons and symptoms leading to hospitalization) and two secondary diagnoses (which can also consist of two separate diagnoses of the reasons and symptoms) for each hospital episode.

In study IV, the information on all visits up to the age of 3 years was received from the Hospital Discharge Register between 1995 and 2009 (the data on inpatient care are available from 1995 to 2009 and data on outpatient care from 1998 to 2009). The diagnoses included the main diagnoses and two secondary diagnoses for each hospital episode and all diagnoses and grouped them into 15 categories according to their severity and frequency. If the main and secondary diagnoses were related to the same ICD category, only one diagnosis was included. If the child was hospitalized more than once because of the same diagnosis, only the diagnoses at the first care episode were included. For morbidity, all diagnoses found in the Hospital Discharge Register were taken into account, focusing on the diseases that were most common during the different age periods. The numbers of admitted children and hospital visits and the length of the episodes were determined from the first day of hospitalization. When outpatient and inpatient care occurred on the same day, it was defined as inpatient care. The Hospital Discharge Register includes all hospital care days, excluding the discharges of healthy newborns from the delivery unit. The infant’s outpatient and inpatient care visits were studied in various age windows (0–6, 7–27, and 28–364 days; 1 year, 2 years, and 2–3 years of age).
The Cause-of-Death Register maintained by Statistics Finland contains data from death certificates and includes all deaths of Finnish citizens and permanent residents of Finland classified according to World Health Organization ICD-10 codes. The data are obtained from the death certificates, which are supplemented with the data from the population information system of the Central Population Register. The data in the Cause-of-Death Register cover persons who have died in Finland or abroad and who at the time of death were domiciled in Finland.

In study IV, the causes of deaths were categorized as ICD-10 codes into four categories: conditions originating from the perinatal period (ICD-10 chapter P), congenital malformations (ICD-10 chapter Q), other diseases and medical conditions (ICD-10 chapters A–R, excluding P and Q), and external causes (ICD-10 chapters V–Y). Furthermore, the deaths of children were counted in the abovementioned age periods.
<table>
<thead>
<tr>
<th>Outcome</th>
<th>Study</th>
<th>Data source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal outcome</td>
<td>II–IV</td>
<td>Medical Birth Register</td>
</tr>
<tr>
<td>Maternal age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of non-viable pregnancies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>miscarriage, ectopic pregnancy, induced abortion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Socioeconomic position (occupation when delivering)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking during pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obstetric outcomes</td>
<td>II–IV</td>
<td>Medical Birth Register</td>
</tr>
<tr>
<td>Induction of labor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mode of delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caesarean section</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placental disturbance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breech presentation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perinatal outcomes</td>
<td>II–IV</td>
<td>Medical Birth Register</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birthweight and length</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGA / LGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ponderal index (kg/m(^3))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Apgar scores at 1 min (0–6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Need for special care</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital care &lt; 7 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perinatal mortality rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Still births</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early neonatal death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infant mortality rate</td>
<td>II, IV</td>
<td>Cause-of-Death Register</td>
</tr>
<tr>
<td>Congenital anomalies</td>
<td>III</td>
<td>Register of Congenital Malformations</td>
</tr>
<tr>
<td>Induced abortions</td>
<td>III</td>
<td>Register of Induced Abortions</td>
</tr>
<tr>
<td>Physical health</td>
<td>IV</td>
<td>Hospital Discharge Register(^1)</td>
</tr>
<tr>
<td>Discharge diagnoses</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital admissions</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.3 Statistical methods

4.3.1 Early pregnancy data (I)

Power analysis was based on the assumption that the serum levels of progesterone are increased by 50% (estimated from the study by Johnson et al. 1994) at pregnancy weeks 5–6 in the fresh ET group in comparison with SC pregnancies (mean 70 nmol/l; with SD 50 nmol/l). The power analysis revealed that 22 subjects would be needed in each group for the study to have 80% power when an error was set at a significance level of 0.05. A total of 28 subjects needed to be recruited in each group after adjusting for a possible 20% dropout rate. The statistical analyses were performed from week 5 onwards since only a few SC pregnancies were recruited at week 4. The general linear model for repeated measures was employed to detect the differences within and between the groups. A p-value of < 0.05 was considered statistically significant. All steroid values in the results are given as the median and inter-quartile range (IQR).

4.3.2 Mother’s and children’s data (II–IV)

For studies on mothers and children, the differences between the groups were examined using tests for relative proportions, t-tests, and chi-square tests. A p-value of < 0.05 was considered statistically significant. Comparisons among the three study groups adjusted for children’s year of birth and maternal age, parity, and socioeconomic status (measured from maternal occupation) were run using logistic regression. Smoking and socioeconomic status are strongly correlated (Jaakkola et al. 2001), and therefore we used socioeconomic status in the logistic regression model. The results were displayed as unadjusted ORs and adjusted ORs (AORs) with 95% confidence intervals (CI). When the physical health of children was studied (study IV), the children were also stratified by premature birth. Subanalyses were also run (study II), comparing FET–IVF versus fresh IVF, FET–ICSI versus fresh ICSI, FET–IVF versus FET–ICSI, and each subgroup versus the SC group.

For study III, the power analysis with data on all major CAs among newborns showed that with 5% alpha error level and power of 80%, the required sample size is 1925 to detect the current difference between cases and controls. The Breslow-Day test was used to study the homogeneity of the ORs by sex, diagnoses, and type of treatment.
4.3.3 Ethical aspects

In study I, informed consent was obtained from each subject who participated in the study and only voluntary women were recruited. This study has been approved by the ethics committee of Oulu University Hospital, Oulu, Finland. The interventions (blood samples, ultrasound examinations) have proven to be safe and not to endanger the developing fetus.

In studies II, III, and IV, the study plan and the use of sensitive health register information were approved by the National Research and Development Center for Welfare and Health and Statistics Finland. For register linkages, the National Data Protection Authority was consulted and permission from the register keepers was obtained. The researchers had access to anonymous health register data only.
5 Results

5.1 Early pregnancy hormone profiles

Figure 6 illustrates mean serum estradiol and progesterone levels in women with pregnancies after COH+ET, FET, and SC in the early trimester. The statistical analyses were performed from weeks 5 to 11.

5.1.1 Estradiol levels

The serum estradiol was initially significantly higher at pregnancy week 5 in the COH+ET group (median, IQR: 4.1, 2.2–6.6 nmol/l) compared with the FET (0.7, 0.6–0.9 nmol/l; p<0.001) and SC groups (1.1, 0.7–1.6 nmol/l; p<0.001). The estradiol level at pregnancy week 11 was higher in the SC group (8.6, 6.9–11.1 nmol/l) than in the COH+ET group (5.3, 3.7–7.7 nmol/l; p<0.001) and the FET group (6.6, 4.1–8.1 nmol/l; p<0.002).

In the COH+ET group, the daily estradiol level between weeks 5 and 11 remained rather stable (change of 0.04, -0.01 to 0.08 nmol/l per day), whereas it increased in the SC group (0.18, 0.14–0.23 nmol/l per day; p<0.001) and FET group (0.12, 0.07–0.17 nmol/l per day; p<0.001); the increase was higher in the SC group than the FET group (p<0.03).

5.1.2 Progesterone levels

The progesterone level at pregnancy week 5 was higher in the COH+ET group than the FET and SC groups (median, IQR: 312, 183–480 nmol/l versus 74, 48–96 nmol/l versus 63, 52–80 nmol/l; p<0.001), and it remained higher than in the FET and SC groups, even at week 11 (189, 124–260 nmol/l versus 101, 78–120 nmol/l versus 115, 80–139 nmol/l; p<0.001).

In the COH+ET group, the daily progesterone level decreased 3.0 (1.3–5.0) nmol/l per day between weeks 5 and 11, whereas it increased in the FET group 0.6 (0.2–1.4) nmol/l and the SC group 0.9 (0.2–1.7) nmol/l per day (p<0.001).
Fig. 6. Mean serum progesterone and estradiol levels (error bars representing SE) from pregnancy weeks 4 to 11 in women with pregnancies after spontaneously conceived pregnancies (SPONT, n=41), controlled ovarian hyperstimulation and fresh embryo transfer (COH+ET, n=39) and frozen embryo transfer in spontaneous menstrual cycle (FET SPONT, n=30).

5.2 Obstetric outcome (II)

Age differences between the FET, fresh ET, and SC mothers were statistically significant, mothers in the FET group being the oldest (p<0.001). The frequency of
nulliparous women was highest in the fresh ET group (72.4% versus 55.0% in the FET group and 51.3% in the SC group, p<0.0001). The SES based on maternal occupation and smoking during pregnancy between FET and fresh ET mothers did not differ (Table 5).

More Caesarean sections were performed in the FET group (28.4%) than in the fresh ET group (27.8%). However, after adjusting for background factors (year of birth, maternal age, parity, and SES), the difference disappeared between the FET and fresh ET groups (AOR 1.01, 95% CI 0.90–1.14). The risk of placental disturbance (placental abruption and placenta previa) did not differ significantly between the two ART groups (AOR 0.75, 95% CI 0.45–1.25), but in comparison with the reference group, an increased risk of placental disturbances was found in the FET (AOR 2.44; CI 1.52–3.93) group. Table 8 illustrates obstetric outcomes after FET, fresh ET, and SC.

Table 8. Obstetric outcomes in singletons born after FET, fresh ET, and SC pregnancies.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>FET (%)</th>
<th>Fresh ET (%)</th>
<th>p-value</th>
<th>SC (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancies, n</td>
<td>1830</td>
<td>2942</td>
<td></td>
<td>31,243</td>
<td></td>
</tr>
<tr>
<td>Induction of labor</td>
<td>360 (19.7)</td>
<td>504 (17.1)</td>
<td>0.027</td>
<td>4730 (15.2)</td>
<td>0.004</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>519 (28.4)</td>
<td>27.8 (27.8)</td>
<td>0.692</td>
<td>5087 (16.3)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Placental abruption</td>
<td>3 (0.2)</td>
<td>6 (0.2)</td>
<td>1.000</td>
<td>55 (0.2)</td>
<td>0.684</td>
</tr>
<tr>
<td>Placenta previa</td>
<td>16 (0.9)</td>
<td>38 (1.3)</td>
<td>0.207</td>
<td>57 (0.2)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

5.3 Perinatal outcomes (II)

**FET versus fresh ET children**

The mean birthweight [3551±585 g (SD) vs 3417 ±605 g (SD), p<0.0001] and mean gestational age [277±14 (SD) days vs 275±15 days, p<0.0001] were higher in singletons born after FET than in singletons born after fresh ET. When stratifying for sex, the mean birthweight was significantly higher for both genders in the FET group than the fresh ET group [FET vs fresh ET boys; 3612± 586 g (SD) vs 3476± 606 g (SD), p<0.001 and FET vs fresh ET girls; 3487± 577 g (SD) vs 3354± 596 g, (SD), p<0.001].

Singletons born after FET had a reduced risk of LBW, PTB, and SGA, but the FET group revealed a significantly increased risk of LGA compared with singletons born after fresh ET. Further, more infants were macrosomic (> 4500 g) in the FET group. The stillbirth, perinatal mortality, and infant mortality rates of singletons did
not differ between the FET and fresh ET groups. The perinatal outcomes in singletons born after FET and fresh ET are shown in Table 9.

In the sub-analysis, where the different treatment types were compared, the singletons born after FET-IVF had a reduced risk for LBW (AOR 0.70, 95% CI 0.57–0.86) and SGA (AOR 0.53, 95% CI 0.39–0.73), but an increased risk for LGA (AOR 1.71, 95% CI 1.12–2.59) compared with singletons born after FET-ICSI. However, no differences were observed regarding LBW (AOR 0.87, 95% CI 0.64–1.19); PTB (AOR 0.76, 95% CI 0.57–1.01), and SGA (AOR 1.00, 95% CI 0.61–1.61) between the FET-ICSI and fresh ICSI singleton groups.

**FET versus SC children**

The FET newborns were 12 g heavier than the SC children [3551±585 g (SD) vs 3539±556 g (SD); p<0.0001] and the mean gestational age was one day shorter in the FET children than in the SC children [277±14 (SD) days vs.278±13 (SD) days, p<0.0001].

Singletons born after FET had an increased risk of PTB and LBW compared with the SC singletons. Further, the neonatal morbidity rate was clearly higher among FET singletons than in the SC singletons when defined as the need for special care and hospital care more than seven days after birth. The perinatal outcomes in singletons born after FET and SC are show in Table 10.
Table 9. Adverse perinatal outcomes for singletons born after FET (n= 1830) and fresh ET (n= 2942).

<table>
<thead>
<tr>
<th>Outcome</th>
<th>FET (%)</th>
<th>SC (%)</th>
<th>p-value</th>
<th>Crude OR</th>
<th>95% CI</th>
<th>AOR 1</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTB, &lt; 32 weeks</td>
<td>17 (0.9)</td>
<td>42 (1.4)</td>
<td>0.130</td>
<td>0.65</td>
<td>0.37–1.14</td>
<td>0.58</td>
<td>0.34–0.97</td>
</tr>
<tr>
<td>LBW, &lt; 2500 g</td>
<td>60 (3.3)</td>
<td>141 (4.8)</td>
<td>0.011</td>
<td>0.67</td>
<td>0.49–0.92</td>
<td>0.68</td>
<td>0.51–0.90</td>
</tr>
<tr>
<td>VLBW, &lt; 1500 g</td>
<td>16 (0.9)</td>
<td>36 (1.2)</td>
<td>0.258</td>
<td>0.71</td>
<td>0.39–1.29</td>
<td>0.79</td>
<td>0.43–1.44</td>
</tr>
<tr>
<td>SGA, &lt; 2SD</td>
<td>28 (1.5)</td>
<td>91 (3.1)</td>
<td>&lt;0.0001</td>
<td>0.49</td>
<td>0.32–0.75</td>
<td>0.51</td>
<td>0.33–0.79</td>
</tr>
<tr>
<td>LGA, &gt; 2 SD</td>
<td>66 (3.6)</td>
<td>60 (2.1)</td>
<td>&lt;0.0001</td>
<td>1.80</td>
<td>1.26–2.56</td>
<td>1.77</td>
<td>1.23–2.53</td>
</tr>
<tr>
<td>Very LGA, &gt; 3 SD</td>
<td>12 (0.7)</td>
<td>13 (0.4)</td>
<td>0.320</td>
<td>1.49</td>
<td>0.68–3.27</td>
<td>1.44</td>
<td>0.65–3.19</td>
</tr>
<tr>
<td>Macrosomia, &gt; 4500 g</td>
<td>69 (3.8)</td>
<td>52 (1.8)</td>
<td>&lt;0.0001</td>
<td>2.18</td>
<td>1.51–3.14</td>
<td>2.15</td>
<td>1.48–3.10</td>
</tr>
<tr>
<td>Need for special care</td>
<td>386 (21.1)</td>
<td>640 (21.9)</td>
<td>0.559</td>
<td>0.96</td>
<td>0.63–1.11</td>
<td>1.12</td>
<td>0.96–1.30</td>
</tr>
<tr>
<td>Ventilator treatment</td>
<td>28 (1.5)</td>
<td>64 (2.2)</td>
<td>0.115</td>
<td>0.70</td>
<td>0.45–1.09</td>
<td>0.72</td>
<td>0.45–1.13</td>
</tr>
<tr>
<td>Hospital care, &lt; 7 days</td>
<td>148 (8.1)</td>
<td>248 (8.6)</td>
<td>0.677</td>
<td>0.96</td>
<td>0.77–1.18</td>
<td>0.95</td>
<td>0.78–1.16</td>
</tr>
<tr>
<td>Stillbirth, ≥ 22 weeks</td>
<td>5 (0.3)</td>
<td>9 (0.3)</td>
<td>1.000</td>
<td>0.89</td>
<td>0.30–2.67</td>
<td>0.94</td>
<td>0.31–2.84</td>
</tr>
<tr>
<td>Perinatal mortality 2</td>
<td>8 (0.4)</td>
<td>16 (0.5)</td>
<td>0.659</td>
<td>0.80</td>
<td>0.34–1.88</td>
<td>0.84</td>
<td>0.36–1.98</td>
</tr>
<tr>
<td>Infant mortality 3</td>
<td>3 (0.16)</td>
<td>7 (0.24)</td>
<td>0.750</td>
<td>0.46</td>
<td>0.15–1.39</td>
<td>0.66</td>
<td>0.30–1.44</td>
</tr>
</tbody>
</table>

1Adjusted for parity, years of birth, maternal age, and SES. 2Stillbirth from gestational age 22+0 to early neonatal death day 0–6. 3From live birth to neonatal death days 0-6. PTB = preterm birth, VTPB = very preterm birth, LBW = low birthweight, VLBW = very low birthweight, SGA = small for gestational age, LGA = large for gestational age
Table 10. Adverse perinatal outcomes for singletons born after FET (n= 1830) and SC (n= 31,243).

<table>
<thead>
<tr>
<th>Outcome</th>
<th>FET (%)</th>
<th>SC (%)</th>
<th>p-value</th>
<th>Crude OR</th>
<th>95% CI</th>
<th>AOR(^1)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTB, &lt; 37 weeks</td>
<td>103 (5.6)</td>
<td>1177 (3.8)</td>
<td>&lt; 0.0001</td>
<td>1.52</td>
<td>1.24–1.87</td>
<td>1.44</td>
<td>1.18–1.76</td>
</tr>
<tr>
<td>VPTB, &lt; 32 weeks</td>
<td>17 (0.9)</td>
<td>236 (0.8)</td>
<td>0.407</td>
<td>1.23</td>
<td>0.75–2.02</td>
<td>1.05</td>
<td>0.66–1.67</td>
</tr>
<tr>
<td>LBW, &lt; 2500 g</td>
<td>60 (3.3)</td>
<td>788 (2.5)</td>
<td>0.047</td>
<td>1.31</td>
<td>1.00–1.71</td>
<td>1.29</td>
<td>1.01–1.65</td>
</tr>
<tr>
<td>VLBW, &lt; 1500 g</td>
<td>16 (0.9)</td>
<td>203 (0.6)</td>
<td>0.250</td>
<td>1.35</td>
<td>0.81–2.25</td>
<td>1.22</td>
<td>0.72–2.06</td>
</tr>
<tr>
<td>SGA, &lt; 2SD</td>
<td>28 (1.5)</td>
<td>661 (2.1)</td>
<td>&lt; 0.0001</td>
<td>0.72</td>
<td>0.49–1.05</td>
<td>0.69</td>
<td>0.47–1.02</td>
</tr>
<tr>
<td>LGA, &gt; 2 SD</td>
<td>66 (3.6)</td>
<td>891 (2.9)</td>
<td>&lt; 0.0001</td>
<td>1.27</td>
<td>0.99–1.64</td>
<td>1.10</td>
<td>0.85–1.43</td>
</tr>
<tr>
<td>Very LGA, &gt; 3 SD</td>
<td>12 (0.7)</td>
<td>149 (0.5)</td>
<td>0.285</td>
<td>1.38</td>
<td>0.76–2.49</td>
<td>1.34</td>
<td>0.73–2.45</td>
</tr>
<tr>
<td>Macrosomia, &gt; 4500 g</td>
<td>69 (3.8)</td>
<td>983 (3.2)</td>
<td>0.139</td>
<td>1.21</td>
<td>0.94–1.55</td>
<td>1.06</td>
<td>0.83–1.37</td>
</tr>
<tr>
<td>Need for special care</td>
<td>386 (21.1)</td>
<td>4015 (12.8)</td>
<td>&lt; 0.0001</td>
<td>1.81</td>
<td>1.61–2.04</td>
<td>1.85</td>
<td>1.63–2.09</td>
</tr>
<tr>
<td>Ventilator treatment</td>
<td>28 (1.5)</td>
<td>317 (1.0)</td>
<td>&lt; 0.0001</td>
<td>1.52</td>
<td>1.03–2.24</td>
<td>1.45</td>
<td>0.97–2.16</td>
</tr>
<tr>
<td>Hospital care, &lt; 7 days</td>
<td>148 (8.1)</td>
<td>1510 (4.8)</td>
<td>&lt; 0.0001</td>
<td>1.73</td>
<td>1.45–2.07</td>
<td>1.61</td>
<td>1.36–1.92</td>
</tr>
<tr>
<td>Stillbirth, ≥ 22 weeks</td>
<td>5 (0.3)</td>
<td>105 (0.3)</td>
<td>1.000</td>
<td>0.81</td>
<td>0.33–2.00</td>
<td>0.87</td>
<td>0.35–2.16</td>
</tr>
<tr>
<td>Perinatal mortality(^2)</td>
<td>8 (0.4)</td>
<td>169 (0.5)</td>
<td>1.000</td>
<td>0.81</td>
<td>0.40–1.64</td>
<td>0.92</td>
<td>0.45–1.90</td>
</tr>
<tr>
<td>Infant mortality(^2)</td>
<td>3 (0.16)</td>
<td>64 (0.3)</td>
<td>0.672</td>
<td>0.64</td>
<td>0.23–1.73</td>
<td>0.82</td>
<td>0.41–1.61</td>
</tr>
</tbody>
</table>

\(^1\)Adjusted for parity, years of birth, maternal age, and SES.  
\(^2\)Stillbirth from gestational age 22+0 to early neonatal death day 0–6.  
\(^3\)From live birth to neonatal death days 0-6. LBW = low birthweight, VLBW = very low birthweight, PTB = preterm birth, VTPB = very preterm birth; SGA = small for gestational age, LGA = large for gestational age.
At least one major CA was reported in 77 cases (4.2%) in the singletons born after FET, in 132 cases (4.5%) in the singletons born after fresh ET, and in 994 cases (3.2%) in the singletons born after SC. In the sub-analyses, the prevalence of major CAs for the four different groups was as follows: FET-ICSI 5.1%, fresh-ICSI 5.0%, fresh-IVF 3.0%, and FET-IVF 3.9%. During the years 1995–2006, the prevalence of terminated pregnancies for major fetal anomalies per all births did not differ between the ART groups (FET; n=13, 0.57% and fresh ET; n= 20, 0.48%). The main indication for terminations in both ART groups was chromosomal anomalies (FET; n=7 and fresh ET n= 13). The number of major CAs and the number of CAs in specific organ systems in FET, fresh ET, and SC singletons are shown in Table 11. The risk for at least one major CA of the singletons born after FET was not increased compared with the singletons born after fresh ET, even after adjusting for maternal age, parity, SES, and year of birth (AOR 0.95, 95% CI 0.71–1.27). When comparing the risk according to the different organ systems affected, no significant increased risk was found between these two ART groups (Table 12).

The risk for at least one major CA was slightly higher in FET singletons than in SC singletons (OR 1.34, 95% CI 1.5–1.69); however, after adjusting for maternal age, parity, SES, and year of birth the risk disappeared (AOR 1.21, 95% CI 0.95–1.54) (Table 11). When comparing the risk for specific organ systems, urogenital anomalies was increased, particularly in FET boys (AOR 2.95, 95% CI 1.08–3.90) as well as fresh ET boys (AOR 1.89, 95% CI 1.09–3.28) (Table 12).
Table 11. Number of major CAs by the organ systems\(^1\) affected in singletons born after FET versus fresh ET and FET versus SC.

<table>
<thead>
<tr>
<th>Variable studied</th>
<th>FET</th>
<th>Fresh ET</th>
<th>p-value</th>
<th>OR</th>
<th>95% CI</th>
<th>SC</th>
<th>p-value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singletons</td>
<td>1830</td>
<td>2942</td>
<td>0.647</td>
<td>0.94</td>
<td>0.70–1.25</td>
<td>31,243</td>
<td>994</td>
<td>0.016</td>
<td>1.34</td>
</tr>
<tr>
<td>Singletons with CA (%)</td>
<td>77 (4.2)</td>
<td>132 (4.5)</td>
<td></td>
<td></td>
<td></td>
<td>994 (3.2)</td>
<td>0.016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All organ systems affected:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central nervous system</td>
<td>1</td>
<td>5</td>
<td>0.274</td>
<td>0.32</td>
<td>0.04–2.75</td>
<td>47</td>
<td>0.295</td>
<td>0.36</td>
<td>0.05–2.63</td>
</tr>
<tr>
<td>Eye, ear, face, and neck</td>
<td>2</td>
<td>9</td>
<td>0.168</td>
<td>0.36</td>
<td>0.08–1.65</td>
<td>44</td>
<td>0.725</td>
<td>0.39</td>
<td>0.05–2.82</td>
</tr>
<tr>
<td>Cardiovascular(^2)</td>
<td>27</td>
<td>37</td>
<td>0.459</td>
<td>1.18</td>
<td>0.71–1.94</td>
<td>320</td>
<td>0.066</td>
<td>1.45</td>
<td>0.97–2.15</td>
</tr>
<tr>
<td>Other circulatory system(^3)</td>
<td>1</td>
<td>3</td>
<td>0.583</td>
<td>0.54</td>
<td>0.06–5.15</td>
<td>4</td>
<td>0.157</td>
<td>4.27</td>
<td>0.48–38.2</td>
</tr>
<tr>
<td>Respiratory system</td>
<td>3</td>
<td>2</td>
<td>0.319</td>
<td>2.41</td>
<td>0.40–14.46</td>
<td>12</td>
<td>0.014</td>
<td>4.27</td>
<td>1.20–15.2</td>
</tr>
<tr>
<td>Cleft palate and cleft lip</td>
<td>6</td>
<td>7</td>
<td>0.562</td>
<td>1.38</td>
<td>0.46–4.11</td>
<td>63</td>
<td>0.250</td>
<td>1.63</td>
<td>0.70–3.77</td>
</tr>
<tr>
<td>Digestive system</td>
<td>5</td>
<td>6</td>
<td>0.627</td>
<td>1.34</td>
<td>0.41–4.40</td>
<td>58</td>
<td>0.404</td>
<td>1.18</td>
<td>0.43–3.25</td>
</tr>
<tr>
<td>Urogenital</td>
<td>16</td>
<td>21</td>
<td>0.681</td>
<td>1.23</td>
<td>0.64–2.36</td>
<td>126</td>
<td>0.007</td>
<td>2.20</td>
<td>1.30–3.70</td>
</tr>
<tr>
<td>Musculoskeletal system</td>
<td>13</td>
<td>25</td>
<td>0.929</td>
<td>0.83</td>
<td>0.43–1.64</td>
<td>191</td>
<td>0.166</td>
<td>1.16</td>
<td>0.66–2.04</td>
</tr>
<tr>
<td>Integument (skin, hair, and nails)</td>
<td>2</td>
<td>7</td>
<td>0.319</td>
<td>0.46</td>
<td>0.10–2.21</td>
<td>47</td>
<td>0.656</td>
<td>0.36</td>
<td>0.05–2.63</td>
</tr>
<tr>
<td>Chromosomal anomalies</td>
<td>10</td>
<td>10</td>
<td>0.283</td>
<td>1.61</td>
<td>0.67–3.88</td>
<td>70</td>
<td>0.006</td>
<td>2.45</td>
<td>1.26–4.75</td>
</tr>
<tr>
<td>Other CAs</td>
<td>3</td>
<td>8</td>
<td>0.449</td>
<td>0.60</td>
<td>0.16–2.27</td>
<td>82</td>
<td>0.418</td>
<td>0.83</td>
<td>0.30–2.27</td>
</tr>
</tbody>
</table>

\(^1\)If a child had a major CA in more than one system, the child appears several times in the table. If the CAs affect the same organ system, the child appears only once in the table. \(^2\)Cardiovascular includes diagnoses codes (ICD–09) 745 Bulbus cordis anomalies and anomalies of cardiac septal closure and 746 Other CAs of heart. \(^3\)Other circulatory system includes diagnose code (ICD–09) 747 Other CAs in circulatory systems.
Table 12. Risk for major CAs in singletons and separately in different organ systems\(^1\) affected in FET and fresh ET groups versus the SC group.

<table>
<thead>
<tr>
<th>Variable studied</th>
<th>Total</th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Singletons with CA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SC</td>
<td>994</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>FET</td>
<td>77</td>
<td>1.34</td>
<td>1.05–1.69</td>
</tr>
<tr>
<td>Fresh ET</td>
<td>132</td>
<td>1.43</td>
<td>1.19–1.72</td>
</tr>
<tr>
<td>Organ systems affected:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular(^3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SC</td>
<td>320</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>FET</td>
<td>27</td>
<td>1.45</td>
<td>0.97–2.15</td>
</tr>
<tr>
<td>Fresh ET</td>
<td>37</td>
<td>1.23</td>
<td>0.87–1.73</td>
</tr>
<tr>
<td>Urogenital</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SC</td>
<td>126</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>FET</td>
<td>16</td>
<td>2.18</td>
<td>1.29–3.67</td>
</tr>
<tr>
<td>Fresh ET</td>
<td>21</td>
<td>1.78</td>
<td>1.12–2.82</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SC</td>
<td>191</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>FET</td>
<td>13</td>
<td>1.16</td>
<td>0.66–2.04</td>
</tr>
<tr>
<td>Fresh ET</td>
<td>25</td>
<td>1.39</td>
<td>0.92–2.12</td>
</tr>
<tr>
<td>Chromosomal anomalies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SC</td>
<td>70</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>FET</td>
<td>10</td>
<td>2.45</td>
<td>1.26–4.75</td>
</tr>
<tr>
<td>Fresh ET</td>
<td>10</td>
<td>3.05</td>
<td>1.85–5.02</td>
</tr>
</tbody>
</table>

\(^1\)If a child had a major CA in more than one system, the child appears several times in the table. If the CAs affect the same organ system, the child appears only once in the table. \(^2\)Adjusted by year of birth, maternal age, parity, and SES. \(^3\)Cardiovascular includes diagnoses codes (ICD 09) 745 Bulbus cordis anomalies and anomalies of cardiac septal closure and 746 Other CAs of heart.
5.5 Physical health of children (IV)

For 15 out of 16 categories of diseases according to ICD–10 classifications, only the risk of mental and behavioral diseases was increased in FET children after adjusting for maternal age, parity, SES, and year of birth (AOR 1.51; 1.19–1.93) when compared with fresh ET children. However, after adding PTBs into the model, the risk disappeared (AOR 0.83; 0.57–1.21). The most common discharge diagnoses, including gastroenteritis and colitis, otitis, upper and lower respiratory diseases, asthma, and allergies, did not significantly differ when compared with FET and fresh ET children up to 3 years of age (Table 13).

A large proportion of FET children (70.1%) and fresh ET children (69.9%) had visited a hospital at least once (p=0.877) until three years of age. The cumulative time spent in a hospital was significantly shorter in the FET than in the fresh ET children (8.8 vs 9.3 days, p<0.001). However, in the adjusted analyses, the risk of hospital admission (both in- and outpatient) did not differ significantly between these study groups after adjusting for maternal age, parity, year of birth, and SES (AOR 0.98, CI 0.85–1.13) and even after adding the prematurity into analyses (AOR 1.01, CI 0.88–1.17). When studying in- and outpatient admissions separately, no increased risks were found between the two ART groups (AOR 1.00, CI 0.89–1.12 for inpatient care and AOR 1.01, CI 0.91–1.13 for outpatient care).
Table 13. The most common discharge diagnoses recorded (according ICD-10 grouping) comparing children born after FET and fresh ET up to three years of age.

<table>
<thead>
<tr>
<th>Variable studied</th>
<th>FET</th>
<th>Fresh ET</th>
<th>p–value</th>
<th>FET vs Fresh ET</th>
<th>FET vs Fresh ET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n¹</td>
<td>%</td>
<td>n¹</td>
<td>%</td>
<td>OR 95% CI</td>
</tr>
<tr>
<td>Singleton live births</td>
<td>1825</td>
<td>2933</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnoses (ICD–10 codes):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastroenteritis and colitis (A08–A09)</td>
<td>153</td>
<td>8.4</td>
<td>267</td>
<td>9.1</td>
<td>0.395</td>
</tr>
<tr>
<td>Otitis (H65–66)</td>
<td>235</td>
<td>12.9</td>
<td>309</td>
<td>10.5</td>
<td>0.014</td>
</tr>
<tr>
<td>Upper respiratory disease (J04–J06)</td>
<td>146</td>
<td>8.0</td>
<td>223</td>
<td>7.6</td>
<td>0.619</td>
</tr>
<tr>
<td>Lower respiratory disease (J12–18, J20–21)</td>
<td>142</td>
<td>7.8</td>
<td>188</td>
<td>6.4</td>
<td>0.070</td>
</tr>
<tr>
<td>Chronic disease of tonsils and adenoids (J35)</td>
<td>82</td>
<td>4.5</td>
<td>131</td>
<td>4.5</td>
<td>0.965</td>
</tr>
<tr>
<td>Asthma (J45–J46)</td>
<td>48</td>
<td>2.6</td>
<td>70</td>
<td>2.4</td>
<td>0.599</td>
</tr>
<tr>
<td>Allergy (L20–L23, L25, L27)</td>
<td>115</td>
<td>6.3</td>
<td>177</td>
<td>6.0</td>
<td>0.709</td>
</tr>
</tbody>
</table>

¹A child is counted only once in the incidence; repeated events are not taken into account. ²Adjusted for year of birth, sex of child, maternal age, parity, socio-economic status, and premature birth. Fresh ET= 1.0.
Figure 7 illustrates the percentage of children with hospital admissions (in- and outpatient) in three study groups according to the children’s age at first admission in 1998–2009. After adjusting for maternal age, parity, SES, year of birth, and premature birth, the hospital admission risk per child in different time windows significantly increased only in FET children during the early neonatal period (0–6 days) compared with fresh ET children (p=0.735, AOR 1.25; CI 1.04–1.49). In the sub-analysis, the term FET children had significantly more respiratory distress as newborns (ICD–10 code P22) than the term fresh ET children (1.8% vs 0.9%, p=0.011) during their first week of life.

The risk of hospital admission (both in- and outpatient) was significantly higher in the children born after ART than after SC pregnancies (69.9% vs 67.5%, p<0.001, AOR 1.12, 95% CI 1.04–1.21). These results did not change even after adjusting for premature births (AOR 1.10, 95% CI 1.02–1.19). In different age windows other than the late neonatal period (7–27 days, p=0.084, AOR 1.06, 95% CI 0.95–1.19), term ART children had a slightly increased risk for hospitalization compared with SC children (data shown in original article IV).

The mortality rate for live births up to the age of three years among singletons did not significantly differ between the FET and fresh ET groups (p=0.278). The
highest mortality was found during the early neonatal period (FET 1.6/1000 vs fresh ET 2.4/1000, p=0.586), and the main causes were conditions originating in the perinatal period and CAs.
6 Discussion

The first child after FET was born over 30 years ago (Zeilmaker et al. 1984) and since then, both the freezing and thawing procedures along with the use of various cryoprotectants have been suspected to cause an increased risk of fetal CA or other adverse obstetric outcomes. To date, the knowledge of perinatal outcomes in children born after FET is reassuring, and the findings of this research confirm and extend the results of previously published studies. However, the data on the early health of children born after FET are still limited, and the present investigation attempted to shed light on this important detail.

6.1 Discussion of the results

6.1.1 The maternal serum hormonal profiles after FET pregnancies

The current study (I, Figure 6) revealed that serum estradiol and progesterone levels in early pregnancy after FET in the natural menstrual cycle closely resemble those during SC pregnancies. However, between pregnancy weeks 8 and 11, serum estradiol levels were lower in FET pregnancies in comparison with SC pregnancies. On the contrary, the progesterone levels did not differ between FET and SC pregnancies. The findings of this research complement prior research (Hu et al. 2014) by emphasizing that FET cycles provide a more physiological environment for early fetal development than fresh ET cycles.

The role of estradiol during human pregnancy is still poorly understood. It has been proposed that estrogen may augment urine blood flow during pregnancy, which has been confirmed in baboons (Aberdeen et al. 2012, Albrecht et al. 2006, Bonagura et al. 2012). When the estradiol levels during the first trimester are elevated, the trophoblastic invasion and remodeling of the uterine spiral arteries are considerably diminished (Bonagura et al. 2012), leading to a disruption in the uteroplacental blood flow (Aberdeen et al. 2012). Early placentation is also regulated through epigenetic mechanisms. Indeed, imprinted gene network, stimulated by fetal signals may modify placental growth and vascularization (Choux et al. 2015). Evidence from animal and human research supports the hypothesis that suboptimal trophoblastic invasion during the early phases of placentation could potentially explain the higher frequency of adverse pregnancy outcomes (Choux et al. 2015). High estradiol levels in women after fresh ET are
associated with an increased risk for SGA and preeclampsia (Hu et al. 2014, Imudia et al. 2012, Pereira et al. 2015).

The clinical significance of slightly lower estradiol levels after FET compared to SC pregnancies during first trimester remains unclear. Both low and high estradiol levels may be harmful for placentation, but in different aspects. In our study (I), the FET pregnancies differed from the SC pregnancies also in that the embryos were cultured in the laboratory environment before freezing as well as after thawing. Some of the earlier studies have shown that the type of culture media (Kleijkers et al. 2014, Nelissen et al. 2012) as well as the duration of the culture period (Dar et al. 2014, Makinen et al. 2013) may affect the birthweight and perinatal outcome of the newborn, reflecting changes in the fetoplacental function and growth. One limitation of our study is that we have not studied estradiol and progesterone levels during substituted FET cycles. Thus, we are not able to discuss potential differences between these two protocols when it comes to obstetric and perinatal outcomes.

No other luteal steroid is considered as essential as progesterone in maintaining pregnancy (Csapo & Pulkkinen 1978). The results of study I (Figure 6) highlighted that the progesterone levels in pregnancies after FET did not differ from those in the SC pregnancies, as expected, since in both of them there was only one single corpus luteum. It has to be remembered that the FET patients received luteal support only for two weeks following the embryo transfer. On the contrary, in the pregnancies after fresh ET, the progesterone levels were five to six times higher at pregnancy week 5. This was expected, as the COH resulted in several corpora luteas, which were activated by hCG. No specific progesterone rise, reflecting a luteoplacental shift, could be detected in the fresh ET pregnancies during the first trimester of pregnancy.

The current hypothesis is that adverse pregnancy outcomes linked to ART are associated with abnormal trophoblastic invasion; one player in this is the hormonal milieu during implantation and early pregnancy. The epigenetic regulation of the placenta evolves during preimplantation development (Nelissen et al. 2011). Ovarian stimulation, which is required in fresh cycles, probably modifies the uterine environment. This is assessed by studies that demonstrated differential expression of genes in the endometrium between stimulated and natural cycles, with a dose-response effect (Choux et al. 2015). After FET cycles the hormonal milieu resembles normal spontaneous pregnancies, which could offer more optimal environment for embryo implantation and fetal development.
6.1.2 Pregnancy complications after FET

In our study (II, Table 9), FET newborns have an increased risk for LGA compared with fresh ET newborns. According to previous study the LGA is a risk factor for term pre-eclampsia (Vatten & Skjaerven 2004). Further the hypertensive disorders have been linked to FET pregnancies compared with fresh ET and SC pregnancies (Ishihara et al. 2014, Sazonova et al. 2012). Opdahl and colleagues (2015) showed that the increased risk for hypertensive disorders in FET pregnancies persisted when analyses were restricted to a comparison of sibling pregnancies in woman who had conceived after fresh ET and FET, and there were no differences in the risk for pregnancies after IVF or ICSI (Opdahl et al. 2015). This may indicate that the association cannot be attributed to maternal characteristics alone. Unfortunately, we did not have data on the whole study group concerning other pregnancy complications shown in Table 8. However, we had data on maternal hospital treated hypertension from year 2004 onwards including FET (n= 698), fresh ET (n=837) and SC (n=7435) pregnancies. No significant differences were seen between the ART groups. When comparing the ART groups with SC group, they had hypertension significantly more often (data not shown). The risk of hypertensive disorders in pregnancies after FET merits further attention.

The rate of placenta previa did not significantly differ between the ART groups (Study II, Table 8). In contrast, the large population based studies from Australia and Sweden showed a lower rate of placenta previa in pregnancies from FET cycles than in fresh ET cycles (Healy et al. 2010, Sazonova et al. 2012). One explanation for these discrepancies may be due to the lack of power, but also country-specific factors including differences in data collection and ART technologies could explain it.

6.1.3 Perinatal outcomes of children born after FET

We were the first study group to prove that children born after FET have better outcomes regarding PTB, LBW, and SGA than children born after fresh ET. However, when compared with SC children, the risk of PTB and neonatal morbidity was higher in children born after FET (Study II, Table 9). These findings are further supported by the other large Scandinavian population-based studies (Pinborg et al. 2010, Sazonova et al. 2012, Wennerholm et al. 2013).

The improved perinatal outcome of FET singletons compared with fresh ET singletons should be considered reassuring even though the explanation has been
elusive. When analyzing the outcomes of children born after FET, it is important to bear in mind that there is a healthy patient effect, as in general these children are born to women with a good ovarian response, resulting in surplus embryos after COH. Furthermore, there is a positive selection of embryos, as only good-quality embryos survive the freezing-thawing procedure (Henningsen & Pinborg 2014). These frozen-thawed embryos have often been replaced during the woman’s natural menstrual cycle or artificial cycle that mimic those in natural cycles. As shown in our study (I), the peri-implantation environment in FET cycles is less disturbed without the potential endocrine disturbance from multiple corpora lutea during implantation and early embryo development.

Our study was the first to explore the risk of being LGA in singletons born after FET compared with singletons born after fresh ET (Study II, Table 9). Subsequent Scandinavian large population-based register studies have confirmed our findings (Pinborg et al. 2014, Sazonova et al. 2012, Wennerholm et al. 2013). Further, the FET children have an increased risk for macrosomia (> 4500g). When comparing FET children with SC children, the Scandinavian studies showed an increased risk for LGA and macrosomia (Pinborg et al. 2014, Sazonova et al. 2012, Wennerholm et al. 2013), but not in our study (II; Table 10). The differences between our and other studies might be attributable to different adjustments for confounders and/or selection of controls.

The causes of the higher risk of LGA after FET are still unknown. It is well known that the mother’s BMI could influence the distribution of fetal weight (Wennerholm et al. 2013). In our data (study II), we were able to compare the study groups only in the subpopulation (born in 2004–2006) in terms of maternal BMI and abnormal oral glucose tolerance test and found no differences between the ART groups. In a later the Swedish study (Sazonova et al. 2011), an adjustment was made for BMI, and still singletons born after FET had a higher rate of LGA and macrosomia than singletons born after fresh ET. These findings indicate that the freezing and thawing procedures may play an independent role in the growth potential of the fetus.

One explanation for the higher rate of LGA in FET singletons could be the possible asynchrony between the embryo and the endometrium, which could alter the subsequent trajectory of fetal growth and development (Grace & Sinclair 2009). Another hypothesis is that ART human offspring may be prone to intrauterine overgrowth, but after COH and fresh ET, the endocrine milieu with high levels of steroid hormones has a negative impact on the early placental and embryonic
growth, which masks this overgrowth potential (Henningsen & Pinborg 2014). However, this does not explain the higher risk of being LGA in FET singletons compared with SC singletons (Pinborg et al. 2014, Sazonova et al. 2012, Wennerholm et al. 2013). Another possible reason for this is the epigenetic changes in the very early embryonic stages during freezing and thawing, which have the potential to alter developmental processes and the intrauterine growth potential of the offspring (Pinborg et al. 2014). In animals, the association between in vitro culture and “large offspring syndrome,” which is closely related to a long list of severe conditions, including organ and placental abnormalities with polyhydramnios, is well known (Grace & Sinclair 2009, Young et al. 1998). However, there is no reason to assume that the higher risk of LGA in FET singletons equals the large offspring syndrome because the risk for CAs was not seen when comparing FET children with fresh ET children (study III, Table 12). The mechanisms behind the epigenetic modification in human embryos and the relation between the freezing/thawing of embryos remain to be explored (Pinborg et al. 2014).

6.1.4 Congenital anomalies of children born after FET

In the current study (III, Table 11), children born after slow freezing have the same prevalence of major CAs as children born after fresh ET. Further, the CA rates did not differ between different FET treatments (IVF or ICSI). These finding are supported by previous studies (Davies et al. 2012, Kallen et al. 2010b, Pinborg et al. 2010) and meta-analyses (Maheshwari et al. 2012, Pinborg et al. 2013a). However, Belva et al. (2008) revealed a higher major CA rate in the FET-ICSI than in the fresh-ICSI and FET-IVF groups (Belva et al. 2008). This finding has not been verified by our or other studies (Davies et al. 2012, Kallen et al. 2010b, Pinborg et al. 2010). These contradictory results are attributable to important aspects of data collection, such as different recruitment periods between the study groups, missing data on confounding factors, a lack of maternal background data, and different definitions of CAs. In our study, the information on all three study groups was extracted in an identical way from the Register of Congenital Malformations which uses multiple data sources.

Certain organ systems seem to be disproportionately affected among the ART groups, with urogenital CAs more common in both FET and fresh ET singleton births than in those after SC pregnancy (Study III, Table 12). We verified the results of an earlier register-based Finnish study (Klemetti et al. 2005), which showed that
the increased risk for male urogenital CAs in both ART singleton births was not explained by the method of treatment (IVF or ICSI). The increased risk of CAs in male relative to female newborns after ART is difficult to explain (Olson et al. 2005). The finding might be due to an ascertainment bias because many female genitourinary birth defects might not be detected before puberty. In the large Swedish study, the increased risk of hypospadias found in the older ICSI cohorts disappeared over time and was not present in the younger cohorts of ICSI children (Kallen et al. 2010b). One explanation for this declining incidence of hypospadias in ICSI boys over time may be a dilution effect, that is, the current indications for ICSI are much wider and therefore severe male factor infertility in the father is less frequent in the younger generations of ICSI boys. However, improvements in the use of the ICSI technique may also have an influence (Kallen et al. 2010b, Pinborg et al. 2013a).

Only three cases with imprinting diseases were identified from our data (study III). One child with Angelman syndrome was born after FET-IVF and two other children were born after SC—one with Prader-Willi syndrome and one with Beckwith-Wiedemann syndrome. The incidence of imprinting diseases is low; therefore, data on imprinting disorders in children born after different ART techniques do not exist. However, if such an association exists, it is weak and at least partly related to the reproductive disease of the parents (Pinborg et al. 2016).

6.1.5 Somatic health of children born after FET

Our study (IV) confirms and extends the results of previously published studies and shows that somatic health does not differ between FET and fresh ET children until the age of three (Table 13). However, knowledge on the early growth, development and morbidity of children born after FET is very limited at the moment. Direct comparisons between the published studies are difficult because of the substantial variation in the study design, diagnostic criteria and the follow-up times.

The results on cancer risk in children born after FET are sparse. In our study, only one FET child had cancer and the result was insignificant. Källen and co-workers (2005), who studied fewer children but over a longer follow-up time, reported that in their nationwide register study including 1474 FET singletons and twins with a median follow-up time of 5.5 years, three of the FET children had a cancer diagnosis, while the expected number was 1.94 (Kallen et al. 2005c).
The long-term health of all ART singletons was slightly worse than that of SC singletons, even after taking into account confounders including premature birth. These results confirm earlier studies in Finland (Klemetti et al. 2006, Koivurova et al. 2003) and elsewhere (Kallen et al. 2005c, Ludwig et al. 2009). It is biologically plausible that ART may cause childhood morbidity. One hypothesis is that the mechanical and hormonal manipulations of the gametes and the embryo induce epigenetic changes that may influence the immune system and reduce resistance to disease. This could indicate that the real underlying cause of morbidity in these children may not be the ART treatment per se, but other patient-related factors like subfertility (Kettner et al. 2015). Therefore, follow-up studies conducted to examine and compare different ART techniques are important.

6.2 Clinical implications and future research

The perinatal outcomes of FET children are similar to, or even better than, those of fresh ET children. However, FET children have a slightly increased risk of being LGA and macrosomic. It remains unclear how the higher risk of being born LGA and macrosomic after FET should be interpreted. Our knowledge on epigenetics and metabolomics on FET in particular is very limited and future research is needed. In addition, future efforts should be made to outline the causal pathways between the freezing and thawing of embryos and growth potential. Second, it is well known that several adverse outcomes, such as stillbirth, asphyxia, shoulder dystocia, hypoglycemia, respiratory distress, and perinatal mortality are increased in macrosomic babies (Henriksen 2008), and macrosomic children have an increased risk of diabetes, overweight, and metabolic syndrome in their later life (Boney et al. 2005). However, studies on the early health of FET children are sparse, and data on FET children’s later childhood are missing. Consequently, controlled, large population-based follow-up studies are needed including data on growth, weight, and mental development during childhood to gain more information on the health of FET children, particularly those whose were born LGA and macrosomic.

It is very important to keep in mind that our studies (II–IV) and other Scandinavian large population-based registry studies (Pinborg et al. 2010, Sazonova et al. 2012, Wennerholm et al. 2013) of children born after FET have been carried out during the years when only slow freezing was used. In the future, the recent shift to the longer embryo culture and vitrification method and its impact on the health of FET children should be studied carefully to develop prevention.
strategies to reduce future ART children’s risk of having unfavorable health outcomes.

Conventionally, most IVF/ICSI embryos are transferred in fresh cycles, with freezing reserved for spare ones. The improvement in cryopreservation facilities over time has encouraged the greater use of this technology with the success rate of FET approaching that associated with fresh ET (Maheshwari & Bhattacharya 2013). Today, the data from large population-based studies, including our large study (II), suggest that perinatal outcomes are better in FET cycles. According to these results, one question is: Should we freeze all embryos after IVF/ICSI treatment with subsequent treatment in a more “physiological environment” with FET cycles to improve perinatal outcomes in children (the freeze-all policy)? However, this question is not without controversy. The lack of consensus regarding the superiority of any protocol for the cryopreservation of human embryos results in substantial differences among centers in the day of embryo cryopreservation, freezing methods, selection criteria for which embryo to freeze, and endometrial preparations for the transfer of frozen embryos (Wong et al. 2014). In the future, a freeze-all policy will require a large multicenter, randomized trial to evaluate the clinical and cost-effectiveness as well as the acceptability of elective FET versus fresh ET (Maheshwari & Bhattacharya 2013).
7 Conclusion

This study provides further evidence on the safety of FET in comparison with fresh ET. This information should further encourage clinicians to implement eSET combined with cryopreservation in their IVF/ICSI programs.

Based on the results of the present study, the following conclusions can be made:

1. In pregnancies after fresh ET, the serum estradiol and progesterone levels were higher in early pregnancy, lasting up to weeks 7–8, than after FET and SC pregnancies, while the hormone levels after FET did not differ from SC pregnancies. Pregnancies after FET during the natural cycle closely resembled the SC pregnancies and seemed to provide a more physiological environment for early fetal development than fresh ET.

2. Children born after FET have improved outcomes regarding PTB, LBW, and SGA compared with children born after fresh ET. Nevertheless, FET children have a slightly increased risk for LGA and macrosomia. The major CAs and the early somatic health until three years of age did not significantly differ between these two ART groups. When compared with SC children, the risk of PTB, LBW, and major CAs was higher in children born after FET. Moreover, the early somatic health of FET children was slightly worse than SC children.
References


89


Kalra SK & Barnhart KT (2011) In vitro fertilization and adverse childhood outcomes: what we know, where we are going, and how we will get there. A glimpse into what lies behind and beckons ahead. Fertil Steril 95(6): 1887–1889.


93


Original publications

This thesis is based on the following publications, which are referenced throughout the text by their Roman numerals:


Reprinted with permission from Oxford University Press (I–IV).

Original publications are not included in the electronic version of dissertation.
135. Selkäälä, Eija (2016) Role of α-methylacyl-CoA racemase in lipid metabolism


137. Oikarinen, Anne (2016) Effects of risk factor targeted lifestyle counselling intervention on quality of lifestyle counselling and on adherence to lifestyle change in stroke patients


140. Räsänen, Päivi (2016) Kotona asuvien ikääntyvien itsestä huolenpito: hoitotieteen keskitason teorian ydinrakenteen testaaminen

141. Hannila, Ilkka (2016) T2 relaxation of articular cartilage: normal variation, repeatability and detection of patellar cartilage lesions

142. Pihlaja, Juha (2016) Treatment outcome of zirconia single crowns and fixed dental prostheses


144. Heikkilä, Vesa-Pekka (2016) New techniques and methods for decreasing healthy tissue dose in prostate cancer radiotherapy, with special reference to rectal doses

145. Aro, Jani (2016) Novel load-inducible factors in cardiac hypertrophy

146. Mäntylä, Mikko (2016) Hypoxia-inducible factor prolyl 4-hydroxylase-2 in Tibetan high-altitude adaptation, extramedullary erythropoiesis and skeletal muscle ischemia


149. Mosorin, Matti-Aleksi (2016) Prognostic impact of preoperative and postoperative critical conditions on the outcome of coronary artery bypass surgery

Book orders:
Granum: Virtual book store
http://granum.uta.fi/granum/
Sari Pelkonen

FROZEN EMBRYO TRANSFER
EARLY PREGNANCY, PERINATAL OUTCOMES,
AND HEALTH OF SINGLETON CHILDREN

UNIVERSITY OF OULU GRADUATE SCHOOL;
UNIVERSITY OF OULU, FACULTY OF MEDICINE;
MEDICAL RESEARCH CENTER OULU;
OULU UNIVERSITY HOSPITAL;
UNIVERSITY OF HELSINKI;
HELSEINKI UNIVERSITY HOSPITAL;
VÄESTÖLIITTO FERTILITY CLINICS IN HELSINKI AND OULU;
NATIONAL INSTITUTE FOR HEALTH AND WELFARE.