ASSISTED REPRODUCTIVE TECHNOLOGY AND ADVERSE PERINATAL OUTCOMES

By

Galit Levi Dunietz

A DISSERTATION

Submitted to
Michigan State University
In partial fulfillment of the requirements
for the degree of

Epidemiology - Doctor of Philosophy

2015

ABSTRACT

ASSISTED REPRODUCTIVE TECHNOLOGY AND ADVERSE PERINATAL OUTCOMES

By

Galit Levi Dunietz

Since its introduction in 1981, Assisted Reproductive Technology (ART) has been the conventional therapy for infertile couples seeking a pregnancy. However, consistent reports linked ART with adverse perinatal and obstetric outcomes. To elucidate this association we examined the risk of preterm birth and suboptimal newborn size among ART-conceived and non-ART singletons. We expanded our analyses to evaluate the risk of preterm birth and suboptimal newborn size along the gestational age continuum and by detailed parental infertility diagnoses and extensive treatment modality.

Our retrospective cohort was assembled using a population-based ART data collected by the National ART surveillance system. The ART data were probabilistically linked to vital records of all live births in Massachusetts and Florida 2000-2010 and Michigan 2000-2009. We restricted our sample to a subset of singleton live births of mothers age 15-60 between 22 and 44 weeks' gestation, resulting in 32,691 ART and 4,263,846 non-ART singletons.

We confirmed previous reports that ART singletons have a significantly increased risk of poor perinatal outcomes, e.g. preterm birth, small for gestational age and suboptimal newborn size. Our results suggested a heterogeneous pattern of preterm birth along the gestational age continuum only for ART singletons born to

mothers with diagnosed infertility, but not other infertility sources. Examination of preterm birth risk, across distinct categories of female infertility, indicated significantly increased preterm birth for all ART singletons, independent of their parental infertility source. However, we were able to detect a different risk magnitude by the underlying infertility cause, such that ART singletons born to mothers with a diagnosis of uterine factor or ovarian disorders had a statistically significant increased risk to be born prematurely compared with ART singletons of other parental infertility cause. Thus, it is plausible that the etiology of adverse perinatal outcomes observed among ART singletons is related to both the infertility cause and the technology of assisted reproduction.

Several ART cycle types and techniques were associated with poor perinatal outcomes among ART-conceived compared with non-ART singletons. Two invasive ART techniques, assisted hatching and intra-cytoplasmic sperm injection, were not associated with an increase the risk of preterm birth and small newborn size among singletons compared with singletons born following the basic ART procedures. This information may be reassuring for infertile couples seeking pregnancy. However, other perinatal risks may be associated with these techniques. The use of frozen, autologous embryos in ART cycles was associated with better perinatal outcomes compared to frozen/donor, fresh/donor and fresh/non-donor ART modalities. The observed protective effect of ART therapy with frozen, autologous embryos on preterm birth and small newborn size may be attributed to the less invasive ART procedures that are associated with frozen embryos, to the immunologic tolerance of autologous cycles or to ART methods applied in embryo cryopreservation.

To my parents for inspiring my dreams
And
To my father-in-law for making my dreams real

ACKNOWLEDGEMENTS

Over the past three years, I have received the support and encouragement of many. I wish to acknowledge, first and foremost, Dr. Claudia Holzman the chair of my doctoral committee, my advisor and mentor. Throughout my training, Dr. Holzman maintained the perfect balance of academic supervision and cultivation of my independence. Her guidance and support helped me overcome many challenges and reach the finish line. But it is Dr. Holzman's innate passion for science and commitment for women's health that made this journey meaningful to me.

I would like to express my deep appreciation to my other committee members, Dr. Michael P. Diamond, Dr. Patricia McKane, Dr. Chenxi Li and Dr. David Todem for sharing with me their knowledge and expertise. I am grateful to Dr. Patricia McKane for introducing me to the CDC's SMART team and for promoting my professional exposure and visibility.

My CDC collaborator, Dr. Yujia Zhang maintained continuous commitment to my project. Dr. Zhang dedicated many hours to run my code and worked closely with me to identify and resolve data related errors and obstacles. His respect to deadlines was crucial to the completion of my project. I want to thank my other collaborators, Dr. Sheree Boulet, Dr. Nicole Talge and Dr. Dmitry Kissin for refining my scientific literacy and providing invaluable insights and guidance throughout our collaboration. Dana Bernson and Glenn Copland were always available to clarify questions related to vital records and review my drafts in a timely manner. I also want to thank Ajay Yesupriya from RDC for his technical support and prompt data processing.

I am forever indebted to my parents, Rena and Avraham Levi, for instilling in me their unique vision and values and for making efforts to create an unusual path for me. I am fortunate to celebrate this milestone with both of them.

My father-in-law, David Dunietz (z”l), is a beacon of endurance, courage and generosity. I thank him for giving me the freedom and confidence to pursue my dreams. I miss him every day.

I am blessed with a close and loving family and friends. My sisters, Anat and Adi, my siblings-in-law, Tamara and Isi and my friends shared my long academic journey with patience and enthusiasm. I thank them for their intellectual and emotional support.

Finally, I would like to thank my husband, Barry, and my children, Liana, Talia and Tom, for being a constant source of love and inspiration.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVATIONS

ART	Assisted Reproductive Technology
ET	Embryo Transfer
AH	Assisted Hatching
ICSI	Intra Cytoplasmic Sperm Injection
PTB	Preterm Birth
VTS	Vanishing Twin Syndrome
LBW	Low Birth Weight
SGA	Small for Gestational Age
GA	Gestational Age
OR	Odds Ratio
aOR	Adjusted Odds Ratio
CI	Confidence Interval
IVF	In-Vitro Fertilization
NASS	National ART Surveillance System
IUI	Intra-Uterine Insemination
SMART	States Monitoring Assisted Reproductive Technology

CHAPTER 1

BACKGROUND LITERATURE AND AIMS

On November 1977, after 9 years of infertility, Lesley and John Brown from Bristol, England, underwent the first successful procedure of Assisted Reproductive Technology (ART) under the care of Dr. Patrick Steptoe, an obstetrician and gynecologist and Dr. Robert Edward, a physiologist from Cambridge University. The birth of their daughter, Louise Joy Brown, on July 25, 1978 at the Royal Oldham Hospital in England, marked the beginning of a new era in the practice of reproductive medicine and brought hope to infertile couples around the world. More than 3 decades later, ART has become a standard treatment for male and female infertility.

1.1 Impaired fertility

Infertility, clinically defined as a disease of the reproductive system is manifested by the failure to conceive a pregnancy after at least 12 months of regular and unprotected sexual intercourse (1). Often used interchangeably, subfertility and infertility are distinguished by Gnoth, based on time to pregnancy (2). He defines subfertility as reduced fertility with long time to pregnancy, less than 48 months, and infertility, as a similar state as sterility, but with a rare chance of achieving a naturally conceived pregnancy. Infertility can be primary, if a pregnancy was never achieved, or secondary, if attempts for a subsequent pregnancy are failed. The prevalence of infertility is estimated at 3.5%-16.7% of reproductive age women in the developed world (3-6) and higher in the developing world (7).

Unlike other diseases, in which the diagnosis is on the individual patient level, infertility affects couples and may be identified even in the absence of pathological evidence. Fertility is inversely associated with age for both men and women, but its natural decline occurs earlier for women (8). However, within the reproductive age range, infertility causes are distributed as follows: 35% of the cases related to female factors, 30% to male infertility, 20% of infertile couples are diagnosed with a combination of female and male factors, and 15% will experience infertility of unexplained nature (9). Infertility diagnosis serves as an umbrella term to multiple abnormalities of the human reproductive system. Thus, female and male infertility diagnoses are further subdivided to categories of hormonal imbalance or mechanical dysfunction resulting from congenital anomalies or exposures to infections, hazardous agents, or co-morbidities.

Several studies have linked untreated infertility, defined as time to pregnancy >1 year, with adverse birth and obstetric outcomes, such as low birth weight, small for gestational age, preterm birth, perinatal death, cesarean delivery, antepartum hemorrhage and hypertensive disorders of pregnancy (10-13). However, further research is needed to examine whether the risk of adverse outcomes differ among infertility diagnoses or characteristics of subfertile populations.

1.2 Assisted reproductive technology

ART, considered the last resort treatment for infertility, offers a wide array of medical procedures, in which both male and female gametes are handled outside the body to achieve conception. In 2012, it was reported that the cumulative number of ART births reached a significant milestone of 5 million infants globally (14). In the US, ART

births accounted for 1.5% of the overall births in 2011, resulting in 61,660 infants (15). These numbers represent one of the lowest rates of ART utilization in the developed world (16). According to a recent report, since 2002, the annual number of ART cycles in the US, has increased from 110,000 to more than 140,000 cycles. However, a more modest rise was observed in the number of ART live deliveries and infants born (17).

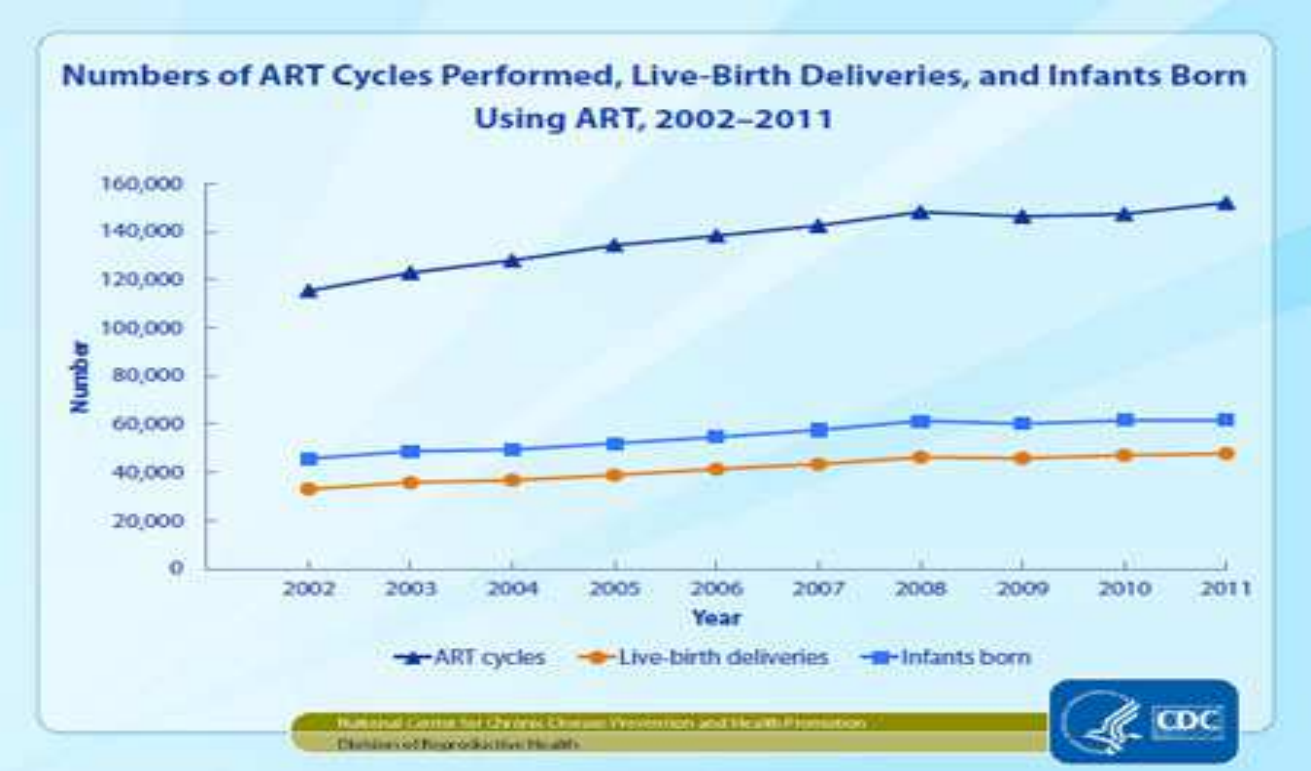

Figure 1: Numbers of ART cycles performed, live-birth deliveries, and infants born 2002-2011

The standard ART procedure involves controlled ovarian hyper-stimulation, followed by surgical retrieval of eggs. The eggs are then placed in a dish with motile sperm to achieve fertilization or undergo Intra-cytoplasmic sperm injection. Once fertilization occurs, an embryologist will be monitoring the development and growth of the zygote. Transfer of the resulting embryo into the uterine environment may take place 1-6 days post fertilization.

Table 1.1: Stages of embryonic growth and development in-vivo (18, 19)

Embryogenesis	Time	Location	Description
Zygote	12-24 hours post ovulation	Oviduct	Single cell combined of maternal and paternal gametes (DNA)
Cleavage	2-3 days post fertilization	Oviduct	First stage of embryogenesis. Repeated division of fertilized ovum
Morula	3 days post fertilization	Oviduct	16-20 cell size ball
Blastocyst	3-6 days post fertilization	Uterus	Contains ~ 50 cells of 2 distinct types: trophoblast (outer cell layer) and embryoblast (inner cell mass) Implantation occurs ~ day 6
Embryo	Post implantation-8 weeks gestation	Uterus	After implantation, blastocyst differentiate into embryonic and placental structures

A 2010 ART surveillance report indicates that 52% of Embryo Transfer (ET) procedures were performed on day 3 post-fertilization, at the cleavage stage, following by 38% day 5 ET, at the blastocyst stage (20), albeit evidence of higher pregnancy rates associated with blastocyst transfer (21, 22) versus reports of comparable outcomes for ET on day 3 and day 5 (23-26).

Gametes source varies across ART cycles; Couples may use autologous or donated gametes, based on their infertility status and medical risk factors (27). The number of ART cycles using donated eggs are positively associated with maternal age; only 4.5% of ART cycles, with female partner at age 35, involve donor eggs compared to 23% at age 42 and nearly 44% at age 44 (17).

In addition to ET timing, a decision regarding the number of embryos to be transferred should be considered, if more than one embryo is available. ART is a major contributor to multi-fetal pregnancies, accounting for almost 20% of all multiples born in 2011 (15). Multiple gestations are considered an undesirable outcome as those pregnancies are linked to adverse outcomes. In an attempt to reduce the incidence of multi-fetal pregnancies, a committee was formed with members of the Society of ART and the American Society of Reproductive Medicine to provide guidelines (28) for the recommended number of ET based on patient's age and prognosis. Elective Single Embryo Transfer is the practice of selection and transfer of one embryo, out of several available embryos, into the uterus (15). 2011 data show that in 70% of fresh non-donor cycles 1-2 embryos were transferred while in the remaining 30% of ART cycles, 3 or more ET occurred (17). Post-ET, if patients have additional embryos of good quality, they may choose to cryopreserve these embryos for a later cycle, discard them, or donate their embryos for research purposes.

A sub-type of the standard ART therapy, frozen ART cycles do not involve egg retrieval, rather, they use thawed embryos or oocytes that were cryopreserved in a previous ART cycle. Descriptive data imply slightly higher rate, 26.8% vs 25.6%, of singleton live birth for frozen compared to fresh autologous cycles, respectively (17).

Intra-cytoplasmic sperm injection (ICSI) is a technique of selecting a single sperm for direct microinjection into an oocyte, rather than placing the gametes in a dish for independent fertilization in a conventional ART (29). Initially, ICSI was applied to treat severs male infertility related to impaired sperm characteristics (30). However, in light of improved outcomes observed outside of the male infertility diagnoses (31-35), ICSI use has expanded to ART cycles independent of the underlying infertility diagnoses. Recent data show that while 36% of ART cycles during 1996-2012, were due to male factor infertility, ICSI was performed in 65% of them, mostly in couples with male infertility, unexplained infertility, advanced maternal age, poor ovarian response and 2 or more unsuccessful ART cycles (36).

Assisted Hatching (AH) is an ART method, usually performed on day 3 post fertilization, to improve pregnancy and implantation rates among subfertile couples seeking treatment (37). During their development, human oocytes are surrounded by a protective membrane called the Zona Pellucida (ZP). Thinning of the ZP is necessary to allow the embryo hatch out of the ZP and attach to the uterine lining. AH methods are aimed at thinning of the ZP, such as drilling with artificial solution, laser photo-ablation and dissection with a glass micro-needle (38). Since those techniques carry potential embryonic damage, their routine use in ART treatments is not recommended.

The use of donor gametes, oocyte, sperm or both, is suggested in ART treatments of subfertile couples with male infertility factor, female infertility involving diminished ovarian reserve, poor oocyte quality, or their combination. According to a National ART data report, in 2010, 12% of ART cycles used donated oocytes or

embryos (20) and 27.5% of these cycles had good perinatal outcomes, defined as full term, live birth of a singleton with birth weight greater than 2,500g (39).

1.3 Access to assisted reproductive technology

In 1979, the UN endorsed the right of women to independently make decisions related to their reproductive health and to have access to exercise these rights, including family planning (40-42). The right to 'procreative liberty' was seen as the basis for the right to reproduce, that is, the right to have children or live childfree (43, 44). Robertson argues that reproductive autonomy is important for freedom and human dignity, and essential for personal identity, meaning, and happiness (45). The access to this right may be different between fertile and infertile couples, as the latter may need to use ART for reproduction. Similar access to reproduction should be provided to infertile couples such that they, too, can exercise their right to have children (46). Unfortunately, access to reproduction autonomy for infertile couples is still not recognized globally. Access is restricted by woman's age (46), marital status, and sexual orientation (47), Access is also unequal by income, education, race, and ethnicity (48-52), as well as knowledge of infertility and ART services (53), infertility cause (54), and geographic availability (48, 50, 55). Even when infertile couples are able to overcome social and spatial barriers, they are still challenged by financial barriers.

Although mandated ART reimbursement is associated with increased access to ART services by infertile couples (50), in the US, it is offered in only 15 states, Arkansas, California, Connecticut, Hawaii, Illinois, Louisiana, Maryland, Massachusetts, Montana, New Jersey, New York, Ohio, Rhode Island, Texas and West Virginia. However, even within this selected group significant variation still exists with respect to

patient eligibility for ART services and their reimbursement (56). Indeed, lower access and utilization of ART services among US infertile couples has been confirmed in multiple studies (57-59). Consistent data suggest that in the US, ART users are a selective group of older, non-Hispanic white women with a higher education level (60).

1.4 Assisted reproductive technology and adverse birth outcomes

1.4.1 Biological plausibility

While the association of ART and poor birth outcomes in singleton births has been repeatedly observed across time and populations, its etiology is still unknown. Current hypotheses of biological pathways have pointed at the underlying infertility, ART procedures and their combination.

Infertility pathophysiology and adverse birth outcomes may have a shared etiology (61). Several causes of infertility, such as impaired ovarian functions, pelvic infection, uterine abnormalities, obesity and stress were linked to preterm birth and small newborn size (13, 62-64). Environmental exposures to pesticides, air pollutants and solvents may alter female hormonal function, contributing to both infertility and poor birth outcomes (62, 65, 66).

ART is a collection of techniques for treating infertility with the purpose of achieving a pregnancy. While ART methods are universal, variation in laboratory protocols and practices exists. For example, preimplantation embryogenesis takes place in culture media that varies in their nutrients, temperature, and along the in-vitro embryonic developmental stages (67). Culture media plays an important role in embryonic growth, development, quality, implantation and birth rates (68, 69). Animal and human studies provided robust evidence of an interaction between post-fertilization

media culture and growth-related epigenetic processes, gene expressions and imprinting within embryos in-vivo (70-77).

The pharmacologic component in ART involves administration of exogenous gonadotrophins during a controlled ovarian hyper-stimulation for the purpose of oocyte production. Consequently, supraphysiological sex hormones are circulating in the bloodstream during the follicular phase and oogensis. Animal and human studies reported the detrimental effect of stimulated ovulation on uterine and endometrial receptivity and development, placental function and development, and embryo quality and growth (78-86). An alternative hypothesis proposes that gonadotrophins administration increases the levels of serum relaxin, that in turn, induces collagen breakdown and cervical ripening that could lead to preterm birth (PTB) (87-92).

Vanishing twin syndrome (VTS), first reported in 1945 by Stoeckel, is an event of embryonic loss and absorption, occurring in a twin gestation, that results in a singleton birth (93). Pinborg estimated that 10% of ART singletons are survivors of a vanishing co-twin (94). Several studies examined the effect of a spontaneous reduction and its timing on birth outcomes of the surviving twin. Compared to ART singletons originating from a singleton gestation, ART singletons, survivors of VTS, have a significantly increased risk to be born at the lowest 10^{th} percentile birth weight for their gestational age, at a low birth weight and after a shorter gestation (82, 94-97). The reported risk was positively correlated with the timing of co-twin demise, such that later losses were associated with poorer outcomes (94). Selective reduction is the complement pathway for a co-twin fetal demise and a subsequent singleton birth. The purpose of selective reduction is to improve perinatal outcomes and survival of a singleton in multi-fetal

gestation (98). However, in conjunction with VTS, selective fetal reduction carries its own risks of PTB and Low Birth Weight (LBW) (99, 100).

1.4.2 Evidence from human studies

While ART alleviates the burden of infertility, it also presents a challenge to public health, as ART pregnancies are associated with several adverse maternal and neonatal outcomes. There is a large body of research to suggest that ART is linked to increased risk of poor birth and obstetric outcomes, among multiples and singletons, such as PTB (101-114), small newborn size (63, 82, 101-103, 107-109, 112, 115, 116), perinatal mortality (104, 107-109, 112, 117), cesarean section (104, 116, 118), placental abnormalities (105, 119-122) and birth defects (123-127).

Preterm birth, defined as a birth occurring before 37 weeks' gestation, accounts for 12% of all US births (128). Based on gestational age, PTB is further classified to extremely early (≤25 weeks), early (<32 weeks), moderate (32-33) and late preterm birth (34-36). Numerous studies have established the role of PTB in neonatal morbidity and mortality (129-132). The reported risk of adverse neonatal outcomes was present along the gestational age of all PTB, including late PTB (133-136). Multi-fetal pregnancies, common in ART pregnancies (137, 138), are an important risk factor for PTB (137-139). But even among ART-conceived singletons, compared to non-ART, a significantly increased risk for PTB was observed (61, 81, 92, 102-105, 109, 110, 112, 114, 116, 140-142). Table 1.2 provides a summary of major studies linking ART and preterm birth.

Table 1.2: Summary of studies linking Assisted Reproductive Technology with Preterm Birth

Author Year	Data Source Years included	Study Design	Study Groups, size	PTB	Covariates	Preterm Birth OR (95% CI)
Bergh 1999	Swedish birth registry 1982-1995	Retrospective cohort	Natural conception 1,505,724 ART 5,856	< 32 < 37	Age, parity, plurality, birth year, IF duration	1.46 (1.10, 1.95) 1.48 (1.30, 1.68)
D'Angleo 2011	PRAMS (6 states: IL, AL,ME,MD,NE, OK) 2000-2003	Population-based surveillance	Natural conception 14,673 ART 920	< 37	Age, race, income, alcohol, smoking, BMI parity, plurality, education, marital status, state, medical risk factors	1.91 (1.31, 2.80)
Dhont 1999	ART Singleton births Flanders, Belgium 1992-1997	Case-control Match: regional registry	Natural conception 3,048 ART 3,048	< 33 33-36 >36	Age, parity, fetal sex	3.48 (2.16, 5.66) 1.92 (1.55, 2.39) 0.45 (0.37, 0.55)
Hayashi 2012	Japanese society OB/GYN Singleton births 2001-2005	Case-control Match: same registry	Natural conception 4,264 ART 4,570	< 34 < 37	Age, parity, height, weight, smoking, alcohol, pre-existing diseases	1.33 (1.13, 1.57) 1.29 (1.16, 1.45)
Helmerhorst 2004	12 studies Singleton births	Systematic Review	Natural conception 7,038 ART 5361	< 32 < 37	Age, parity	3.27 (2.03, 5.28) 2.04 (1.80, 2.32)
Henningsen 2011	Denmark National birth registry Singleton births 1994-2006	Population-based Crossover	3,879 women with ART birth after natural conception birth	< 32 < 37	Age, parity, birth year, infant sex	1.1 (0.7, 1.8) 1.3 (1.1, 1.6)
Jackson 2004	14 studies Singleton births	Meta-Analysis	Natural conception 410,690 ART 12,114	< 37	Age, parity, birth date	1.95 (1.73, 2.20)
Koudstaal 2000	4 ART centers Singleton births The Netherlands Before 1992	Case-control Match: same hospital registry	Natural conception 307 ART 307	< 37	Age, parity, height, weight, ethnicity, birth date, clinic, smoking, obstetric, medical history	2.8 (1.6, 5.0)
Marino 2014	South Australia ART registry linked to birth data 1986-2002	Population-based	[1]No ART, fertile 298,952 [2]OI, infertile 767 [3]ART 5,949	< 32 < 37	Plurality, age, parity, infant sex	2.30 (1.82, 2.90) [3 vs 1] 6.96 (4.88, 9.92) [2 vs 1] 1.64 (1.46, 1.84) [3 vs 1] 2.76 (2,17, 3.52) [2 vs 1]

Table 1.2 (cont'd)						
McDonald 2005	10 case-control Studies Singleton births	Systematic Review and Meta-Analysis	Natural conception 4,044 ART 3,055	< 32 32-36 < 37	Age, parity	2.99 (1.54, 5.80) 2.30 (1.00, 5.28) 1.93 (1.36, 2.74)
McDonald 2010	15 studies Singleton births	Systematic Review and Meta-Analysis	Natural conception 1,532,188 ART 12,070	< 32 32-36 < 37	Age, parity*	2.27 (1.73, 2.97) 1.52 (1.01, 2.30) 1.84 (1.54, 2.21)
McGovern 2004	27 studies Singleton births	Systematic Review and Meta-Analysis	n/a**	< 37	Age and/or parity	1.98 (1.77, 2.22)
Pandey 2012	7 cohort studies Singleton births	Systematic Review and Meta-Analysis	Natural conception 614,440 ART 27,819	< 37	Age, parity	1.54 (1.47, 1.62)
Perri 2001	Medical center Singleton births Israel 1996	Case-control Match: births from same hospital	Natural conception 190 ART 95	< 37	Age, parity, ethnicity, gravidity	5.69 (2.39, 13.55)
Romundstad 2008	Norway birth registry Singleton births 1984-2006	Population-based Crossover	[1]Natural conception 1,127,739 [2]Both ART & Natural 2,204 [3]ART 7,474	< 37	Age, parity, sex, birth year, inter-pregnancy interval, previous perinatal death	1.69 (1.55, 1.85) [3 vs 1] 1.20 (0.90, 1.61) [2]
Sazonova 2011	ART data linked to Swedish birth registry 2002-2006	Population based Retrospective cohort	Natural conception 587,009 ART 13,544	< 28 < 32 < 37	Age, parity, smoking, BMI, IF duration, birth year, plurality	1.69 (1.34, 2.14) 1.70 (1.50, 1.94) 1.80 (1.70, 1.90)
Schieve 2004	ART surveillance from U.S. IF clinics Singleton births 1996-2000	Population based	Reference population: singleton births from the 2000 U.S. Natality File ART 62,5551	< 37	Age, parity, race	1.41 (1.32, 1.51) Observed vs. expected
Wisborg 2010	Medical Center Denmark Singleton births 1989-2006	Prospective cohort	[1]Natural conception [(<1 yr)] 16,464 [2]Natural conception [(>1 yr)] 2,009 [3]OI, IUI 877 [4]ART 730	< 32 < 37	Parity, age, education, marital status, BMI, smoking, alcohol, coffee	1.10 (0.62, 1.94) [2 vs 1] 0.95 (0.38, 2.37) [3 vs 1] 2.33 (1.17, 4.65) [4 vs 1] 1.19 (0.98, 1.44) [2 vs 1] 1.16 (0.87, 1.56) [3 vs 1] 1.53 (1.15, 2.04) [4 vs 1]

ART=assisted reproductive technology; PTD=preterm birth, defined in Gestational Weeks; GA=gestational age; IF=infertility; IUI-Intra Uterine Insemination; OI=ovulation induction; OR=odds ratio

* adjusted in all but one study **included estimates rather than actual number of participants

Newborn size is another important indicator for fetal and infant health (135, 143, 144). Information on fetal growth may be generated directly with imaging technology, or indirectly using neonatal anthropometric measurements, such as birth weight and length, and head or chest circumference. Birth weight is widely used in perinatal research, categorically, with 2,500 grams as the cutoff between normal and LBW, or continuously as mean birth weight. There are several reasons for the frequent use of birth weight as health indicators; first, birth weight is readily available and accurately measured in birth records; second, it is associated with immediate and later-in-life health status of individuals; and lastly, mean birth weight is linked to population health (145-147). Paradoxically, the simplicity of birth weight measurement conceals the complexity of its interpretation, as LBW may be attributed to short gestation, poor fetal growth or their combination. It is, thus, important to identify and distinguish the distinct etiology of LBW.

Indices that match the gestational age with the birth weight measurements, have been gradually replacing LBW in the scientific literature. The first, birth weight z-score, or standard scores, quantifies the distance and direction of a measurement (x) from its population mean (μ) at a given gestational age in units of standard deviation (σ).

$$\rightarrow Z=x-\mu/\sigma$$

Z-score transforms an observation to its corresponding value in a standard normal distribution with μ =0 and σ=1, such that a z-score < 0 represent a value that is below the population mean while a z-score > 0 describe a value above the population mean. In terms of birth weight, z-score expresses the distance in standard deviations of an observed birth weight from the mean birth weight of an appropriate reference population

based on their sex and gestational age at birth. For example, z=1 reflects a baby's birth weight that is 1 standard deviation larger than counterparts born at the same gestational age and of the same sex.

One of the advantages of z-score is its property as a standardized quantity that allows comparison across sexes and individuals at any gestational age. A second advantage stems from the definition of z-score as a continuous variable that may be summarized using statistical functions such as mean and standard deviation. However, the complex concept of z-score and its indirect interpretation pose challenges for wider use (148, 149).

Percentiles are the second index that incorporates gestational-age-for-birth-weight measurements, as they assess the relative position of an observation in a sex-specific and gestational age-specific reference population. Assuming normal distribution, newborn size is often measured using percentiles, most commonly, 3rd, 5th, 50th (median), 95th, 97th and 99th. Alternatively, the cut off for suboptimal size may be defined as 2 standard deviations below the mean birth weight for gestational age. For example, Small for Gestational Age (SGA), is expressed as birth weight below the 10th or 5th percentile or 2 standard deviations below the mean birth weight for a given gestational age within a sex specific population.

The majority of ART studies examined LBW (<2,500 g) and very low birth weight (<1,500 g) of ART-conceived infants compared to naturally conceived singletons (61, 63, 81, 82, 92, 101-109, 114-116, 119, 141, 150, 151). Fewer studies associated ART with newborn size in terms of mean birth weight (106, 115, 150, 152). ART was also linked to SGA infants, whose birth weight was lower than the 10th, 5th percentile for their

gestational age, or 2 standard deviations below the mean birth weight (61, 92, 103, 107, 108, 112, 116, 119, 141, 153-161). Relative to LBW, mean birth weight and SGA, newborn size, measured as a birth weight z-score, was rarely used to study birth outcomes in ART populations (162). Table 1.3 presents major findings on SGA risk in ART births.

Table 1.3: Summary of studies linking ART with Newborn Size (Small for Gestational Age)

Author Year	Data Source Years included	Study Design	Study Groups, size	Newborn Size Definition	Covariates	SGA OR (95% CI)
D'Angelo 2011	PRAMS (6 states: AL, IL, ME, MD, NE, OK) 2000-2003	Population-based surveillance	[1] Natural conception 14,673 [2]Ovulation induction 904 [3]ART 920	<10th percentile Adjusting for race, sex, ga, bw	Income, education, age, race, alcohol, smoking, parity, marital status, BMI, state, medical risk factors	1.71 (1.09, 2.69) [2 vs 1] 1.98 (1.21, 3.24) [3 vs 1]
Doyle 1992	ART registry Singleton births England 1978-1987	Population-based	ART 648	<10th percentile By ga	Maternal age, infant sex	17% vs 10% Observed vs Expected P<0.01
Fujii 2010	Perinatal database Singleton births Japan 2006	Cross sectional	Non-ART 53,566 ART 1,396	<10th percentile By ga	Age, placenta previa, maternal characteristics	1.12 (0.95, 1.31)
Helmerhurst 2004	6 matched studies Singleton births	Systematic Review	Non-ART 2,290 ART 1,507	<10th percentile	Age, parity	1.40 (1.15, 1.71)
Isaksson 2002	ART clinic births Finland 1993-1999	Case-control Match: Finnish birth registry	[1]Natural conception 345 [2]ART/unexplained IF 69 [3]All ART 1,901	<2 sd of mean bw by sex, ga	Maternal age, parity, residence, birth year, plurality,	[1]2.9% [2]2.9% [3]2.4% p>0.05
Jackson 2004	7 studies Singleton births	Meta-Analysis	Natural conception 2,208 ART 1,889	<10th percentile by ga	Age, parity	1.60 (1.25, 2.04)
Koudstaal 2000	4 ART centers The Netherlands Singleton births Before 1992	Case-control Match: same hospital registry	Natural conception 307 ART 307	<10th percentile by sex, ga	Age, parity, height, weight, ethnicity, delivery date, clinic, smoking, obstetric, medical history	2.29 (1.37, 3.84)
Maman 1998	Medical Center, Israel Singleton births 1989-1994	Case-control Match: same medical center	[1]Natural conception 469 [2]ART 169	<10th percentile by ga	Age, parity, ga	1.03 (0.53, 2.00)
Marino 2014	South Australia ART registry linked to birth data 1986-2002	Population-based	[1]No ART, fertile 298,952 [2]OI, infertile 767 [3]ART 5,949	<10th percentile <3rd percentile by sex, ga	Plurality, age, parity, infant sex	1.34 (1.06, 1.69) [2 vs 1] 1.22 (1.11, 1.33) [3 vs 1]

Table 1.3 (cont'd)						
McDonald 2005	9 case-control Studies (6 low quality studies) Singleton births	Systematic Review and Meta-Analysis	Natural conception 3,009 ART 1,823	<10th percentile By ga	7 studies did not adjust for parity	1.59 (1.20, 2.11)
Olivennes 1993	Medical Center France Singleton births 1987-1989	Retrospective cohort	[1]Natural conception 5,096 [2]Ovulation induction 263 [3]ART 162	<10th percentile by ga	Age, parity, pregnancy complications, delivery site, date	1.90 (1.26, 2.87) [2 vs 1] 1.99 (1.21, 3.31) [3 vs 1]
Panday 2012	7 cohort studies Singleton births	Systematic Review and Meta-Analysis	Natural conception 580,810 ART 13,207	<10th, <3rd percentile by ga	Age, parity No heterogeneity	1.39 (1.27, 1.53)
Reubinoff 1997	Medical Center Israel Singleton births 1983-1993	Case-control Match: same medical center	Natural conception 260 ART 260	<10th percentile by ga	Age, parity, ethnicity, delivery date, location	0.97 (0.57, 1.62)
Romundstad 2008	Birth registry, Norway Singleton births 1984-2006	Population-based Crossover	[1]Natural conception 1,127,739 [2] ART & Natural 2,204 [3]ART 7,474	<2 sd of mean bw by sex, ga	Age, parity, sex, birth year, inter-pregnancy interval, previous perinatal death	1.26 (1.10, 1.44) [3 vs 1] 0.99 (0.62, 1.57) [2]
Tan 1992	ART clinic England 1978-1987	Case- control Match: same hospital registry	Non-ART 978 ART 494	<10th percentile by ga	Maternal age, plurality	1.97 (1.45, 2.69)
Verlaenen 1995	Medical center Births, Belgium 1988-1994	Case-control Match: same medical center	Natural Conception 140 ART 140	<10th percentile By sex, ga	Age, parity, height, weight, IF history, plurality	2.28 (0.90, 5.78)
Wang 1994	Medical Center South Australia Singleton births 1982-1991	Retrospective cohort	Non-ART 21,547 ART 465	<10th percentile by ga	Age, parity, ga, IF cause, pregnancy complications	1.60 (1.28, 1.97)
Zhu 2007	National birth cohort, Denmark Singleton births 1997-2003	Population-based	[1]No ART 51,041 [2]Infertile, no ART 5,787 [3]ART 4,317	<5th percentile by sex, ga	Age, smoking, parity	1.24 (1.10–1.40) [2 vs1] 1.40 (1.23, 1.60) [3 vs 1]

ART=assisted reproductive technology; bw=birth weight; ga=gestational age; IF=infertility; OI=ovulation induction; OR=odds ration; sd=standard deviation

1.5 Current literature: limitations and gaps

Multiple studies have observed an increased risk of adverse perinatal and obstetric outcomes among ART pregnancies compared with spontaneous conceptions. However, methodological limitations of previous studies may lead to an inherent bias of their findings. We identified five potential sources of bias:

1) Composition of ART group combines the effect of infertility and treatment, thus comparison of non-ART with ART pregnancies does not provide an opportunity to disentangle effects associated with infertility versus effects of ART treatment.
2) Unmeasured confounding in the general population resulting from infertility that is undiagnosed, untreated and unreported.
3) Misclassification of exposed individuals as controls due to unreported use of non-ART infertility treatments.
4) Distinct distribution of baseline characteristics between the exposed and unexposed groups, such as age, education, race/ethnicity, plurality and parity. Those confounders are often unaccounted for in studies of ART outcomes.
5) Heterogeneity of methods for outcomes assessment between the ART and non-ART groups. For example, in the general population, gestational age at birth is determined based on last menstrual period or clinical estimate, while within ART studies, it is more precisely calculated using the date of embryo transfer.

Several attempts have been made to address these limitations, most notably, separating the effect of the underlying infertility from the technology effect. A recent meta-analysis (13) reported that a history of infertility in spontaneous pregnancies is associated with a moderate risk for PTD, LBW and SGA. These results reinforce the

problem of confounding by infertility associated factors and motivate the need for alternative approaches to assess the *sole* effect of ART in relation to adverse birth outcomes.

The selection of the male infertility diagnosis as a comparison group was proposed in other studies. A significantly shorter gestation was observed among singletons born to females with any infertility diagnosis, or with tubal factor infertility, compared to those born to subfertile males (151, 163). Conversely, Schieve et al reported no difference between expected and observed PTB cases among singletons with paternal infertility (164).

Two Scandinavian studies used a crossover design to examine the risk of PTB and SGA in ART-conceived compared to naturally conceived singletons within the same women (106, 112). While the first study detected an increased risk for PTB [aOR 1.3 (95% CI 1.1, 1.6)], the latter, reported a similar PTB risk for singletons born to the same women independent of their conception mode.

Finally, birth outcomes of ART singletons born to subfertile couples were compared with those born following low technology infertility treatments, e.g. ovulation induction or stimulation. A significantly increased risk of LBW and PTB was observed among assisted conceptions compared with unassisted conceptions (108, 111, 152, 165). Two cohort studies reported an increased risk of PTB among low technology infertility treatments, RR=1.68 (95% CI 1.18, 2.40) and OR=1.72 (95% CI 1.16, 2.56) (108, 165), whereas a meta-analysis found lower PTB odds, OR=1.45 (95% CI 1.21, 1.74) (111).

In addition to the confounded infertility and treatment effect on birth outcomes, another source of ambiguity surrounding ART risk is related to the heterogeneity of infertility treatments and diagnoses. Recent studies have attempted to elucidate the conundrum of ART risk by classifying infertility diagnoses and treatment profiles into separate categories.

Consistent evidence suggests better birth outcomes following cryopreserved embryo transfer compared to fresh cycles (81, 108, 141, 151, 164, 166, 167). A recent meta- analysis and systematic review reported lower risk of SGA [RR 0.45 (95% CI 0.30, 0.66)] and PTB [RR 0.84 (95% CI 0.78, 0.90)] in cryopreserved cycles compared with fresh ART cycles (167). With spontaneous pregnancies as a reference group, PTB risk was lower in cycles with frozen or fresh embryo transfer [OR 2.02 (95% CI 1.49, 2.75) and OR 2.20 (95% CI 1.79, 2.70)], respectively (108).

Intra-cytoplasmic sperm injection (ICSI), a technique of selecting a single sperm for direct microinjection into an oocyte, is now routinely used in ART cycles (36). The popularity of ICSI has raised questions with respect to its safety prompting investigation of birth outcomes following ICSI/ART treatments. A protective effect for PTB among ICSI/ART singletons compared to conventional ART was observed in several studies (111, 168, 169), while other studies showed similar PTB (112, 166) or SGA risk (112). An increased PTB risk was evident in ART/ICSI singleton pregnancies relative to those in the general population (106, 108) and when expected versus observed PTB cases were compared [RR 1.24 (95% CI 1.12, 1.36)] (164).

Assisted Hatching (AH) techniques carry a theoretical embryonic damage, thus, their routine use in ART treatments is not recommended. Fewer studies found a positive

effect of ART/AH on clinical pregnancy, but not live births, mostly among subfertile women with poor prognosis (170-172). So far, only one study examined the effect of laser-AH on adverse obstetric and neonatal outcomes, reporting similar mean gestational age and birth weight for ART/AH/cryopreserved compared to ART/cryopreserved live births (173). However, small sample size and unaccounted heterogeneity among study subgroups limit the interpretation of these findings. Finally, an association of AH and monozygotic twins or multiple gestations was suggested by some studies (173-178) but not observed in others (179-181). It is plausible, although, yet to be shown, that multifetal gestations may mediate the associations of AH/ART and PTB or small newborn size.

Adverse obstetric and neonatal outcomes were observed in pregnancies with donor oocytes relative to unassisted conceptions, but the effect was attenuated after accounting for preeclampsia (182). Several small studies examined the association of poor birth outcomes among ART-conceived pregnancies with autologous versus donor oocytes, provided inconclusive evidence (183-186). Larger studies are needed to investigate the implications of using donor oocyte in ART pregnancies.

Although ART has been a conventional treatment for subfertile couples in the last three decades, there is scarcity of sufficient evidence to establish their safety with respect to maternal and child health. It is still unclear, whether adverse outcomes such as PTB and small newborn size are attributed to the treatment, the underlying infertility or their combination. As newer methods and techniques are being introduced into the practice of reproductive medicine, larger studies of high data quality with robust

assessment of exposures and outcomes are needed to evaluate their safety across infertility diagnoses for the benefit of clinicians and patients alike.

1.6 SMART collaborative project

States Monitoring Assisted Reproductive Technology (SMART) is a collaborative project, established in 2001 as a surveillance system of infertility and ART outcomes by the Centers for Disease Control and Prevention and public health agencies of member states, Massachusetts, Connecticut, Michigan, and Florida (187). Initially, SMART team has created a population-based dataset that linked ART data to birth records of member states with the purpose of studying ART outcomes. Later, SMART linkage was extended to hospital discharge data, birth defects and cancer registries. The current SMART dataset is used by investigators around the world to examine the association of ART with a variety of outcomes across the life course of its users.

In 2012, I submitted a proposal to study birth outcomes among ART-conceived singletons. The proposal was approved by the SMART stirring committee and the Institutional Review Boards of Massachusetts, Michigan, Florida and the CDC. A subset of the SMART data was created for my study and was stored at the Research Data Center, according to the CDC practices for restricted data. External investigators are encouraged to access the data through a remote automated system (ANDRE). For this analysis, I wrote the code in SAS, according to ANDRE guidelines, then, submitted it electronically. Within 24-48 hours, the output and log were emailed back to me.

The SMART subset used for this analysis included a total of 4,296,537 singleton live births, of which 4,263,846 were non-ART and 32,691 were ART related births,

occurred in Florida and Massachusetts from 2000-2010, and Michigan from 2000-2009 (188).

1.7 Study aims

1. Estimate the effect of infertility diagnoses above the ART treatment effect, by investigating the risk of preterm birth among four mutually exclusive subgroups of ART users: female infertility, male infertility, combined male and female infertility and unexplained infertility.
2. Examine whether the risk of preterm birth is homogenous along the gestational age continuum, including early term births, for each of the ART subgroups.
3. Investigate the risk of small newborn size using two indices, percentiles and birth weight z-score, among ART population classified to subgroups based on their infertility diagnoses.
4. Evaluate the risk of adverse birth outcomes e.g. preterm birth and newborn size for ART users across distinct diagnoses of female infertility.
5. Examine the risk of preterm birth and small newborn size across treatment profiles, e.g. ICSI, assisted hatching, fresh and cryopreserved cycles, donor and autologous embryos.
6. Estimate the ART technology effect by comparing newborn size between non-ART population and ART users with male infertility that conceived a pregnancy with donor sperm. This comparison would allow separating the effect of parental infertility from treatment effect, as the pregnancy was conceived using fertile gametes and was carried by fertile females.

CHAPTER 2

ASSISTED REPRODUCTIVE TECHNOLOGY AND THE RISK OF PRETERM BIRTH AMONG PROMIPARAS

2.1 Introduction

Assisted Reproductive Technology (ART) is a group of medical procedures for treating infertility in which both male and female gametes are handled outside the body to achieve conception. Since its introduction in 1978, ART has contributed to the birth of more than 5 million infants worldwide (14). In 2009, European registries reported that 109,239 infants were born following ART (189), while in the US, a total of 61,564 infants were born in 2010, representing, on average, 1.5% of its total births (190). Although ART may help infertile couples achieve pregnancy, it also presents a public health challenge because of reported associations with adverse birth outcomes such as preterm birth (PTB), low birth weight and small for gestational age (82, 103, 140). Multi-fetal pregnancies are common in pregnancies achieved through ART (190), and are an important risk factor for PTB (109, 191). However, the association between ART and PTB was also observed in singletons (105, 192). Numerous studies have found a two-fold risk increase for PTB in ART-conceived compared to non-ART conceived singleton pregnancies (107, 140). The explanation for this ART-PTB association remains unclear; the effect may be fully or partially confounded by other factors such as causes of underlying infertility. One of the methodological limitations of previous studies has been the composition of the control group, typically non-ART pregnancies. The comparison of non-ART pregnancies with ART pregnancies does not provide an opportunity to disentangle effects associated with infertility versus effects of ART treatment. A recent meta-analysis (13) reported that a history of infertility among couples who conceived

spontaneously is associated with a moderate risk for preterm birth, low birth weight, and small for gestational age. These results reinforce the problem of confounding by fertility-associated factors and demonstrate the need for alternative approaches to try and assess more specifically the effects of ART on adverse birth outcomes.

To elucidate ART link to adverse birth outcomes, we used data from the States Monitoring Assisted Reproductive Technology (SMART), a collaborative project of ART surveillance initiated by the Centers for Disease Control and Prevention (CDC) and the Massachusetts, Florida and Michigan public health agencies. As previously described (187), the SMART Collaborative was established to monitor and enhance ART surveillance within states and study health outcomes among ART users. The SMART dataset has been previously described. Briefly, it is a population-based dataset of vital records of Massachusetts, Michigan and Florida probabilistically linked to National ART Surveillance System (NASS) data of all ART associated deliveries (188).

The goals of this study were to: 1) confirm previous investigations linking increased PTB risk with ART among singleton pregnancies; 2) extend this line of inquiry by considering if excess PTB risk is confined to couples with female infertility; and 3) examine the ART-related excess risk of PTB across the gestational age continuum.

2.2 Materials and methods

For the current study, a subset of SMART Collaborative data, with all singleton live births to primiparous women occurring in Massachusetts and Florida between the years 2000-2010 and Michigan 2000-2009 was used to examine gestational age at birth across study groups. We restricted the dataset to primiparous women with singleton

deliveries to avoid including multiple live births to the same woman, which would have resulted in correlated, non-independent data. We excluded deliveries of women younger than 15 years of age or women older than 60 years due to lack of comparable ART and non-ART groups, respectively.

Details on infertility diagnosis are part of the NASS dataset and were used in the linked data file to create five mutually exclusive groups based on ART status and reason for infertility among ART patients, i.e. non-ART (n=1,804,100), ART female infertility (n =9,891), ART male infertility (n=4,819), ART combined (male and female) infertility (n=3,688), and ART unexplained infertility (n=2,930). The outcome of interest, gestational age (GA) at birth, was based on the clinical estimate obtained from birth files.

To avoid small cell sizes in contingency tables, our adjusted models included collapsed race/ethnicity and education categories. Race/ethnicity categories of 'Hispanic' and 'Asian/other' were grouped into one, and similarly, both categories of 'high school diploma or GED' and 'less than high school education were merged.

Initial analyses were conducted by comparing socio-demographic and pregnancy-relevant factors among the ART and non-ART groups, with the purpose of identifying potential confounders. We used basic inferential statistical methods such as t-test, chi square and linear regression models. In the regression models, odd ratios were calculated for preterm (<37 weeks gestation) and preterm/early term (<39 weeks gestation) births. Binary logistic regression models compared PTB odds for each ART

subgroup with that of non-ART births. The male infertility only group was of particular interest as a means of examining ART outcomes in the absence of female infertility.

To gain more insight into the distribution of PTB risk, we further sub-classified gestational age into 5 and 6 categories, with ≥37 and >39 weeks as the referent for PTB and preterm/early term birth, respectively. This strategy recognizes both uncertainties in gestational age dating and recent concerns for adverse outcomes even among 'early term' births (37-38 weeks gestation) (193). Recently, the American College of Obstetricians and Gynecologists suggested new definitions for full term births as ≥39 to <41 gestation weeks (194).

Using multinomial logistic regression models, we next studied odds of preterm (< 37 weeks) versus term (≥ 37 weeks) birth along the continuum of preterm gestational ages, i.e. <28, 28-30, 31-33, and 34-36, as well as the odds of preterm/early term (<39 weeks) birth along the GA continuum of <28, 28-30, 31-33, 34-36, and 37-38 weeks relative to ≥39 weeks' gestation. Finally we evaluated the association between infertility diagnosis and PTB among ART births, using female infertility as the referent. This comparison was of particular interest as a means of assessing the likelihood of PTB according to infertility type among a subfertile population. Crude and adjusted odds ratios and 95% confidence intervals were calculated (SAS 9.3, Cary, NC); all adjusted models included maternal age, education, race, state and year which were derived from the birth certificate data. The study received approval from the Institutional Review Boards of Massachusetts, Michigan, Florida and CDC.

2.3 Results

During the study period there were 21,328 (1.2%) singleton deliveries to primiparous ART users and 1,804,100 (98.8%) singleton deliveries to primiparous non-ART users (Table 2.1). The ART users were more likely to be older, Non-Hispanic white and have a higher level of education compared to their non-ART counterparts ($p< 0.01$ for each comparison). ART-conceived pregnancies were of shorter duration (mean GA 38.3 – 38.7 weeks) than pregnancies not conceived through ART (mean GA 38.8 weeks; $p<0.01$) and a higher percentage of ART-conceived pregnancies resulted in PTB (10% – 14% among ART births versus 9% non-ART births, $p<0.01$).

In both crude and adjusted models for PTB <37 weeks' gestation, the odds were significantly higher among all ART groups, compared to the non-ART referent group. Similarly, when 39 weeks' gestation was used as the cutoff to assess risk of preterm and early term birth combined, all ART groups had significantly higher PTB odds compared with the non-ART deliveries (Table 2.2). After adjusting for maternal age, education, race state and year, we found that of all four infertility groups, female infertility had the highest adjusted odds ratio (aOR) for PTB [OR=1.60, 95% CI (1.50, 1.70)]. The adjusted odds ratios for couples with both female and male infertility and those with male infertility only were 1.49 (95% CI 1.35, 1.64) and 1.24 (95% CI 1.13, 1.37), respectively. Finally, the adjusted odds ratio for couples with unexplained infertility was 1.26 (95% CI 1.12, 1.43), Models using preterm/early term birth, defined as <39 weeks' gestation, produced similar results with mostly attenuated aORs.

Table 2.1: Maternal and Pregnancy characteristics for ART and non-ART primiparas with live births of singletons in Florida and Massachusetts 2000-2010 and Michigan 2000-2009

	Non-ART	ART				P value
		Female Infertility	Male Infertility	Combined Infertility	Unexplained Infertility	
Sample Size N (%)	1,804,100 (98.8)	9,891 (0.5)	4,819 (0.3)	3,688 (0.2)	2,930 (0.2)	
Maternal Age, mean (s.d)	25.5 (6.1)	35.6 (5.2)	33.4 (4.1)	34.8 (5.0)	35.3 (4.1)	**<0.01**
Race/Ethnicity						
Non-Hispanic White	1,0587,748 (58.7)	7,781 (78.7)	3,775 (78.3)	2,787 (75.6)	2,455 (83.8)	**<0.01**
Non-Hispanic Black	292,407 (16.2)	468 (4.7)	138 (2.9)	163 (4.4)	53 (1.8)	
Hispanic	334,055 18.5)	831 (8.4)	496 (10.3)	466 (12.6)	140 (4.8)	
Asian/Other	104,808 (5.8)	660 (6.7)	355 (7.4)	225 (6.1)	222 (7.6)	
Missing/Unknown	14,082 (0.8)	151 (1.5)	55 (1.1)	47 (1.3)	60 (2)	
Education						
High school or lower	832,697 (46)	1,268 (13)	585 (12)	497 (13)	236 (8)	**<0.01**
Some college	432,698 (24)	2,045 (20.5)	976 (20)	817 (22)	475 (16)	
Bachelor's or higher	523,035 (29)	6,532 (66)	3,236 (67)	2,346 (64)	2,211 (75)	
Missing/Unknown	15,670 (1)	46 (0.5)	22 (<1)	28 (1)	8 (<1)	
GA at Birth						
Mean	38.8	38.3	38.7	38.4	38.7	**<0.01***
Median (IQR)**	39 (2)	39 (2)	39 (2)	39 (2)	39 (2)	
PTB <37 weeks	154,247 (9)	1,345 (14)	489 (10)	472 (13)	296 (10)	**<0.01**
Term Birth ≥37 weeks	1,647,045 (91)	8,350 (86)	4,323 (90)	3,211 (87)	2,633 (90)	
State						
Florida	956,532 (53)	3,479 (35)	1,647 (34)	1,583 (43)	558 (19)	
Massachusetts	368,346 (20)	4,684 (47)	2,245 (47)	1,178 (32)	2,150 (73)	
Michigan	479,222 (27)	1,728 (18)	927 (19)	927 (25)	222 (8)	**<0.01**

Table 2.1 (cont'd)						
Year						
2000	164,646 (9)	704 (7)	304 (6)	222 (6)	139 (5)	
2001	163,900 (9)	821 (8)	315 (7)	297 (8)	185 (6)	
2002	162,287 (9)	867 (9)	401 (8)	327 (9)	233 (8)	
2003	165,761 (9)	927 (9)	408 (8)	292 (8)	232 (8)	
2004	169,049 (9)	898 (9)	413 (9)	308 (8)	296 (10)	
2005	169,691 (9)	893 (9)	427 (9)	300 (8)	287 (10)	
2006	176,043 (10)	949 (10)	481 (10)	404 (11)	283 (10)	
2007	177,249 (10)	995 (10)	498 (10)	394 (11)	290 (10)	
2008	172,799 (10)	978 (10)	517 (11)	432 (12)	295 (10)	
2009	165,962 (9)	1,038 (11)	572 (12)	438 (12)	333 (11)	**<0.01**
2010	116,713 (7)	821 (8)	483 (10)	274 (7)	290 (12)	

*Non parametric test for the mean (Kruskal-Wallis)
** Inter-Quartile Range

Table 2.2: Estimated effect of ART and infertility on PTB and preterm/early term births among primiparas. Population-based data of all singleton live births in Florida and Massachusetts 2000-2010 and Michigan 2000-2009

	Non-ART	Female Infertility	Male Infertility	Combined Infertility	Unexplained Infertility
PTB <37 weeks					
cOR (95% CI)	Referent	**1.69 (1.59, 1.79)**	**1.21 (1.10, 1.33)**	**1.58 (1.44, 1.74)**	**1.21 (1.08, 1.37)**
*aOR (95% CI)	Referent	**1.60 (1.50, 1.70)**	**1.24 (1.13, 1.37)**	**1.49 (1.35, 1.64)**	**1.26 (1.12, 1.43)**
PTB/early term <39 weeks					
cOR (95% CI)	Referent	**1.47 (1.41, 1.53)**	**1.12 (1.06, 1.19)**	**1.44 (1.35, 1.54)**	**1.08 (1.00, 1.17)**
*aOR (95% CI)	Referent	**1.48 (1.42, 1.54)**	**1.19 (1.12, 1.26)**	**1.39 (1.30, 1.49)**	**1.23 (1.14, 1.33)**

*Adjusted for age, race, education, state and year

Next we explored the odds of preterm birth along GA intervals (<28, 28-30, 31-33, 34-36 weeks' gestation with a referent category of ≥37 weeks gestation) among infants conceived through ART, by type of parental infertility, as compared to those not conceived through ART. Adjusted ORs for the female infertility group were 1.71 (95% CI 1.38, 2.13), 1.95 (95% CI 1.59, 2.39), 1.56 (95% CI 1.34, 1.81), and 1.57 (95% CI 1.46, 1.68), for birth before 28 weeks' gestation, between 28- 30 weeks, 31-33 weeks and 34-36 weeks, versus ≥ 37 weeks, respectively (Table 2.3). A similar pattern was observed in the changes of GA-specific point estimates for combined infertility. We then used this approach to examine the odds of preterm and early term birth along the same GA intervals, with the exception that the later GA group was 37-38 weeks and ≥39 weeks was the referent GA; results were similar, with slightly higher PTB odds ratio estimates for all infertility groups (table 2.3).

Table 2.3: Estimated effect of infertility and ART on PTB and preterm/early term births among primiparous women with a singleton live births. Population-based data of all singleton deliveries in Florida and Massachusetts 2000-2010 and Michigan 2000-2009

PTB < 37					
GA weeks (N)	Non-ART	Female Infertility	Male Infertility	Combined Infertility	Unexplained Infertility
<28 (13,226)	Reference	**1.71 (1.38, 2.13)**	**1.19 (0.83, 1.72)**	1.27 (0.86, 1.87)	0.95 (0.55, 1.64)
28-30 10,114	Reference	**1.95 (1.59, 2.39)**	1.34 (0.94, 1.91)	**2.21 (1.62, 3.00)**	1.35 (0.86, 2.13)
31-33 22,766	Reference	**1.56 (1.34, 1.81)**	**1.28 (1.01, 1.62)**	**1.70 (1.36, 2.14)**	**1.52 (1.15, 1.99)**
34-36 112,202	Reference	**1.57 (1.46, 1.68)**	**1.23 (1.10, 1.38)**	**1.41 (1.25, 1.58)**	**1.23 (1.07, 1.43)**
≥37 1,670,340	Reference	Referent	Referent	Referent	Referent
Wald Test P value		0.22	0.96	**0.02**	0.39
Preterm/early term birth < 39					
GA weeks (N)	Non-ART	Female Infertility	Male Infertility	Combined Infertility	Unexplained Infertility
<28 13,226	Reference	**1.88 (1.51, 2.33)**	**1.23 (0.86, 1.79)**	1.37 (0.93, 2.03)	0.99 (0.57, 1.71)
28-30 10,114	Reference	**2.13 (1.73, 2.62)**	1.39 (0.97, 1.98)	**2.39 (1.76, 3.26)**	1.41 (0.89, 2.22)
31-33 22,766	Reference	**1.71 (1.47, 1.98)**	**1.33 (1.05, 1.68)**	**1.84 (1.47, 2.31)**	**1.58 (1.20, 2.08**)
34-36 112,202	Reference	**1.72 (1.60, 1.84)**	**1.28 (1.14, 1.43)**	**1.52 (1.35, 1.72)**	**1.29 (1.11, 1.49)**
37-38 429,919	Reference	**1.38 (1.31, 1.44)**	**1.15 (1.08, 1.23)**	**1.31 (1.22, 1.42)**	**1.20 (1.09, 1.31)**
≥39 1,240,421	Reference	Referent	Referent	Referent	Referent
Wald Test P value		**< 0.01**	0.34	**< 0.01**	0.27

Adjusted for maternal age, race, education, state and year

We used the Wald test to assess whether PTB odds ratios displayed a heterogeneous pattern along the early GA continuum; only the combined infertility group showed a statistically significant heterogeneous pattern (p=0.02). For the outcome of preterm/early term birth (<39 weeks' gestation), a heterogeneous pattern was detected for both female infertility and combined infertility groups (<0.01). Figure 2 provides a graphic display of the odds ratios for preterm/early term birth by GA.

Table 2.4: Estimated effect of infertility on PTB and preterm/early term deliveries for primiparous, ART-users. Population-based data of all singleton live births in Florida and Massachusetts 2000-2010 and Michigan 2000-2009 linked to ART data

	Female Infertility	Male Infertility	Combined Infertility	Unexplained Infertility
Sample Size	N=9,891	N=4,819	N=3,688	N=2,930
PTB <37 weeks				
cOR (95% CI)	Reference	**0.72 (0.64, 0.80)**	0.94 (0.84, 1.05)	**0.72 (0.63, 0.82)**
*aOR (95% CI)	Reference	**0.78 (0.70, 0.87)**	0.93 (0.83, 1.05)	**0.79 (0.69, 0.91)**
Preterm/Early Term Birth <39 weeks				
cOR (95% CI)	Reference	**0.76 (0.71, 0.82)**	0.98 (0.91, 1.06)	**0.74 (0.68, 0.81)**
*aOR (95% CI)	Reference	**0.80 (0.75, 0.87)**	0.94 (0.87, 1.02)	**0.83 (0.76, 0.91)**

*Adjusted for age, race, education, state and year

For analyses within the ART population, the female infertility group served as the referent. We observed significantly lower odds for PTB < 37 weeks in both the male infertility group, aOR= 0.78 (95% CI 0.70, 0.87), and the unexplained infertility group, aOR=0.79 (95% CI 0.69, 0.91) (Table 2.4) when compared with the female infertility group. Models with PTB/early term birth defined as <39 weeks' gestation showed

significantly lower PTB odds across all ART groups relative to the female infertility group.

2.4 Discussion

We found that, among singleton deliveries to primiparous women, use of ART was associated with increased odds of PTB for couples with identified male infertility but without female infertility, when compared with births to non-ART users. These findings suggest that even in the absence of female infertility, the use of ART increases the risk for PTB. In addition, we found that the odds of PTB were higher among couples with female infertility only or those with combined female and male infertility, than those of the male infertility group, thereby indicating that factors related to female infertility may further increase the risk of preterm birth among primiparous ART users. Indeed, comparisons within the ART population showed that singletons conceived with ART due to male infertility had a lower risk of preterm or preterm/early term birth compared to singletons born to mothers with female infertility.

When the preterm groups were further subdivided into 5 or 6 categories, the increased risk associated with ART was not confined to late preterm and included the most vulnerable early preterm births, regardless of whether >37 weeks or >39 weeks was used as the referent category. When >37 weeks was used as the referent, variations in PTB odds ratios over the different gestational age categories were not statistically significant for the female and male infertility groups; however they were significant for the combined infertility group. When >39 weeks' gestation was used as the referent category for preterm/early term birth, the same heterogeneous pattern of odds ratios was observed in two groups - the female infertility and combined infertility

groups. The observed variations in PTB odds across various gestational age thresholds may be due to pregnancy complications related to the infertility itself, the use of ART procedures, or their combination, as well as other unmeasured factors. As far as we know, our study is the first to report variability in PTB odds along the gestational age continuum and within specific ART subgroups involving female infertility or combined infertility diagnosis.

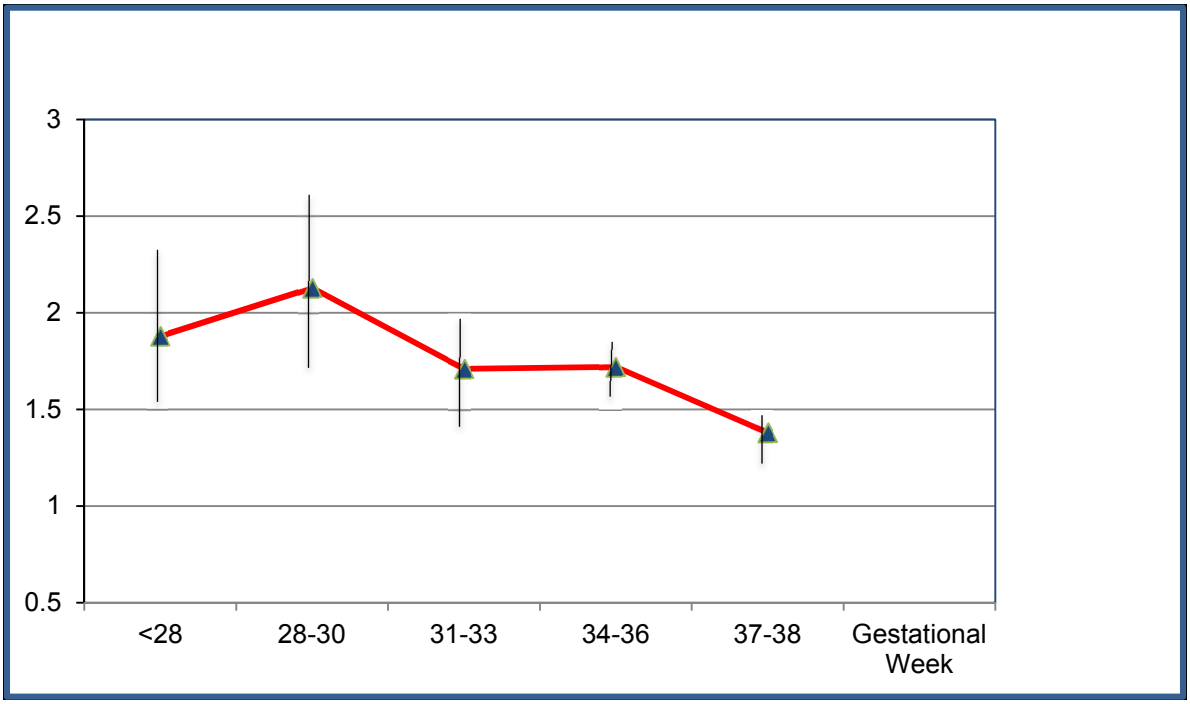

Figure 2: Odds ratio estimates for preterm/early term birth (<39 weeks) by GA for primiparous ART users with female infertility diagnosis

Biological explanations for our findings are likely heterogeneous and complex. One intriguing lead may come from a well-studied biomarker, relaxin. Several studies have indicated that relaxin, a polypeptide hormone produced during pregnancy by the corpus luteum and decidua, could play a role in PTB because of its effect on collagen breakdown (87, 90). In animal models, it was shown that the involvement of relaxin in cervical collagenolysis decreased cervical resistance and led to consequent cervical ripening (88). Among uncomplicated, non-ART pregnancies, maternal blood relaxin levels typically decrease with advancing gestation (195). However, compared to naturally conceived pregnancies, those conceived through ovulation induction, had higher circulating levels of relaxin, not only in the first trimester (91) but also in the second and third trimesters (89). Finally, higher relaxin levels, observed at the third trimester were significantly associated with PTB (90). Other suggested explanations for the higher PTB risk among ART singletons include placental abnormalities (107, 196), a co-twin fetal reabsorption (94) transfer of fresh versus frozen embryo (167) and use of donor versus autologous oocytes (182).

Our study has several strengths. *First*, while previous studies compared PTB risk between pregnancies achieved with and without the use of ART, we were able to divide the ART population into four distinct groups with respect to their infertility diagnosis thereby providing specific PTB risk by infertility diagnosis. *Second*, the SMART Collaborative database is comprehensive and unique as it includes numerous variables related to both exposures and outcomes. ART variables are of particularly high quality because they are collected via a federally mandated reporting system with approximately 7-10% of reporting clinics randomly selected for validation each year.

Consequently, the data provide us with the opportunity to refine previous study questions and conduct novel analyses among distinct subgroups of the population. Finally, by linking multiple years of data, sample size and thus statistical power are increased.

There are also limitations to consider. *First*, the probabilistic linkage method of birth files and NASS data resulted in high success rate, but is not free of matching errors (188). *Second*, some women in the unexposed group, the non-ART group, may have been exposed to hormones, through non-IVF ovulation induction or ovarian stimulation protocols. In addition a small percentage of couples in the non-ART group may have experienced sub-fertility similar to the ART group, but continued to attempt conception without ART and were then successful. This would likely have the effect of attenuating odds ratios of PTB related to infertility. *Third*, the female infertility group is not homogeneous with respect to the infertility diagnosis, rather, it is a collection of different conditions related to several mechanical or hormonal dysfunctions. Similarly the infertility groups are heterogeneous with respect to the specifics of ART procedures. Therefore, the PTB risk may differ based on underlying condition of female infertility diagnosis and on ART treatments. Fourth, the gestational age at birth was based on birth certificate data for both non-ART and ART pregnancies. It is possible that birth file GA estimates are better informed (more accurate) among ART pregnancies when embryo transfer dates are known. Finally, the large size of our dataset allows detection of small differences that are statistically significant but may not be of clinical importance.

2.5 Conclusions

The risk for PTB for ART conceived pregnancies, even in the absence of female infertility, is higher than for pregnancies in the general population. A female infertility diagnosis, as opposed to male infertility alone, may be associated with a greater increase in the likelihood of PTB in ART births.

The increased risk of PTB associated with ART is not confined to late preterm births, and among the ART subset with female infertility or combined male and female infertility, the risk is inversely related to gestational age at birth.

CHAPTER 3

ASSISTED REPRODUCTIVE TECHNOLOGY AND NEWBORN SIZE IN SINGLETONS

3.1 Introduction

Singletons conceived with the use of Assisted Reproductive Technology (ART) are at increased risk for preterm birth compared with singletons in the general population (107, 114, 140, 197). In addition, ART singletons are found to have higher risk of low and very low birth weight, classified as <2,500g and <1,500g, respectively, compared with non-ART singletons (82, 103, 116, 198). Low Birth Weight (LBW) has long been used as an indicator for child health, however, its interpretation is unclear because LBW may be related to short gestation, poor fetal growth or their combination (147, 199-202). Therefore, indicators that distinguish LBW infants resulting from short gestation or poor fetal growth by matching the gestational age to the birth weight measurement provide a more informative measure of risk by reducing this confounding. Two such indicators are birth weight z-score and small for gestational age (SGA).

The definition of SGA can vary across studies and may include infants whose birth weight is below the 10^{th} or 5^{th} percentile for gestational age or whose birth weight is 2 or more standard deviations below the mean birth weight for gestational age. The use of inconsistent definitions can hamper the comparison of findings across studies. For example, two recent studies did not detect an increased risk of SGA (defined as $<10^{th}$ percentile) for ART compared to non-ART singletons (105, 119), whereas, other studies reported significantly increased risk of SGA ($<10^{th}$ percentile) among ART singletons, with odds ratios ranging from 1.22- 1.98 (103, 107, 108, 116, 141). In one study, ART singletons were found to have a 40% higher odds for SGA (5^{th} percentile)

compared with their non-ART counterparts (161). Finally, when defined as 2 standard deviations below the mean birth weight for gestational age, SGA risk for ART singletons was similar to that of singletons in the general population (81, 112). These conflicting results may be attributed to the variety of SGA definitions across studies, or to other sources of heterogeneity such as sample size and/or the approach to potential confounders and effect modifiers, e.g. plurality, social factors and sources of the underlying infertility.

Reporting birth weight z-score, constructed as a continuous and standardized measure, allows the comparison of newborn size for infants across gestational ages, sexes and birth weights, representing the same *relative* birth weight for infants, rather than their *absolute* weight. Compared to LBW, newborn size measured as birth weight z-score has rarely been used to investigate birth outcomes in ART populations (203). In 2008, Shih et al reported a higher birth weight z-score for non-ART singletons [-0.061 (SD=1.099)] relative to those born as a result of ART with fresh, non-frozen embryos [-0.163 (SD=1.004)] (162).

The inconsistent findings across studies of ART and newborn size warrant additional investigations of this potential association. The purpose of the current study was to use data from the States Monitoring Assisted Reproductive Technology (SMART) collaborative to examine: 1) whether ART singletons are at higher risk of small newborn size, measured by both SGA (10^{th} and 5^{th} percentile) and birth weight z-score as indicators, compared with singletons in the general population; and 2) whether an association between ART and newborn size is modified by the source of infertility.

3.2 Materials and methods

3.2.1 Study population

We used a population-based dataset of birth files from three states linked to the National ART Surveillance System (NASS) by the SMART collaborative project. After initial pilot project in Massachusetts that started in 2001, SMART Collaborative was established by the Centers for Disease Control and Prevention and public health agency of Massachusetts, Michigan, and Florida to evaluate maternal and perinatal outcomes of ART and to improve state-based ART surveillance (187). Our sample included all live births in Florida and Massachusetts from 2000-2010, and Michigan from 2000-2009 linked to ART cycles in the respective states, using a probabilistic matching method with a high linkage rate (87.8%) and good validity (188).

We restricted our sample to singletons born to mothers age 15-60 between 22 and 44 weeks' gestation. We then excluded records with implausible combinations of birth weight and gestational age. To do this, we began by applying the criteria described in Table 1 of Alexander et al (204). We then applied a sex-specific and gestational age-specific reference to generate birth weight z-scores based on the United States population (205). The distribution of z-scores continued to suggest the presence of outliers. We therefore applied additional, published criteria to identify implausible values. For full-terms, outliers included z-scores beyond -5 and 5; for preterms, outliers included z-scores beyond -4 and 3 (205, 206). This approach resulted in exclusion of 4,188 records. After all exclusions, our final dataset included a total of 4,296,537 singleton live births, of which 4,263,846 (99%) were non-ART (1%) births and 32,691 were ART related births.

The study received approval from the Institutional Review Boards of Massachusetts, Michigan, Florida and the CDC

3.2.2 Infertility groups

Infertility diagnoses for ART users were identified through linked data from birth files with their records in NASS. We further divided the ART users into four mutually exclusive subgroups based on their infertility diagnosis, as recorded by the NASS: female infertility only (n=15,713), male infertility only (n=6,982), combined male and female infertility (n=5,536) and unexplained infertility (n=4,460). Women were classified in the non-ART group, if no match was found between their live birth record and NASS data.

3.2.3 Newborn size

Gestational age, based on clinical estimate, birth weight, and sex, were abstracted from birth certificates. We then used this information in conjunction with a sex and gestational age specific, population-based reference to calculate categorical (small for gestational age (SGA)) and continuous (birth weight z-score) measures of newborn size (205). We defined SGA according to two different thresholds (10^{th} and 5^{th} percentiles, SGA/10^{th} and SGA/5^{th}, respectively). Birth weight z-score was computed using the following formula:

$$\text{Birth weight z-score} = \frac{(\text{Infant's birth weight} - \text{mean birth weight in the reference population})}{\text{Standard deviation of the reference population}}$$

3.2.4 Covariates

In unadjusted analyses race/ethnicity was classified into 5 categories: non-Hispanic white, non-Hispanic black, Hispanic, Asian/Pacific Islander or other (including American Indian) and maternal education was modeled as a four level variable: less than high school, high school or GED diploma, some college education including associate's degree and a bachelor's or postgraduate degree. Adjusted models required some collapsing of categories within these variables to meet requirements of cell sizes >10 in contingency tables. Specifically, we collapsed race/ethnicity categories of 'Hispanic' and 'non-Hispanic Asian/other' into one group, and maternal education categories of 'high school diploma or GED' and 'less than high school into one group. Maternal age at the time of the birth and parity, specified as the number of prior live births, were recorded on birth files as continuous variables. The state variable had three categories, Florida, Massachusetts and Michigan. Delivery year had 11 categories from year 2000 to 2010.

3.2.5 Statistical analysis

We used basic descriptive statistics, chi-square tests and linear regression for complex data, to compare the distributions of maternal and infant characteristics among ART and non-ART groups, before and after excluding those with implausible birthweight and gestational age combinations (see *Study Population*). To evaluate the unadjusted association between ART and newborn size, using categorical and continuous measures of growth, we constructed logistic and linear regression models, respectively, with robust variance estimators to address the correlation between infants delivered by the same mother during the study period. We then repeated the above analyses

adjusting for parity, maternal age, race, education, state of residence and delivery year as the covariates. To investigate whether newborn size was influenced by female infertility diagnosis, above the ART effect, we performed a second set of regression analyses among all ART users with the male infertility group as a referent.

Finally, we repeated the analyses of SGA/10th and ART after removing preterm births. The construct of SGA/10th for preterm infants has some inherent bias because infants born preterm tend to be smaller than their counterparts who remain in-utero. By comparing only full-term ART and non-ART, we examine the robustness of our results absent this potential preterm bias.

SAS 9.3 (Cary, NC) was used for logistic models analysis. Linear regression models compared birth-weight-z-scores across our study groups ($p<0.05$) were generated with the PROC REGRESS of SUDAAN 11 (RTI) statistical software.

3.3 Results

After excluding infants with implausible combinations of birth weight and gestational age (1% of the total births), the final sample included 4,263,846 infants. Frequencies of maternal and infant characteristics were similar in samples with and without the excluded births (Table 3.1).

Compared to the non-ART mothers, ART mothers were significantly older, more educated and more likely to be Non-Hispanic white and primiparas ($p<0.01$). Infants born to non-ART mothers had a similar sex distribution, 51% male and 49% female, as that found among infants of ART mothers, but ART infants' mean birth weight was 24 grams lower ($p<0.01$).

Table 3.1: Maternal and Infant characteristics for ART and non-ART singleton live births in Massachusetts and Florida 2000-2010 and in Michigan 2000-2009: before and after exclusion of implausible birth weight for gestational age

Maternal, infant Characteristics	**Before Exclusion**			**After Exclusion**		
	Non-ART	ART	*P value	Non-ART	ART	*P value
Sample Size N (%)	4,287,315 (99)	32,847 (<1)	<0.01	4,263,846 (99)	32,691 (<1)	<0.01
Maternal Age (mean)	27.7	35.5	<0.01	27.7	35.5	<0.01
Maternal Race/Ethnicity						
Non-Hispanic White	2,459,087 (58)	26,250 (81)		2,447,489 (58)	26,136 (81)	
Non-Hispanic Black	764,997 (18)	1,273 (4)		759,051 (18)	1,263 (4)	
Hispanic	807,398 (19)	2,808 (9)	<0.01	803,781 (19)	2,798 (9)	<0.01
Asian/Other	223,101 (5)	2,021 (6)		222,012 (5)	2,012 (6)	
Maternal Education						
High school or lower	2,054,842 (48)	4,260 (13)		2,042,263 (48)	4,230 (13)	
Some college	1,050,003 (25)	6,789 (21)	<0.01	1,044,839 (25)	6,747 (21)	<0.01
Bachelor's or higher	1,143,164 (27)	21,619 (66)		1,138,459 (27)	21,538 (66)	
Parity						
0	1,805,559 (42)	21,400 (65)		1,794,285 (42)	21,284 (65)	
1	1,398,243 (33)	8,473 (26)	<0.01	1,392,288 (33)	8,446 (26)	<0.01
2	672,521 (16)	2,058 (6)		669,158 (16)	2,046 (6)	
≥3	410,992 (10)	916 (3)		408,115 (10)	915 (3)	
Newborn Sex						
Male	2,196,386 (51)	16,853 (51)	0.78	2,184,140 (51)	16,770 (51)	0.79
Female	2,090,838 (49)	15,994 (49)		2,079,706 (49)	15,921 (49)	
Mean Birth weight (g) (se)	3,318 (0.3)	3,295 (3.5)	<0.01	3,320 (0.3)	3,296 (3.4)	<0.01

*P values computed for correlated data

There were 2,795 (8.5%) and 400,220 (9.4%) infants born SGA/10th in the ART and non-ART groups, respectively. In unadjusted analyses, the percentage of SGA/10th and SGA/5th Infants was significantly lower in the ART versus the non-ART group (Table 3.2). Newborn size measured as birth weight z-score was 0.08 for ART and 0.03 for non-ART, suggesting that infants born to couples undergoing ART were born with higher birth weight than their non-ART counterparts (p<0.01).

Table 3.2: Newborn size measures for ART and non-ART groups with singleton live births in Florida and Massachusetts 2000-2010 and in Michigan 2000-2009

Newborn Size	Non-ART (%)	ART (%)	P value*
SGA <10th percentile	400,220 (9.4)	2,795 (8.5)	
Non-SGA ≥10th percentile	3,863,626 (90.6)	29,896 (91.5)	<0.01
SGA <5th percentile	193,192 (4.5)	1,337 (4.1)	
Non-SGA ≥5th percentile	4,070,654 (95.5)	31,354 (95.9)	<0.01
Birth weight z-score (se)	0.03 (0.0005)	0.08 (0.006)	<0.01

SGA=Small for Gestational Age;
*P value computed for complex data

The associations between ART treatment and newborn size (SGA/10th and SGA/5th) for the pooled ART group, as well as for each of the exclusive infertility subgroups, are presented in table 3.3. In adjusted analyses, the odds of an SGA/10th or SGA/5th infant were significantly greater in the ART combined group than in the non-ART group [aOR 1.15 (95% CI (1.11, 1.20) and aOR 1.13 (95% CI (1.07, 1.20), respectively]. Each ART subgroup had significantly increased odds of delivering an SGA/10th infant relative to the non-ART group; adjusted odds ratio for female infertility [1.15 (95% CI 1.08, 1.22)], male infertility [1.14 (95% CI 1.05, 1.25)], combined male and female infertility [1.11 (95% CI 1.01, 1.23)] and unexplained infertility [1.24 (95% CI

1.11, 1.38)]. Similar results were observed in adjusted SGA/5th models with the exception of male infertility diagnosis.

Table 3.4 depicts the likelihood of delivering an SGA infant for the ART subgroups using male infertility as the referent. There was little difference between subgroups when SGA was defined by the 10th percentile. However, couples with 'unexplained infertility' had a barely significant increase in risk of delivering an SGA/5th infant [1.22 (95% CI 1.00-1.48)] compared with couples with a diagnosis of male factor infertility.

Table 3.3: Associations between ART and delivery of SGA infant using non-ART as the referent group; Population-based data of all singleton live births in Florida and Massachusetts 2000-2010 and in Michigan 2000-2009

ART/Infertility Type	N			SGA* / 10th percentile		SGA* / 5th percentile	
	Total	SGA <10th	SGA <5th	cOR (95% CI)	**aOR (95% CI)	cOR (95% CI)	**aOR (95% CI)
Non-ART	4,263,846	400,220	193,192	Reference	Reference	Reference	Reference
ART (all users)	32,691	2,795	1,337	**0.90 (0.86, 0.93)**	**1.15 (1.11, 1.20)**	**0.89 (0.84, 0.95)**	**1.13 (1.07, 1.20)**
- ART/Female	15,713	1,340	634	**0.90 (0.85, 0.95)**	**1.15 (1.08, 1.22)**	**0.88 (0.81, 0.95)**	**1.11 (1.02, 1.21)**
- ART/Male	6,982	599	260	**0.91 (0.83, 0.99)**	**1.14 (1.05, 1.25)**	**0.82 (0.72, 0.93)**	1.03 (0.91, 1.17)
- ART/Combined	5,536	462	247	**0.88 (0.80, 0.97)**	**1.11 (1.01, 1.23)**	0.98 (0.86, 1.11)	**1.23 (1.08, 1.40)**
- ART/Unexplained	4,460	394	196	0.94 (0.84, 1.04)	**1.24 (1.11, 1.38)**	0.96 (0.83, 1.12)	**1.26 (1.08, 1.46)**

SGA-small for gestational age; cOR-crude odds ratio; aOR- adjusted odds ratio; CI-confidence interval
* Sex specific ** adjusted for parity, age race and education level, state of residence and delivery year

Table 3.4: Associations between ART and delivery of SGA infant using ART male infertility as the referent group. Population-based data of all singleton live births in Florida and Massachusetts 2000-2010 and Michigan 2000-2009

ART/Infertility Type	N	Small for Gestational Age* 10th percentile		Small for Gestational Age* 5th percentile	
		cOR (95% CI)	**aOR (95% CI)	cOR (95% CI)	**aOR (95% CI)
Non ART	4,263,846	N/A	N/A	N/A	N/A
ART/Female	15,713	0.99 (0.90, 1.10)	1.00 (0.91, 1.11)	1.09 (0.94, 1.26)	1.08 (0.93, 1.25)
ART/Male	6,982	Reference	Reference	Reference	Reference
ART/Combined	5,536	0.97 (0.85, 1.10)	0.97 (0.85, 1.10)	**1.21 (1.01, 1.44)**	1.19 (0.99, 1.43)
ART/Unexplained	4,460	1.03 (0.90, 1.18)	1.09 (0.95, 1.25)	1.19 (0.98, 1.44)	**1.22 (1.00, 1.48**)

SGA-small for gestational age; cOR-crude odds ratio; aOR- adjusted odds ratio; CI-confidence interval
* Sex specific ** Adjusted for parity, age race and education, state of residence and delivery year

After excluding all preterm births from our cohort, the statistically significant association between ART and small newborn size remained. Full term ART singletons were more likely to be SGA/10th relative to full term singletons in the non-ART group [aOR 1.19 (95% CI 1.14, 1.24)]. Similar results were observed when each ART subgroup was compared to the non-ART group (data not shown).

Next, newborn size was modeled as a continuous variable (i.e., birth weight z-score) and non-ART singletons were the referent group (Table 3.5). ART singletons appeared larger (higher mean z-score) in unadjusted analyses, but in adjusted analyses the ART singletons were significantly smaller (lower mean z-score). This pattern held for all ART infertility subgroups and mimicked the contrasting results we found between unadjusted and adjusted models with SGA.

We observed that all ART subgroups had a negative mean birth weight z-score (female infertility= -0.09, male infertility= -0.07, combined male and female infertility= -0.09 and couples with unexplained infertility diagnosis= -0.12,), indicating birth weight means below the mean of the reference population. The mean birth weight z-score of singletons in the non-ART group was above that of the reference population. In analyses focused on comparisons across ART subgroups, male infertility again served as the referent subgroup. The mean birth weight z-score was significantly lower among the 'unexplained infertility' group only.

Table 3.5: Associations between ART and infant mean Birth Weight z-score using non-ART as the referent group; Population-based data of all singleton live births in Florida and Massachusetts 2000-2010 and Michigan 2000-2009

ART/ Infertility Type	N (%)	Birth Weight z-score* crude (CI)	Birth Weight z-score* adjusted** (CI)	Regression coefficient adjusted** (CI)	P Value**	P Value**
Non-ART	4,263,846	0.033 (0.032, 0.034)	0.035 (0.034, 0.036)	Reference	Referent	N/A
ART/Female	15,713	0.085 (0.069, 0.101)	-0.060 (-0.089, -0.056)	-0.09 (-0.11, -0.08)	<0.01	0.06
ART/Male	6,982	0.089 (0.064, 0.113)	-0.032 (-0.076, -0.044)	-0.07 (-0.98, -0.05)	<0.01	Referent
ART/Combined	5,536	0.088 (0.051, 0.106)	-0.052 (-0.079, -0.025)	-0.09 (-0.11, -0.06)	<0.01	0.28
ART/Unexplained	4,460	0.066 (0.036, 0.097)	-0.084 (-0.115, -0.053)	-0.12 (-0.15, -0.09)	<0.01	0.01

CI-confidence interval
* Gestational age and sex specific
** Adjusted for parity, age, race and education level, state of residence and delivery year

3.4 Discussion

Our study is one of the largest to investigate newborn size among singleton infants born to ART users, with 2,795 and 1,337 SGA infants at the 10th and 5th percentiles, respectively. We found that ART singletons had increased odds of being SGA/10th regardless of whether infertility was diagnosed in the female, male or both. The increased odds ranged from 11% to 24%, with the greatest risk in the unexplained infertility group.

Our estimated effect sizes were more modest in comparison to some previous reports of 1.4 to 2-fold risk of being born SGA/10th among ART compared to non-ART singleton infants (92, 103, 107, 116, 141). However, a recent Australian data linkage cohort study found that the risk of SGA/10th in ART singletons vs. non-ART singletons was more modest as well, [aOR 1.22 (95% CI 1.11, 1.33)] (108) and aligned with our result for all ART subgroups combined [aOR 1.15 (95% CI 1.11, 1.20)]. Inconsistent results across studies of ART and newborn size might be explained by multiple heterogeneous elements. SGA has been variably defined as SGA/10th, SGA/5th (161) or SGA less than two standard deviations below a growth standard of a reference population (81, 112, 154). Some studies did not control for important confounders, such as multi-fetal pregnancies and socioeconomic factors (81, 158, 160). The larger effect sizes were often observed among non-US populations. e.g. Danish (161), Swedish (81), British (158) and Dutch (92). One US study reporting a nearly two-fold increased risk of SGA among ART singletons included a relatively small sample size and self-reported information on infertility and use of treatments (103). Self-report of ART use have been shown to overestimate ART singleton births (207).

We showed that singletons born to ART users with 'unexplained infertility' were at greatest risk of SGA, not only compared to infants in the non-ART group, but also compared to other ART singletons. Similar findings of adverse obstetric and neonatal outcomes among couples with unexplained infertility were previously reported (151, 208, 209), suggesting this is a group that merits further investigation.

The birth weight z-score is a standardized measure that allows group comparisons of newborn size when including infants born at varying gestational weeks. Although z-scores are recommended for reporting perinatal outcomes among ART populations (203, 210), such studies are rare. In 2008, Shih et al, used birth weight z-score, based on British growth reference data, to examine whether newborn size was associated with different types of ART treatment (162). While their findings suggested lower mean birth weight z-scores among infants born to couples who used ART with fresh embryos, -0.163 (1.004), compared to infants of non-ART couples, -0.061 (1.099), both groups had mean birth weight z-scores below the expected mean. In our analysis we used two indicators, SGA and birth weight z-score, to measure gestational age-specific and sex-specific newborn size both categorically and continuously. To minimize measurement errors, both indicators were carefully constructed after exclusion of birth records with implausible birth weight and gestational age combinations using established and recently published criteria and algorithms (204-206). In contrast to Shih (162), our results showed that the non-ART group had mean birth weight z-scores slightly above that of the reference population, whereas the ART group's means were below the referent.

Aspects related to ART treatment and/or the underlying infertility might explain suboptimal newborn size among ART infants. These include the effects of ovarian stimulation (211, 212), endometrial or placental abnormalities (119, 213-216) and imprinting disorders and altered expression of genes (H19, IGF1, IGF2) involved in human development (76, 77). The practice of multiple embryo transfer may result in implantation of more than one embryo and early fetal loss of a co-twin may have a detrimental effect on the surviving fetus (96). Untreated maternal infertility and its related characteristics, namely, advanced age and primiparity were also suggested to play a role in infant size (10, 11, 13, 115, 217-219); these latter covariates were accounted for in our analytic models.

In our study design and analyses we tried to address limitations of previous studies, however, some limitations remain. Although SGA is a better indicator of newborn size than LBW, it still represents a heterogeneous group, i.e. those who are constitutionally small and those with pathologically poor fetal growth. In addition, fetuses who begin as appropriate size and then experience poor growth may not meet the 10^{th} percentile cutoff at birth, thus using SGA as an outcome could result in some misclassification of suboptimal newborn size for infants above and below the cut point (146). SGA is constructed using population-based references of birth weight, while excluding unborn fetuses. As a result, SGA formulation for preterm infants may be prone to bias resulting from birth weight differences between preterm infants (often smaller) and those who remain in-utero. In an attempt to minimize this observational bias, we repeated our analysis using only full term infants. Our results indicated a

similar risk of small newborn size for ART singletons versus non-ART infants born at term.

The SMART dataset includes two distinct populations, ART and non-ART couples. We acknowledge that data quality with respect to timing of conception may vary by the mode of conception, and may be more accurate for the ART population. To create the SMART dataset, a probabilistic method was used to link birth files and ART surveillance data. While highly successful, with reported high linkage rate of 87.8% and a good validity, this method is not free of matching errors (188).

We investigated the association of ART and newborn size using one of the largest datasets of ART data linked to birth records. Our outcomes, SGA and birth weight z-score, are the most informative and least biased compared to other frequently used measures, e.g. LBW and mean birth weight. As few studies defined SGA using the 5^{th} percentile cutoff, we defined SGA both at the 10^{th} and 5^{th} percentiles and examined the risk of SGA singleton births among all ART users as one group, compared to non-ART singletons. We then created four mutually exclusive infertility groups, to identify whether newborn size within ART users is influenced by parental infertility diagnosis. Our second selected outcome, birth weight z-score, has innate properties that allow uniform, standardized reporting, essential for unbiased comparisons of newborn size across studies. Although birth weight z-score was recommended as a preferred measure of newborn size, it was rarely incorporated in previous reports.

Overall, we found an increased risk of small newborn size in ART versus non-ART singleton infants. Although statistically significant, the magnitude of excess risk overall was small, which is reassuring. Similarly, the excess risk of small newborn size within

ART subgroups defined by infertility source (male, female), was not large. Greater subgroup heterogeneity in newborn size may be detected when male and female infertility groups are further assessed by underlying infertility causes and subtypes of ART procedures.

CHAPTER 4

ASSISTED REPRODUCTIVE TECHNOLOGY AND ADVERSE PERINATAL OUTCOMES; ASSOCIATIONS WITH INFERTILITY DIAGNOSES AND TREATMENT MODALITIES

4.1 Introduction

Since its introduction to the US in 1981, Assisted Reproductive Technology (ART) has become a conventional treatment for infertile couples who desire a pregnancy. ART therapies usually involve the retrieval of male and female gametes and fertilization outside of the body. However, to maximize pregnancy rate, conventional ART treatment may be integrated with additional techniques and procedures, e.g. the use of donor gametes or embryos, transfer of fresh or frozen embryos, intra-cytoplasmic sperm injection (ICSI), insertion of a single sperm into an oocyte, and assisted hatching (AH) which creates a hole in the embryo's zona pellucida to promote embryonic implantation. The modality of ART regimen depends on couples' characteristics, infertility diagnosis, treatment history and factors related to cost and availability of insurance coverage.

Studies have consistently detected an increased risk of shorter gestation and smaller newborn size among ART pregnancies compared with non-ART pregnancies, independent of plurality (107, 197). Explanations for these findings are the subject of debate. It is unclear if the increased risk is present irrespective of the ART techniques employed. In addition, there is the challenge of disentangling excess risk associated with ART and excess risk connected to underlying causes of infertility. Studies attempting to separate the ART technology effect from the infertility effect have found a mild increased risk of preterm birth (PTB) and of small newborn size (10, 13) in relation to ART. There is remarkably little information on risk of adverse birth outcomes among

ART users in association with sources of infertility (male, female), the specific causes of infertility, and the modalities of ART. Reports on the risk of poor birth outcomes by infertility causes among untreated couples are likewise limited (82, 163, 220).

Therefore, we performed a population-based retrospective cohort study to elucidate the associations among ART modality, infertility diagnoses, and poor birth outcomes, i.e. preterm birth and small newborn size.

4.2 Materials and methods

4.2.1 Data source and study population

The States Monitoring Assisted Reproductive Technology (SMART) project was established to create state-based surveillance of ART, with the purpose of monitoring and studying health outcomes associated with ART (187). The SMART consortium includes the Centers for Disease Control and Prevention (CDC) and the Massachusetts, Florida and Michigan Public Health agencies. SMART constructed a population-based dataset that linked ART data from the National Assisted Surveillance System (NASS) with birth files of all live births occurring in Massachusetts, Florida and Michigan in 2000-2010 (188). The probabilistic linkage method used to match NASS and birth records was successfully implemented, achieving a high linkage rate of 87.8% and a good validity (188).

After restricting our study to singletons births of mothers age 15-60 between 22 and 44 weeks' gestation, the resulting sample size contained 4,263,846 non-ART and 32,691 ART mother-infant pairs. Within the ART population we assigned mother-infant pairs to study groups first by infertility source and later by treatment characteristics. There were 10 exclusive categories of infertility: male infertility, unexplained infertility,

endometriosis, diminished ovarian reserve, tubal disease, tubal ligation not reversed, ovulation disorders, uterine factor, other infertility factor (defined as immunologic, chromosomal, cancer chemotherapy or other systemic disease) and more than one infertility diagnoses. We examined various ART treatment modalities by considering: 1) ART technique components for fresh, autologous cycles – ART/basic, ART/ICSI, ART/AH and ART/ICSI/AH; 2) ART type and embryo source – fresh autologous embryos, fresh donor embryos/oocytes, frozen autologous embryos and frozen donor embryos/oocytes and 3) ART/male infertility by semen source, e.g. partner or donor. The donor semen category was merged with a third semen source containing mixed semen of partner and donor.

4.2.2 Birth outcomes

Preterm birth (PTB) was first constructed as a binary variable using 37 completed weeks' gestation as a cutoff to classify preterm births. To investigate the risk pattern along earlier gestations, we further separated PTB to early PTB (<34 weeks' gestation) and later PTB (34-36 weeks) while ≥37 weeks remained the referent category. Newborn size was defined as a continuous measure, birth weight z-score, computed with the formula:

$$\frac{(\text{Infant's birth weight - mean birth weight in the reference population})}{\text{Standard deviation of the reference population}}$$

where birth weight, gestational age and sex were abstracted from birth records and compared with a sex and gestational age specific US population-based reference (205). Finally, we defined singletons as Small for Gestational Age (SGA) if their birth weight was below the 10^{th} percentile for gestational age, or as non-SGA, otherwise.

Our study obtained an approval from the Institutional Review Boards of the Public Health Agencies of Massachusetts, Michigan, and Florida and from the CDC.

4.2.3 Statistical analysis

Descriptive statistics, chi square and linear regression for complex data, were used to compare the distributions of maternal and infant characteristics between ART and non-ART groups. We applied statistical methodology for clustered data, e.g. robust variance estimators, to account for more than one singleton birth to the same mother within the time interval.

The associations among infertility source, ART treatment profiles and perinatal outcomes were examined using regression models; linear regression for the continuous birth weight z-score and logistic or multinomial for SGA and PTB, as binary or a three-category variables, respectively. In all models, we first estimated the crude and then the adjusted effect of ART and infertility adding parity, maternal age, race, education level, state of residence and delivery year as covariates. To control for infertility causes, an additional covariate, infertility source, was included in all adjusted analyses of ART cycle types (fresh/frozen and ICSI/AH), and excluded otherwise.

We modeled our outcome of newborn size by using the continuous measure of birth weight z-scores, with the exception of one analysis where we had adequate group sample sizes and also assessed SGA. PTB was treated as a categorical outcome throughout but could not be investigated with relation to donor, non-donor semen due to limited sample size.

Our initial set of analyses compared newborn size and odds of PTB among non-ART and ART grouped by infertility cause. To assess whether particular infertility

causes had a distinct association with PTB, we conducted a series of contrast tests among pairs of infertility groups.

Next, we examined the same outcomes, newborn size and PTB, in relation to ART techniques. First we compared each ART technique group with the non-ART group, then we conducted comparisons across ART treatment groups using ART/basic group as the referent.

ART cycle types (fresh, frozen, non-donor and donor embryos or oocytes), and their associations with newborn size and PTB were studied using non-ART as a referent group and then frozen/non-donor as the referent group.

Finally, to assess treatment associations separated from infertility associations, we compared newborn size between singletons born to non-ART and ART groups using partner or donor semen. The latter is a unique set of infants born to couples with a diagnosis of male infertility, but conceived with fertile gametes to fertile mothers. In our last set of analyses we assigned ART singletons conceived by couples with male infertility using autologous semen as the referent group with the purpose of assessing whether semen source, donor versus partner, influences newborn size of ART singletons.

SAS 9.3 (Cary, NC) was used for logistic models analysis. Linear regression and multinomial models were generated with SUDAAN 11 (RTI) statistical software.

4.3 Results

4.3.1 Maternal and birth characteristics

Our sample included 4,263,846 non-ART and 32,691 ART-associated singleton births (Table 4.1). ART mothers were more likely to be non-Hispanic white, significantly

older and primaparas, and to have attained a higher education level as compared to non-ART mothers. In unadjusted analyses that grouped all ART together, ART was associated with an increased risk of delivering preterm, but a decreased risk of delivering an SGA infant. We observed a similar sex distribution among ART and non-ART singletons.

Table 4.1: Maternal and Infant characteristics of ART and non-ART singleton live births in Three States; Massachusetts and Florida 2000-2010, Michigan 2000-2009

Maternal, infant Characteristics	Non-ART (%)	ART (%)	*P value
Sample Size N	4,263,846 (99)	32,691 (<1)	<0.01
Mean Maternal Age (se)	27.7 (0.003)	35.5 (0.03)	<0.01
Maternal Race/Ethnicity			
Non-Hispanic White	2,447,489 (58)	26,136 (81)	
Non-Hispanic Black	759,051 (18)	1,263 (4)	
Hispanic	803,781 (19)	2,798 (9)	<0.01
Asian/Other	222,012 (5)	2,012 (6)	
Maternal Education			
High school or lower	2,042,263 (48)	4,230 (13)	
Some college	1,044,839 (25)	6,747 (21)	<0.01
Bachelor's or higher	1,138,459 (27)	21,538 (66)	
Parity			
0	1,794,285 (42)	21,284 (65)	
1	1,392,288 (33)	8,446 (26)	<0.01
2	669,158 (16)	2,046 (6)	
≥3	408,115 (10)	915 (3)	
Newborn Sex			
Male	2,184,140 (51)	16,770 (51)	0.79
Female	2,079,706 (49)	15,921 (49)	
Mean Birth weight (g) (se)	3,320 (0.3)	3,296 (3.4)	<0.01
SGA <10th percentile	400,220 (9.4)	2,795 (8.5)	
Non-SGA ≥10th percentile	3,863,626 (90.6)	29,896 (91.5)	<0.01
Birth weight z-score (se)	0.03 (0.0005)	0.08 (0.006)	<0.01
PTB <37 weeks	342,355 (8)	3,776 (11.6)	
Term Birth ≥37 weeks	3,921,491 (92)	28,915 (88.4)	<0.01

*P value computed for complex data; SGA=Small for Gestational Age; PTD=preterm birth

4.3.2 Newborn size and risk of preterm birth in relation to ART and infertility source

In adjusted analyses of newborn size we found significantly lower mean birth weight z-scores, below the mean of the reference population, among infants in each ART group relative to infants in the non-ART group. As with PTB, we observed ART group variations in newborn size with a mean birth weight z-score range of (-0.11) to (-0.02) representing infants born to ART users with diminished ovarian reserve and tubal disease, respectively.

The adjusted odds of PTB were significantly increased among all ART infants, in any infertility subgroup, compared to non-ART infants (Table 4.2). A spectrum of PTB odds was observed across the ART subgroups ranging from 1.42 (95% CI 1.30, 1.54) to 2.33 (95% CI 1.64, 3.30) for couples with male infertility and uterine factor infertility, respectively. In contrast analysis, the ART male infertility group had significantly lower PTB odds relative to other ART infertility subgroups ($p<0.05$). ART users with diminished ovarian reserve or ovulatory disorders had significantly increased PTB odds relative to ART users with unexplained infertility or with endometriosis.

Table 4.2: Associations among infertility diagnoses and birth outcomes in singleton live births in Three States; Massachusetts and Florida 2000-2010, Michigan 2000-2009

Newborn Size – Birth Weight z-score Mean and Standard Errors					
ART/Infertility Type	N	Birth Weight z-score* crude (SE)	Birth Weight z-score* adjusted** (SE)	Regression coefficient adjusted** (CI)	P Value**
Non-ART	4,263,846	0.03 (0.0006)	0.03 (0.0006)	Referent	Referent
Female Infertility					
Endometriosis	1,825	0.10 (0.02)	-0.04 (0.02)	-0.08 (-0.12, -0.03)	**<0.01**
Diminished ovarian reserve	2,337	0.09 (0.02)	-0.11 (0.02)	-0.15 (-0.19, -0.11)	**<0.01**
Tubal Disease	2,807	0.08 (0.02)	-0.02 (0.02)	-0.06 (-0.10, -0.02)	**<0.01**
Tubal Ligation	443	0.11 (0.05)	-0.08 (0.05)	-0.12 (-0.21, -0.02)	**0.01**
Ovulation Disorder	1,997	0.08 (0.02)	-0.03 (0.02)	-0.07 (-0.12, -0.02)	**<0.01**
Uterine Factor	286	0.06 (0.06)	-0.10 (0.06)	-0.13 (-0.25, -0.02)	**0.02**
Other	2,829	0.07 (0.02)	-0.10 (0.02)	-0.13 (-0.17, -0.09)	**<0.01**
>1 infertility diagnosis	8,546	0.08 (0.01)	-0.05 (0.01)	-0.08 (-0.11, -0.06)	**<0.01**
Male Infertility	6,982	0.09 (0.01)	-0.03 (0.01)	-0.07 (-0.09, -0.04)	**<0.01**
Unexplained Infertility	4,460	0.07 (0.02)	-0.08 (0.02)	-0.12 (-0.15, -0.09)	**<0.01**

Table 4.2 (cont'd)					
Preterm Birth - Odds Ratio and 95% Confidence Intervals					
ART/Infertility Type	N	PTB (%)	Odds Ratio crude (CI)	Odds Ratio adjusted** (CI)	Contrast (p<0.05)
[1]Non-ART	4,263,846	346,022 (8.1)	Referent	Referent	All groups
Female Infertility					
[2]Endometriosis	1,825	191 (10.5)	**1.32 (1.14, 1.54)**	**1.65 (1.41, 1.92)**	1,10
[3]Diminished ovarian reserve	2,337	328 (14.0)	**1.85 (1.64, 2.08)**	**2.05 (1.81, 2.31)**	1,2,10,11
[4]Tubal Disease	2,807	69 (13.8)	**1.81 (1.41, 2.33)**	**1.95 (1.51, 2.53)**	1,10
[5]Tubal Ligation	443	71 (16.0)	**2.15 (1.67, 2.77)**	**2.00 (1.54, 2.61)**	1,10
[6]Ovulation Disorder	1,997	271 (13.6)	**1.78 (1.56, 2.03)**	**2.24 (1.97, 2.56)**	1,2,10,11
[7]Uterine Factor	286	41 (14.3)	**1.89 (1.35, 2.64)**	**2.33 (1.64, 3.30)**	1,10
[8]Other	2,829	329 (11.6)	**1.49 (1.32, 1.67)**	**1.92 (1.70, 2.16)**	1,10
[9]>1 infertility diagnosis	8,546	1,056 (12.7)	**1.65 (1.54, 1.76)**	**1.90 (1.77, 2.03)**	1,10,11
[10]Male Infertility	6,982	646 (9.2)	**1.15 (1.06, 1.25)**	**1.42 (1.30, 1.54)**	All groups
[11]Unexplained Infertility	4,460	450 (10.1)	**1.27 (1.15, 1.40)**	**1.68 (1.52, 1.86)**	1,3,6,9,10

* Gestational age and sex specific ** Adjusted for parity, age, race and education level, state of residence and delivery

Table 4.3: Associations among fresh, autologous ART types and birth outcomes of singleton live births in Three States; Massachusetts and Florida 2000-2010, Michigan 2000-2009

Newborn Size – Birth Weight z-score Mean and Standard Errors						
ART/cycle Type	N	Birth Weight z-score* crude (SE)	Birth Weight z-score* adjusted** (SE)	Regression coefficient adjusted** (CI)	P Value**	P Value**
Non-ART	4,263,846	0.033 (0.0006)	0.03 (0.0006)	Referent	Referent	N/A
ART/Basic	7,971	0.029 (0.012)	-0.10 (0.01)	-0.13 (-0.15, -0.11)	**<0.01**	Referent
ART/ICSI	9,781	0.030 (0.010)	-0.09 (0.01)	-0.12 (-0.14, -0.10)	**<0.01**	0.57
ART/AH	2,457	0.054 (0.021)	-0.11 (0.02)	-0.14 (-0.18, -0.10)	**<0.01**	0.72
ART/ICSI/AH	4,762	0.025 (0.015)	-0.11 (0.02)	-0.14 (-0.17, -0.12)	**<0.01**	0.48
Preterm Birth - Odds Ratio** and 95% Confidence Intervals Non-ART Referent						
ART/cycle Type	N	≥37 weeks' gestation	34-36 weeks' gestation	<34 weeks' gestation	P Value**	
Non-ART	4,263,846	Referent	Referent	Referent	Referent	
ART/Basic	7,971	Referent	**1.70 (1.55, 1.83)**	**2.07 (1.81, 2.36)**	<0.01	
ART/ICSI	9,781	Referent	**1.62 (1.50, 1.74)**	**1.95 (1.73, 2.20)**	<0.01	
ART/AH	2,457	Referent	**1.70 (1.47, 1.97)**	**1.98 (1.56, 2.50)**	<0.01	
ART/ICSI/AH	4,762	Referent	**1.63 (1.47, 1.82)**	**1.73 (1.45, 2.06)**	<0.01	
Preterm Birth - Odds Ratio** and 95% Confidence Intervals ART/Basic Referent						
ART/cycle Type	N	≥37 weeks' gestation	34-36 weeks' gestation	<34 weeks' gestation	P Value**	
Non-ART	4,263,846	N/A	N/A	N/A	N/A	
ART/Basic	7,971	Referent	Referent	Referent	Referent	
ART/ICSI	9,781	Referent	0.96 (0.86, 1.07)	0.94 (0.79, 1.13)	>0.05	
ART/AH	2,457	Referent	1.01 (0.85, 1.19)	0.96 (0.73, 1.25)	>0.05	
ART/ICSI/AH	4,762	Referent	0.97 (0.85, 1.11)	0.84 (0.67, 1.04)	>0.05	

* Gestational age and sex specific

** Adjusted for parity, age, infertility source, race and education level, state of residence and delivery year

4.3.3 Newborn size and risk of preterm birth in relation to ART modality

4.3.3.1 Intra-cytoplasmic sperm injection and assisted hatching

Singletons in all ART subgroups were significantly smaller, mean birth weight z-score below the mean of the reference population, compared with non-ART singletons (Table 4.3). When ART/basic was used as the referent group, PTB odds and newborn size were similar across treatment modalities, e.g. AH, ICSI or their combination. The adjusted odds ratios for PTB (at <34 weeks and 34-36 weeks' gestation) were significantly increased in each ART treatment group, compared to the non-ART group.

4.3.3.2 Fresh or frozen cycles with non-donor or donor embryos/oocytes

In analyses of newborn size, measured as birth weight z-score or SGA, we observed a lower mean newborn size among 2 ART subgroups, fresh/non-donor and fresh/donor compared to non-ART infants (table 4.4). When ART singletons in the frozen/non-donor were used as the comparison group, we found significantly lower mean birth weight z-scores among singletons in all other ART subgroups. The adjusted PTB odds were significantly increased for all ART treatment groups, fresh/non-donor, fresh/donor, frozen/donor and frozen/non-donor, compared with the non-ART group. When ART frozen/non-donor group served as the referent, we observed significantly increased PTB odds among two of the ART groups, fresh/donor and frozen/donor. Finally, the odds of delivering a singleton SGA were significantly increased in two ART groups, fresh/non-donor and fresh/donor, relative to the non-ART group. These same two ART groups had a higher risk of delivering a singleton SGA infant when compared with the ART frozen/non-donor group.

Table 4.4: Associations among ART types and birth outcomes of singleton live births in Three States; Massachusetts and Florida 2000-2010, Michigan 2000-2009

Newborn Size – Birth Weight z-score and Standard Errors						
ART/Cycle Type	N (%)	Birth Weight z-score* crude (SE)	Birth Weight z-score* adjusted** (SE)	Regression coefficient adjusted* (CI)	P Value*	P Value*
Non-ART	4,263,846	0.033 (0.0006)	0.035 (0.0006)	Referent	Referent	N/A
Fresh/Non-donor	25,054	0.031 (0.031)	-0.077 (0.013)	-0.11 (-0.14, -0.09)	**<0.01**	**<0.01**
Fresh/Donor	2,905	0.103 (0.019)	-0.077 (0.024)	-0.11 (-0.16, -0.07)	**<0.01**	**<0.01**
Frozen/Donor	840	0.230 (0.035)	0.031 (0.038)	0.00 (-0.08, 0.07)	0.93	**<0.01**
Frozen/Non-donor	3,879	0.364 (0.017)	0.244 (0.020)	0.21 (0.17, 0.25)	**<0.01**	Referent
Preterm Birth - Odds Ratio and 95% Confidence Intervals						
ART/Cycle Type	N	PTB cOR (95% CI)	PTB aOR** (95% CI)	PTB cOR (95% CI)	PTB aOR** (95% CI)	
Non-ART	4,263,846	Referent	Referent	N/A	N/A	
Fresh/Non-donor	25,054	**1.41 (1.36, 1.47)**	**1.42 (1.30, 1.55)**	1.00 (0.90, 1.12)	1.03 (0.92, 1.15)	
Fresh/Donor	2,905	**2.13 (1.92, 2.35)**	**1.81 (1.58, 2.08)**	**1.52 (1.32, 1.75)**	**1.31 (1.13, 1.52)**	
Frozen/Donor	840	**2.45 (2.05, 2.93)**	**2.19 (1.79, 2.69)**	**1.75 (1.42, 2.15)**	**1.59 (1.29, 1.96)**	
Frozen/Non-donor	3,879	**1.40 (1.27, 1.55)**	**1.38 (1.22, 1.57)**	Referent	Referent	

Table 4.4 (cont'd)					
Small for Gestational Age - Odds Ratio and 95% Confidence Intervals					
ART/cycle Type	N (%)	SGA/10th cOR (95% CI)	SGA/10th aOR* (95% CI)	SGA/10th cOR (95% CI)	SGA/10th aOR* (95% CI)
Non-ART	4,263,846	Referent	Referent	N/A	N/A
Fresh non-donor	25,054	0.99 (0.95, 1.03)	**1.23 (1.13, 1.34)**	**2.08 (1.18, 2.42)**	**2.03 (1.73, 2.38)**
Fresh/Donor	2,905	0.87 (0.76, 0.99)	**1.20 (1.01, 1.42)**	**1.83 (1.50, 2.23)**	**1.98 (1.61, 2.43)**
Frozen/Donor	840	0.60 (0.45, 0.80)	0.83 (0.61, 1.13)	1.26 (0.91, 1.74)	1.37 (0.98, 1.91)
Frozen/Non-donor	3,879	**0.48 (0.41, 0.55)**	**0.61 (0.51, 0.72)**	Referent	Referent

* Gestational age and sex specific

** Adjusted for parity, age, infertility source, race and education level, state of residence and delivery year

Table 4.5: Associations among ART, Male Infertility, Semen Source and Newborn Size of Singleton Live Births in Three States; Massachusetts and Florida 2000-2010, Michigan 2000-2009

ART/Male Infertility by semen source	N (%)	Birth Weight z-score* crude (SE)	Birth Weight z-score* adjusted** (SE)	Regression coefficient adjusted** (CI)	P Value**	P Value**
Non-ART	4,263,846	0.033 (0.0006)	0.034 (0.0006)	Referent	Referent	N/A
ART/Male/Partner	5,791	0.044 (0.0135)	-0.077 (0.0136)	-0.11 (-0.138, -0.084)	**<0.01**	Referent
ART/Male/Donor	241	0.019 (0.0643)	-0.098 (0.0645)	-0.13 (-0.259, -0.006)	**0.04**	0.75

* Gestational age and sex specific

** Adjusted for parity, age, race and education level, state of residence and delivery year

4.3.3.3 Male Infertility with partner or donor sperm

Mean birth weight z-scores for ART singletons, born to couples with male infertility only, and independent of semen source, were significantly lower relative to that of non-ART singletons (table 4.5). However, we did not detect a significant difference in mean newborn size among ART singletons that were conceived with donor or partner's semen.

4.4 Discussion

In this large population-based cohort we examined infertility source and ART techniques in association with two perinatal outcomes of interest, newborn size and preterm birth. Consistent with previous reports, (107, 197), we found that after adjusting for important confounders such as maternal age, race and parity, ART singletons born to subfertile couples were more likely to be smaller and born preterm. We continued in this line of investigation by grouping ART according to underlying infertility diagnoses and ART modalities to examine risk by group. Our results showed that each female infertility ART group had an increased risk of adverse outcomes, i.e. preterm birth and smaller newborn size, whether compared to the non-ART group or to the male infertility group.

Only a limited number of studies have investigated whether the heterogeneity of infertility source and treatments among ART populations influences the risk of poorer perinatal outcomes. One study observed a significantly increased number of PTB cases compared to expected among ART singletons born to couples with tubal factor infertility (164). ART singletons of couples diagnosed with female infertility in general, or tubal

factor infertility in particular, had an increased risk for PTB compared with those born to couples with male infertility (151, 163).

Our study also considered perinatal outcomes across groups exposed to different ART techniques. Basic ART cycles are often combined with ICSI, AH techniques or both, to improve pregnancy rate. In previous studies, ART/ICSI treatments were frequently linked with higher rates of PTB and smaller newborn size compared with the non-ART group (106, 108, 111, 141). However, these studies did not always consider confounders such as type of cycle (fresh or frozen) and infertility source. One study investigated the added PTB risk of ICSI relative to the basic ART among singletons and reported a protective effect for ICSI [aOR 0.80 (95% CI 0.69, 0.93)], but since ART therapies with fresh or frozen embryos were not separated, the protective effect may have been driven by frozen cycles (111). Some studies have examined associations between AH alone, or combined with ICSI, and outcomes such as clinical pregnancy, live birth or implantation rate (38, 221). Our report is unique in its modeling of ICSI, AH and their combination, to show that ART couples exposed to these techniques have risks of PTB and small newborn size that are comparable to the risks among couples exposed to the 'basic' ART technique.

We also found that all combinations of ART types, fresh or frozen and donor or non-donor embryos/oocytes, were associated with a significantly increased risk of PTB and small newborn size among ART compared to non-ART groups. Comparisons across ART groups revealed that non-donor cycles, whether fresh or frozen, were associated with a smaller excess risk of PTB compared to that of groups with donor embryos or oocytes. It is plausible that oocyte donation induces immunological

response that may play a role in higher rates of hypertensive disorders and subsequent poor perinatal outcomes in ART pregnancy with donated compared with autologous oocytes (222-224).

Our results align with previous reports on the increased risk for PTB among ART singletons conceived with frozen embryos relative to non-ART singletons (107, 197). Our data also suggested that risks of PTB and small newborn size may be greater for the ART singletons conceived following a fresh versus a frozen embryo/oocyte cycle; this has been noted by others as well (111, 151, 167). Several hypotheses related to cycle and patients' characteristics, were suggested to explain the protective effect on poor birth outcomes observed in frozen versus fresh cycles; reduced ovarian stimulation, lack of oocyte retrieval, larger number of oocytes and of higher quality are more likely to result in cryopreservation among healthier patients (85, 167).

ART with donor gametes is the recommended treatment for patients with poor ovarian reserve, oocyte quality and/or severe male infertility. One small study observed an increased risk of PTB among ART singletons conceived with donated oocytes compared with ART autologous oocytes [aOR 1.8 (95% CI 1.2, 2.7)] or compared with non-ART singletons [aOR 3.4 (95% CI 2.3, 4.9)] (182). Perhaps due to its small sample size, this study did not further classify treatments as fresh or frozen embryo ART cycles. In our population-based study we were able to group ART couples by donor or non-donor oocytes/embryos and by fresh or frozen cycles. Data from the four resulting ART groups suggest that embryo/oocyte source is a factor more strongly associated with increased risk of PTB and small newborn size.

There is limited evidence on the effect of donor male gametes on PTB and small newborn size. A recent study did not detect significantly increased risks for PTB or small newborn size among singletons born following an Intra Uterine Insemination (IUI) using donor compared with partner semen. However, compared with ART singletons, IUI-conceived singletons had a lower risk of PTB (225). Among ART couples, similar crude odds of low birth weight or PTB were found in those using donor compared with partner sperm (226). In our study, sperm source was of interest for two reasons: 1) to assess associations with risk of suboptimal newborn size; and 2) to separate the infertility effect from the technology effect. We did this by comparing the non-ART group and the ART group, diagnosed with male but not female infertility. In this latter group, the combination of donor sperm and fertile woman has no infertility effect and therefore what remains is the effect of ART technology. Our results suggested that among ART users, semen source did not increase the risk for small newborn size. However, ART singletons, conceived with partner or donor sperm, had a significantly increased risk for smaller newborn size compared with non-ART. Most importantly, we found that even in the absence of parental infertility, singletons conceived through ART with fertile gametes were at a significantly increased risk for small newborn size ($p<0.04$); this would imply the presence an ART technology association that is no not confounded by underlying infertility.

Although we were able to examine birth outcomes, newborn size and PTB across ART treatment profiles and infertility diagnoses, while adjusting for most important confounders, we did not have information on whether the cause of early births was spontaneous or iatrogenic due to perinatal complications. In addition, our ART treatment

group with donor semen was small (n=241) and had insufficient statistical power to detect uncommon birth outcomes among ART singletons such as preterm birth and small for gestational age. Finally, although the probabilistic linkage method achieved a high linkage rate (87.8%) and good validity, it contains some matching errors (188).

In conclusion, our cohort study demonstrated a heterogeneous risk pattern for PTB and small newborn size among ART couples grouped by infertility source and ART modality. ART techniques added to basic ART therapy, did not appear to confer excess risk of these adverse perinatal outcomes over and above that found in association with basic ART. Our data support a small ART treatment association with smaller newborn size, independent of the underlying infertility.

CAHPTER 5

SUMMARY

Rapid technological advances of assisted reproduction that offer novel infertility therapies, and the steady increase of ART users, pose a pressing public health challenge. Thus, it is important to assess what is the health impact of ART on its direct and indirect users, e.g. mothers and infants.

This dissertation has examined the risk of adverse perinatal outcomes among singletons conceived with Assisted Reproductive Technology (ART) compared with non-ART singletons. Three perinatal outcomes were assessed; preterm birth, birth before 37 weeks' gestation; birth weight z-score, a gestational age and sex specific birth weight measure and small for gestational age, defined as birth weight below the 10^{th} or 5^{th} percentile for gestational age. The risk of preterm birth and small newborn size was investigated among distinct infertility diagnoses, female, male, combined and unexplained infertility, and across treatment modalities.

We found that ART singletons, in all infertility diagnoses groups, had an increased risk of preterm birth compared to non-ART singletons. However, among ART singletons, those born to subfertile mothers as opposed to subfertile fathers, may have the greatest increase of preterm birth risk. We also observed a heterogeneous risk of preterm birth along the gestational age continuum, but only among ART singletons born to couples with female infertility only or combined with male infertility. These findings suggest that the risk of preterm birth among ART couples may be mostly attributed to factors associated with female infertility.

Suboptimal newborn size, measured as birth weight z-score and small for gestational age at the 10th and 5th percentile, was also associated with ART compared with non-ART singletons. However, the magnitude of the estimated effect was mild and similar within all ART subgroups. These findings may be reassuring for infertile couples seeking ART treatment.

We found that ART-conceived singletons born to mothers with various infertility diagnoses were at increased risk for preterm birth and small newborn size, but the observed risk was heterogeneous among infertility subgroups, suggesting that infertility is a collection of various conditions with a differential effect on newborn size and gestational age at birth.

Assessment of preterm birth and small newborn size risk by modalities of ART treatment, indicated no excess risk among ART singletons conceived using intra-cytoplasmic sperm injection, assisted hatching or a combination of both techniques, compared to singletons conceived with basic ART therapy. While our data suggested that these ART techniques were not detrimental for preterm birth or suboptimal newborn size, more studies are needed to confirm these results as well as additional investigations on the effect of intra-cytoplasmic sperm injection and assisted hatching on other adverse outcomes.

ART cycle types, using fresh or frozen, donor or autologous oocytes/embryos were associated with preterm birth and suboptimal newborn size. However, our data suggest that the source of embryos/oocytes may play a more influential role in the risk of preterm birth than the transfer of fresh or frozen embryos. Compared to all other cycle types, frozen non-donor cycles had protective effects on perinatal outcomes,

perhaps because of their frozen and autologous components that are associated with less invasive and immunologic tolerant ART cycles.

We found that ART singletons conceived by couples without female infertility but with male infertility, using donor sperm, were more likely to be smaller at birth compared with non-ART singletons. This finding indicated a significant technology effect, separated from the infertility effect, on newborn size.

While our population-based cohort study confirmed previous reports on the risk of preterm birth and small newborn size among ART-conceived singletons compared with non-ART singletons, it was the first to expand and elaborate current knowledge by delineating the risk diversity across detailed treatment modalities and comprehensive parental infertility diagnoses. Future studies are needed to corroborate our findings and provide insight into ART effect on adverse perinatal outcomes among other populations.

REFERENCES

REFERENCES

1. Zegers-Hochschild FA, GD. de Mouzon, J. Ishihara, O. Mansour, R. Nygren, K. Sullivan, E. Vanderpoel, S. International Committee for Monitoring Assisted Reproductive Technology; World Health Organization. The International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology, 2009. Hum Reprod. 2009;Vol.24 (No.11):2683–7.

2. Gnoth C, Godehardt E, Frank-Herrmann P, Friol K, Tigges J, Freundl G. Definition and prevalence of subfertility and infertility. Hum Reprod. 2005;20(5):1144-7.

3. Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod. 2007;22(6):1506-12.

4. Stephen EH, Chandra A. Declining estimates of infertility in the United States: 1982-2002. Fertility and sterility. 2006;86(3):516-23.

5. Thoma ME, McLain AC, Louis JF, King RB, Trumble AC, Sundaram R, et al. Prevalence of infertility in the United States as estimated by the current duration approach and a traditional constructed approach. Fertil Steril. 2013;99(5):1324-31.e1.

6. Wilkes S, Chinn DJ, Murdoch A, Rubin G. Epidemiology and management of infertility: a population-based study in UK primary care. Family Practice. 2009;26(4):269-74.

7. Rutstein SOS, Iqbal H. Infecundity, Infertility, and Childlessness in Developing Countries In: Development USAfI, editor.: DHS Comparative Reports 2004.

8. Dunson DB, Colombo B, Baird DD. Changes with age in the level and duration of fertility in the menstrual cycle. Hum Reprod. 2002;17(5):1399-403.

9. Poppe K, Velkeniers B. Female infertility and the thyroid. Best Pract Res Clin Endocrinol Metab. 2004;18(2):153-65.

10. Basso O, Baird DD. Infertility and preterm delivery, birthweight, and Caesarean section: a study within the Danish National Birth Cohort. Hum Reprod. 2003;18(11):2478-84.

11. Henriksen TB, Baird DD, Olsen J, Hedegaard M, Secher NJ, Wilcox AJ. Time to pregnancy and preterm delivery. Obstet Gynecol. 1997;89(4):594-9.

12. Jaques AM, Amor DJ, Baker HW, Healy DL, Ukoumunne OC, Breheny S, et al. Adverse obstetric and perinatal outcomes in subfertile women conceiving without assisted reproductive technologies. Fertil Steril. 2010;94(7):2674-9.

13. Messerlian C, Maclagan L, Basso O. Infertility and the risk of adverse pregnancy outcomes: a systematic review and meta-analysis. Hum Reprod. 2013;28(1):125-37.

14. The World's Number of IVF and ICSI Babies Has Now Reached a Calculated Total of 5 million [press release]. http://www.eshre.eu/ESHRE/English/Press-Room/Press-Releases/Press-releases-2012/5-million-babies/page.aspx/1606 European Society of Human Reproduction and Embyology, July 2012 2012.

15. Sunderam S, Kissin DM, Crawford SB, Folger SG, Jamieson DJ, Barfield WD. Assisted reproductive technology surveillance--United States, 2011. Morbidity and mortality weekly report Surveillance summaries (Washington, DC : 2002). 2014;63(10):1-28.

16. Sullivan EA, Zegers-Hochschild F, Mansour R, Ishihara O, de Mouzon J, Nygren KG, et al. International Committee for Monitoring Assisted Reproductive Technologies (ICMART) world report: assisted reproductive technology 2004. Hum Reprod. 2013;28(5):1375-90.

17. Centers for Disease Control and Prevention ASfRM, Society for Assisted Reproductive Technology. 2011 Assisted Reproductive Technology National Summary Report. In: Services UDoHaH, editor. Atlanta (GA)2013.

18. Lechniak D, Pers-Kamczyc E, Pawlak P. Timing of the first zygotic cleavage as a marker of developmental potential of mammalian embryos. Reproductive biology. 2008;8(1):23-42.

19. Niakan KK, Han J, Pedersen RA, Simon C, Pera RA. Human pre-implantation embryo development. Development (Cambridge, England). 2012;139(5):829-41.

20. Centers for Disease Control and Prevention ASfRM, Society for Assisted Reproductive Technology. 2010 Assisted Reproductive Technology National Summary Report In: Services USDoHaH, editor. Atlanta (GA)2012.

21. Glujovsky D, Blake D, Farquhar C, Bardach A. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. The Cochrane database of systematic reviews. 2012;7:Cd002118.

22. Milki AA, Hinckley MD, Fisch JD, Dasig D, Behr B. Comparison of blastocyst transfer with day 3 embryo transfer in similar patient populations. Fertil Steril. 2000;73(1):126-9.

23. Coskun S, Hollanders J, Al-Hassan S, Al-Sufyan H, Al-Mayman H, Jaroudi K. Day 5 versus day 3 embryo transfer: a controlled randomized trial. Hum Reprod. 2000;15(9):1947-52.

24. Fernando D, Halliday JL, Breheny S, Healy DL. Outcomes of singleton births after blastocyst versus nonblastocyst transfer in assisted reproductive technology. Fertil Steril. 2012;97(3):579-84.

25. Huisman GJ, Fauser BC, Eijkemans MJ, Pieters MH. Implantation rates after in vitro fertilization and transfer of a maximum of two embryos that have undergone three to five days of culture. Fertil Steril. 2000;73(1):117-22.

26. Scholtes MC, Zeilmaker GH. A prospective, randomized study of embryo transfer results after 3 or 5 days of embryo culture in in vitro fertilization. Fertil Steril. 1996;65(6):1245-8.

27. ASRM. Recommendations for gamete and embryo donation: a committee opinion. Fertil Steril. 2013;99(1):47-62.

28. ASRM. Criteria for number of embryos to transfer: a committee opinion. Fertil Steril. 2013;99(1):44-6.

29. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet. 1992;340(8810):17-8.

30. Palermo GD, Cohen J, Alikani M, Adler A, Rosenwaks Z. Intracytoplasmic sperm injection: a novel treatment for all forms of male factor infertility. Fertil Steril. 1995;63(6):1231-40.

31. Bhattacharya S, Hamilton MP, Shaaban M, Khalaf Y, Seddler M, Ghobara T, et al. Conventional in-vitro fertilisation versus intracytoplasmic sperm injection for the treatment of non-male-factor infertility: a randomised controlled trial. Lancet. 2001;357(9274):2075-9.

32. Kim HH, Bundorf MK, Behr B, McCallum SW. Use and outcomes of intracytoplasmic sperm injection for non-male factor infertility. Fertil Steril. 2007;88(3):622-8.

33. Devroey P, Van Steirteghem A. A review of ten years experience of ICSI. Hum Reprod Update. 2004;10(1):19-28.

34. Fishel S, Aslam I, Lisi F, Rinaldi L, Timson J, Jacobson M, et al. Should ICSI be the treatment of choice for all cases of in-vitro conception? Hum Reprod. 2000;15(6):1278-83.

35. AFS. Assisted reproductive technology in the United States and Canada: 1992 results generated from the American Fertility Society/Society for Assisted Reproductive Technology Registry. Fertil Steril. 1994;62(6):1121-8.

36. Boulet SL, Mehta A, Kissin DM, Warner L, Kawwass JF, Jamieson DJ. Trends in use of and reproductive outcomes associated with intracytoplasmic sperm injection. JAMA. 2015;313(3):255-63.

37. ASRM. The role of assisted hatching in in vitro fertilization: a review of the literature. A Committee opinion. Fertil Steril. 2008;90(5 Suppl):S196-8.

38. ASRM. Role of assisted hatching in in vitro fertilization: a guideline. Fertil Steril. 2014;102(2):348-51.

39. Kawwass JF, Monsour M, Crawford S, Kissin DM, Session DR, Kulkarni AD, et al. Trends and outcomes for donor oocyte cycles in the United States, 2000-2010. JAMA. 2013;310(22):2426-34.

40. Shalev C. Rights to Sexual and Reproductive Health: The ICPD and the Convention on the Elimination of All Forms of Discrimination against Women. Health and Human Rights. 2000;4(2):38-66.

41. Tinker C. Human Right for Women: The UN Convention on the Elimination of All Forms of Discrimination Against Women Hum Rts: 1981.

42. UN. Convention to Eliminate All Forms of Discrimination Against Women (CEDAW) United Nation Entity for gender Equality and the Empowerment of Women, 1979.

43. Chan CCW, Ho PC. Infertility, assisted reproduction and rights. Best Pract Res Clin Obstet Gynaecol. 2006;20(3):369-80.

44. Robertson JA. Children of Choice: Freedom and the New Reproductive Technologies: Princeton University Press; 1996 1996. 296 p.

45. Robertson J. Liberalism and the Limits of Procreative Liberty: A Response to My Critics. Washington and Lee Law Review. 1995;52(1).

46. Goold I. Should older and postmenopausal women have access to assisted reproductive technology? Monash Bioeth Rev. 2005;24(1):27-46.

47. Peterson MM. Assisted reproductive technologies and equity of access issues. J Med Ethics. 2005;31(5):280-5.

48. Ata B, Seli E. Economics of assisted reproductive technologies. Curr Opin Obstet Gynecol. 2010;22(3):183-8.

49. Bitler MP. Effects of Increased Access to Infertility Treatment on Infant and Child Health Outcomes. Working Papers: RAND Corporation; 2006.

50. Hammoud AO, Gibson M, Stanford J, White G, Carrell DT, Peterson M. In vitro fertilization availability and utilization in the United States: a study of demographic, social, and economic factors. Fertility and Sterility. 2009;91(5):1630-5.

51. Seifer DB, Frazier LM, Grainger DA. Disparity in assisted reproductive technologies outcomes in black women compared with white women. Fertility and Sterility. 2008;90(5):1701-10.

52. Vahratian A. Prevalence of Overweight and Obesity among Women of Childbearing Age. Matern Child Health J. 2009;13(2):268-73.

53. Daniluk JC, Koert E, Cheung A. Childless women's knowledge of fertility and assisted human reproduction: identifying the gaps. Fertility and sterility. 2012;97(2):420-6.

54. Bouzayen R, Eggertson L. In vitro fertilization: A private matter becomes public. CMAJ. 2009;181(5):243-.

55. Nangia AK, Likosky DS, Wang D. Access to assisted reproductive technology centers in the United States. Fertility and sterility. 2010;93(3):745-61.

56. Henne MB, Bundorf MK. Insurance mandates and trends in infertility treatments. Fertil Steril. 2008;89(1):66-73.

57. Chandra A, Stephen EH. Impaired fecundity in the United States: 1982-1995. Fam Plann Perspect. 1998;30(1):34-42.

58. Smith JF, Eisenberg ML, Glidden D, Millstein SG, Cedars M, Walsh TJ, et al. Socioeconomic disparities in the use and success of fertility treatments: analysis of data from a prospective cohort in the United States. Fertility and sterility. 2011;96(1):95-101.

59. Vahratian A. Utilization of fertility-related services in the United States. Fertility and sterility. 2008;90(4):1317-9.

60. Jain T. Socioeconomic and racial disparities among infertility patients seeking care. Fertil Steril. 2006;85(4):876-81.

61. McDonald SD, Murphy K, Beyene J, Ohlsson A. Perinatel outcomes of singleton pregnancies achieved by in vitro fertilization: a systematic review and meta-analysis. J Obstet Gynaecol Can. 2005;27(5):449-59.

62. Baird DD, Wilcox AJ, Kramer MS. Why might infertile couples have problem pregnancies? Lancet. 1999;353(9166):1724-5.

63. Camarano L, Alkon A, Nachtigall RD, Schembri M, Weiss S, Croughan MS. Preterm delivery and low birth weight in singleton pregnancies conceived by women with and without a history of infertility. Fertil Steril. 2012;98(3):681-6.e1.

64. Dickey RP. Doppler ultrasound investigation of uterine and ovarian blood flow in infertility and early pregnancy. Hum Reprod Update. 1997;3(5):467-503.

65. Mendola P, Messer LC, Rappazzo K. Science linking environmental contaminant exposures with fertility and reproductive health impacts in the adult female. Fertil Steril. 2008;89(2 Suppl):e81-94.

66. Windham G, Fenster L. Environmental contaminants and pregnancy outcomes. Fertil Steril. 2008;89(2 Suppl):e111-6; discussion e7.

67. Pool TB. An update on embryo culture for human assisted reproductive technology: media, performance, and safety. Semin Reprod Med. 2005;23(4):309-18.

68. Gruber I, Klein M. Embryo culture media for human IVF: which possibilities exist? Journal of the Turkish German Gynecological Association. 2011;12(2):110-7.

69. Xella S, Marsella T, Tagliasacchi D, Giulini S, La Marca A, Tirelli A, et al. Embryo quality and implantation rate in two different culture media: ISM1 versus Universal IVF Medium. Fertil Steril. 2010;93(6):1859-63.

70. Dumoulin JC, Land JA, Van Montfoort AP, Nelissen EC, Coonen E, Derhaag JG, et al. Effect of in vitro culture of human embryos on birthweight of newborns. Hum Reprod. 2010;25(3):605-12.

71. Khosla S, Dean W, Brown D, Reik W, Feil R. Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. Biology of reproduction. 2001;64(3):918-26.

72. Natale DR, De Sousa PA, Westhusin ME, Watson AJ. Sensitivity of bovine blastocyst gene expression patterns to culture environments assessed by differential display RT-PCR. Reproduction. 2001;122(5):687-93.

73. Nelissen EC, Van Montfoort AP, Coonen E, Derhaag JG, Geraedts JP, Smits LJ, et al. Further evidence that culture media affect perinatal outcome: findings after transfer of fresh and cryopreserved embryos. Hum Reprod. 2012;27(7):1966-76.

74. Sinclair KD, Young LE, Wilmut I, McEvoy TG. In-utero overgrowth in ruminants following embryo culture: lessons from mice and a warning to men. Hum Reprod. 2000;15 Suppl 5:68-86.

75. Lonergan P, Rizos D, Gutierrez-Adan A, Fair T, Boland MP. Effect of culture environment on embryo quality and gene expression - experience from animal studies. Reprod Biomed Online. 2003;7(6):657-63.

76. Ishida M, Moore GE. The role of imprinted genes in humans. Molecular aspects of medicine. 2013;34(4):826-40.

77. Katari S, Turan N, Bibikova M, Erinle O, Chalian R, Foster M, et al. DNA methylation and gene expression differences in children conceived in vitro or in vivo. Human molecular genetics. 2009;18(20):3769-78.

78. Deng S, Xu J, Zeng J, Hu L, Wu Y. Ovarian stimulation leads to a severe implantation defect in mice. Reprod Biomed Online. 2013;27(2):172-5.

79. Ertzeid G, Storeng R. The impact of ovarian stimulation on implantation and fetal development in mice. Hum Reprod. 2001;16(2):221-5.

80. Santos MA, Kuijk EW, Macklon NS. The impact of ovarian stimulation for IVF on the developing embryo. Reproduction. 2010;139(1):23-34.

81. Sazonova A, Kallen K, Thurin-Kjellberg A, Wennerholm UB, Bergh C. Obstetric outcome after in vitro fertilization with single or double embryo transfer. Hum Reprod. 2011;26(2):442-50.

82. Schieve LA, Meikle SF, Ferre C, Peterson HB, Jeng G, Wilcox LS. Low and very low birth weight in infants conceived with use of assisted reproductive technology. N Engl J Med. 2002;346(10):731-7.

83. Kalra SK, Ratcliffe SJ, Coutifaris C, Molinaro T, Barnhart KT. Ovarian stimulation and low birth weight in newborns conceived through in vitro fertilization. Obstet Gynecol. 2011;118(4):863-71.

84. Kapiteijn K, de Bruijn CS, de Boer E, de Craen AJ, Burger CW, van Leeuwen FE, et al. Does subfertility explain the risk of poor perinatal outcome after IVF and ovarian hyperstimulation? Hum Reprod. 2006;21(12):3228-34.

85. Kondapalli LA, Perales-Puchalt A. Low birth weight: is it related to assisted reproductive technology or underlying infertility? Fertil Steril. 2013;99(2):303-10.

86. Johnson MR, Irvine R, Hills F, Bolton VN, Abbas AA, Brooks AA, et al. Superovulation, IGFBP-1 and birth weight. Eur J Obstet Gynecol Reprod Biol. 1995;59(2):193-5.

87. Bryant-Greenwood GD, Yamamoto SY, Lowndes KM, Webster LE, Parg SS, Amano A, et al. Human decidual relaxin and preterm birth. Annals of the New York Academy of Sciences. 2005;1041:338-44.

88. Luque EH, Munoz de Toro MM, Ramos JG, Rodriguez HA, Sherwood OD. Role of relaxin and estrogen in the control of eosinophilic invasion and collagen remodeling in rat cervical tissue at term. Biology of reproduction. 1998;59(4):795-800.

89. Mushayandebvu TI, Goldsmith LT, Von Hagen S, Santoro N, Thurston D, Weiss G. Elevated maternal serum relaxin concentrations throughout pregnancy in singleton gestations after superovulation. Obstet Gynecol. 1998;92(1):17-20.

90. Petersen LK, Skajaa K, Uldbjerg N. Serum relaxin as a potential marker for preterm labour. British journal of obstetrics and gynaecology. 1992;99(4):292-5.

91. Weiss G, Goldsmith LT, Sachdev R, Von Hagen S, Lederer K. Elevated first-trimester serum relaxin concentrations in pregnant women following ovarian stimulation predict prematurity risk and preterm delivery. Obstet Gynecol. 1993;82(5):821-8.

92. Koudstaal J, Braat DD, Bruinse HW, Naaktgeboren N, Vermeiden JP, Visser GH. Obstetric outcome of singleton pregnancies after IVF: a matched control study in four Dutch university hospitals. Hum Reprod. 2000;15(8):1819-25.

93. Landy HJ, Keith LG. The vanishing twin: a review. Hum Reprod Update. 1998;4(2):177-83.

94. Pinborg A, Lidegaard O, la Cour Freiesleben N, Andersen AN. Consequences of vanishing twins in IVF/ICSI pregnancies. Hum Reprod. 2005;20(10):2821-9.

95. De Sutter P, Delbaere I, Gerris J, Verstraelen H, Goetgeluk S, Van der Elst J, et al. Birthweight of singletons after assisted reproduction is higher after single- than after double-embryo transfer. Hum Reprod. 2006;21(10):2633-7.

96. Luke B, Brown MB, Grainger DA, Stern JE, Klein N, Cedars MI. The effect of early fetal losses on singleton assisted-conception pregnancy outcomes. Fertil Steril. 2009;91(6):2578-85.

97. Shebl O, Ebner T, Sommergruber M, Sir A, Tews G. Birth weight is lower for survivors of the vanishing twin syndrome: a case-control study. Fertil Steril. 2008;90(2):310-4.

98. Wapner RJ, Davis GH, Johnson A, Weinblatt VJ, Fischer RL, Jackson LG, et al. Selective reduction of multifetal pregnancies. Lancet. 1990;335(8681):90-3.

99. Cheang CU, Huang LS, Lee TH, Liu CH, Shih YT, Lee MS. A comparison of the outcomes between twin and reduced twin pregnancies produced through assisted reproduction. Fertil Steril. 2007;88(1):47-52.

100. Luke B, Brown MB, Nugent C, Gonzalez-Quintero VH, Witter FR, Newman RB. Risk factors for adverse outcomes in spontaneous versus assisted conception twin pregnancies. Fertil Steril. 2004;81(2):315-9.

101. Allen VM, Wilson RD, Cheung A. Pregnancy outcomes after assisted reproductive technology. J Obstet Gynaecol Can. 2006;28(3):220-50.

102. Bergh T, Ericson A, Hillensjo T, Nygren KG, Wennerholm UB. Deliveries and children born after in-vitro fertilisation in Sweden 1982-95: a retrospective cohort study. Lancet. 1999;354(9190):1579-85.

103. D'Angelo DV, Whitehead N, Helms K, Barfield W, Ahluwalia IB. Birth outcomes of intended pregnancies among women who used assisted reproductive technology, ovulation stimulation, or no treatment. Fertil Steril. 2011;96(2):314-20.e2.

104. Dhont M, De Sutter P, Ruyssinck G, Martens G, Bekaert A. Perinatal outcome of pregnancies after assisted reproduction: a case-control study. Am J Obstet Gynecol. 1999;181(3):688-95.

105. Hayashi M, Nakai A, Satoh S, Matsuda Y. Adverse obstetric and perinatal outcomes of singleton pregnancies may be related to maternal factors associated with infertility rather than the type of assisted reproductive technology procedure used. Fertil Steril. 2012;98(4):922-8.

106. Henningsen AK, Pinborg A, Lidegaard O, Vestergaard C, Forman JL, Andersen AN. Perinatal outcome of singleton siblings born after assisted reproductive technology and spontaneous conception: Danish national sibling-cohort study. Fertil Steril. 2011;95(3):959-63.

107. Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. Obstet Gynecol. 2004;103(3):551-63.

108. Marino JL, Moore VM, Willson KJ, Rumbold A, Whitrow MJ, Giles LC, et al. Perinatal outcomes by mode of assisted conception and sub-fertility in an Australian data linkage cohort. PloS one. 2014;9(1):e80398.

109. McDonald SD, Han Z, Mulla S, Ohlsson A, Beyene J, Murphy KE. Preterm birth and low birth weight among in vitro fertilization twins: a systematic review and meta-analyses. Eur J Obstet Gynecol Reprod Biol. 2010;148(2):105-13.

110. Perri T, Chen R, Yoeli R, Merlob P, Orvieto R, Shalev Y, et al. Are singleton assisted reproductive technology pregnancies at risk of prematurity? Journal of assisted reproduction and genetics. 2001;18(5):245-9.

111. Pinborg A, Wennerholm UB, Romundstad LB, Loft A, Aittomaki K, Soderstrom-Anttila V, et al. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis. Hum Reprod Update. 2013;19(2):87-104.

112. Romundstad LB, Romundstad PR, Sunde A, von During V, Skjaerven R, Gunnell D, et al. Effects of technology or maternal factors on perinatal outcome after

assisted fertilisation: a population-based cohort study. Lancet. 2008;372(9640):737-43.

113. Wang JX, Norman RJ, Kristiansson P. The effect of various infertility treatments on the risk of preterm birth. Hum Reprod. 2002;17(4):945-9.

114. Wisborg K, Ingerslev HJ, Henriksen TB. In vitro fertilization and preterm delivery, low birth weight, and admission to the neonatal intensive care unit: a prospective follow-up study. Fertil Steril. 2010;94(6):2102-6.

115. Cooper AR, O'Neill KE, Allsworth JE, Jungheim ES, Odibo AO, Gray DL, et al. Smaller fetal size in singletons after infertility therapies: the influence of technology and the underlying infertility. Fertil Steril. 2011;96(5):1100-6.

116. Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. Bmj. 2004;328(7434):261.

117. Henningsen AA, Wennerholm UB, Gissler M, Romundstad LB, Nygren KG, Tiitinen A, et al. Risk of stillbirth and infant deaths after assisted reproductive technology: a Nordic study from the CoNARTaS group. Hum Reprod. 2014;29(5):1090-6.

118. Kozinszky Z, Zadori J, Orvos H, Katona M, Pal A, Kovacs L. Risk of cesarean section in singleton pregnancies after assisted reproductive techniques. The Journal of reproductive medicine. 2003;48(3):160-4.

119. Fujii M, Matsuoka R, Bergel E, van der Poel S, Okai T. Perinatal risk in singleton pregnancies after in vitro fertilization. Fertil Steril. 2010;94(6):2113-7.

120. Romundstad LB, Romundstad PR, Sunde A, von During V, Skjaerven R, Vatten LJ. Increased risk of placenta previa in pregnancies following IVF/ICSI; a comparison of ART and non-ART pregnancies in the same mother. Hum Reprod. 2006;21(9):2353-8.

121. Shevell T, Malone FD, Vidaver J, Porter TF, Luthy DA, Comstock CH, et al. Assisted reproductive technology and pregnancy outcome. Obstet Gynecol. 2005;106(5 Pt 1):1039-45.

122. Takemura Y, Osuga Y, Fujimoto A, Oi N, Tsutsumi R, Koizumi M, et al. Increased risk of placenta previa is associated with endometriosis and tubal factor infertility in assisted reproductive technology pregnancy. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology. 2013;29(2):113-5.

123. Hansen M, Kurinczuk JJ, de Klerk N, Burton P, Bower C. Assisted reproductive technology and major birth defects in Western Australia. Obstet Gynecol. 2012;120(4):852-63.

124. Hansen M, Kurinczuk JJ, Milne E, de Klerk N, Bower C. Assisted reproductive technology and birth defects: a systematic review and meta-analysis. Hum Reprod Update. 2013;19(4):330-53.

125. Kelley-Quon LI, Tseng CH, Janzen C, Shew SB. Congenital malformations associated with assisted reproductive technology: a California statewide analysis. Journal of pediatric surgery. 2013;48(6):1218-24.

126. Klemetti R, Gissler M, Sevon T, Koivurova S, Ritvanen A, Hemminki E. Children born after assisted fertilization have an increased rate of major congenital anomalies. Fertil Steril. 2005;84(5):1300-7.

127. Wen J, Jiang J, Ding C, Dai J, Liu Y, Xia Y, et al. Birth defects in children conceived by in vitro fertilization and intracytoplasmic sperm injection: a meta-analysis. Fertil Steril. 2012;97(6):1331-7.e1-4.

128. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371(9606):75-84.

129. Markestad T, Kaaresen PI, Ronnestad A, Reigstad H, Lossius K, Medbo S, et al. Early death, morbidity, and need of treatment among extremely premature infants. Pediatrics. 2005;115(5):1289-98.

130. Martin JA, Osterman MJ. Preterm births - United States, 2006 and 2010. Morbidity and mortality weekly report Surveillance summaries (Washington, DC : 2002). 2013;62 Suppl 3:136-8.

131. Tucker J, McGuire W. Epidemiology of preterm birth. Bmj. 2004;329(7467):675-8.

132. Wen SW, Smith G, Yang Q, Walker M. Epidemiology of preterm birth and neonatal outcome. Seminars in fetal & neonatal medicine. 2004;9(6):429-35.

133. Dong Y, Yu JL. An overview of morbidity, mortality and long-term outcome of late preterm birth. World journal of pediatrics : WJP. 2011;7(3):199-204.

134. Gouyon JB, Iacobelli S, Ferdynus C, Bonsante F. Neonatal problems of late and moderate preterm infants. Seminars in fetal & neonatal medicine. 2012;17(3):146-52.

135. McIntire DD, Leveno KJ. Neonatal mortality and morbidity rates in late preterm births compared with births at term. Obstet Gynecol. 2008;111(1):35-41.

136. Raju TN. Epidemiology of late preterm (near-term) births. Clinics in perinatology. 2006;33(4):751-63; abstract vii.

137. Blondel B, Kaminski M. Trends in the occurrence, determinants, and consequences of multiple births. Semin Perinatol. 2002;26(4):239-49.

138. Reynolds MA, Schieve LA, Martin JA, Jeng G, Macaluso M. Trends in multiple births conceived using assisted reproductive technology, United States, 1997-2000. Pediatrics. 2003;111(5 Pt 2):1159-62.

139. Blondel B, Kogan MD, Alexander GR, Dattani N, Kramer MS, Macfarlane A, et al. The impact of the increasing number of multiple births on the rates of preterm birth and low birthweight: an international study. American journal of public health. 2002;92(8):1323-30.

140. McGovern PG, Llorens AJ, Skurnick JH, Weiss G, Goldsmith LT. Increased risk of preterm birth in singleton pregnancies resulting from in vitro fertilization-embryo transfer or gamete intrafallopian transfer: a meta-analysis. Fertil Steril. 2004;82(6):1514-20.

141. Pandey S, Shetty A, Hamilton M, Bhattacharya S, Maheshwari A. Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. Hum Reprod Update. 2012;18(5):485-503.

142. Schieve LA, Rasmussen SA, Buck GM, Schendel DE, Reynolds MA, Wright VC. Are children born after assisted reproductive technology at increased risk for adverse health outcomes? Obstet Gynecol. 2004;103(6):1154-63.

143. Fanaroff AA, Stoll BJ, Wright LL, Carlo WA, Ehrenkranz RA, Stark AR, et al. Trends in neonatal morbidity and mortality for very low birthweight infants. Am J Obstet Gynecol. 2007;196(2):147.e1-8.

144. McCormick MC. The contribution of low birth weight to infant mortality and childhood morbidity. N Engl J Med. 1985;312(2):82-90.

145. Basso O, Wilcox AJ, Weinberg CR. Birth weight and mortality: causality or confounding? Am J Epidemiol. 2006;164(4):303-11.

146. Urquia ML, Ray JG. Seven caveats on the use of low birthweight and related indicators in health research. Journal of epidemiology and community health. 2012;66(11):971-5.

147. Wilcox AJ. On the importance--and the unimportance--of birthweight. International journal of epidemiology. 2001;30(6):1233-41.

148. Mei Z, Grummer-Strawn LM. Standard deviation of anthropometric Z-scores as a data quality assessment tool using the 2006 WHO growth standards: a cross country analysis. Bulletin of the World Health Organization. 2007;85(6):441-8.

149. Wang YC, H. Handbook of Anthropometry. Physical Measures of Human Form in Health and Disease: Springer New York; 2012 2012.

150. Henningsen AA, Gissler M, Skjaerven R, Bergh C, Tiitinen A, Romundstad LB, et al. Trends in perinatal health after assisted reproduction: a Nordic study from the CoNARTaS group. Hum Reprod. 2015.

151. Wang YA, Sullivan EA, Black D, Dean J, Bryant J, Chapman M. Preterm birth and low birth weight after assisted reproductive technology-related pregnancy in Australia between 1996 and 2000. Fertil Steril. 2005;83(6):1650-8.

152. Nakashima A, Araki R, Tani H, Ishihara O, Kuwahara A, Irahara M, et al. Implications of assisted reproductive technologies on term singleton birth weight: an analysis of 25,777 children in the national assisted reproduction registry of Japan. Fertil Steril. 2013;99(2):450-5.

153. Doyle P, Beral V, Maconochie N. Preterm delivery, low birthweight and small-for-gestational-age in liveborn singleton babies resulting from in-vitro fertilization. Hum Reprod. 1992;7(3):425-8.

154. Isaksson R, Gissler M, Tiitinen A. Obstetric outcome among women with unexplained infertility after IVF: a matched case-control study. Hum Reprod. 2002;17(7):1755-61.

155. Maman E, Lunenfeld E, Levy A, Vardi H, Potashnik G. Obstetric outcome of singleton pregnancies conceived by in vitro fertilization and ovulation induction compared with those conceived spontaneously. Fertil Steril. 1998;70(2):240-5.

156. Olivennes F, Rufat P, Andre B, Pourade A, Quiros MC, Frydman R. The increased risk of complication observed in singleton pregnancies resulting from in-vitro fertilization (IVF) does not seem to be related to the IVF method itself. Hum Reprod. 1993;8(8):1297-300.

157. Reubinoff BE, Samueloff A, Ben-Haim M, Friedler S, Schenker JG, Lewin A. Is the obstetric outcome of in vitro fertilized singleton gestations different from natural ones? A controlled study. Fertil Steril. 1997;67(6):1077-83.

158. Tan SL, Doyle P, Campbell S, Beral V, Rizk B, Brinsden P, et al. Obstetric outcome of in vitro fertilization pregnancies compared with normally conceived pregnancies. Am J Obstet Gynecol. 1992;167(3):778-84.

159. Verlaenen H, Cammu H, Derde MP, Amy JJ. Singleton pregnancy after in vitro fertilization: expectations and outcome. Obstet Gynecol. 1995;86(6):906-10.

160. Wang JX, Clark AM, Kirby CA, Philipson G, Petrucco O, Anderson G, et al. The obstetric outcome of singleton pregnancies following in-vitro fertilization/gamete intra-fallopian transfer. Hum Reprod. 1994;9(1):141-6.

161. Zhu JL, Obel C, Hammer Bech B, Olsen J, Basso O. Infertility, infertility treatment, and fetal growth restriction. Obstet Gynecol. 2007;110(6):1326-34.

162. Shih W, Rushford DD, Bourne H, Garrett C, McBain JC, Healy DL, et al. Factors affecting low birthweight after assisted reproduction technology: difference between transfer of fresh and cryopreserved embryos suggests an adverse effect of oocyte collection. Hum Reprod. 2008;23(7):1644-53.

163. Kawwass JF, Crawford S, Kissin DM, Session DR, Boulet S, Jamieson DJ. Tubal factor infertility and perinatal risk after assisted reproductive technology. Obstet Gynecol. 2013;121(6):1263-71.

164. Schieve LA, Ferre C, Peterson HB, Macaluso M, Reynolds MA, Wright VC. Perinatal outcome among singleton infants conceived through assisted reproductive technology in the United States. Obstet Gynecol. 2004;103(6):1144-53.

165. Messerlian C, Platt RW, Tan SL, Gagnon R, Basso O. Low-technology assisted reproduction and the risk of preterm birth in a hospital-based cohort. Fertil Steril. 2015;103(1):81-8.e2.

166. Kallen B, Finnstrom O, Nygren KG, Olausson PO. In vitro fertilization (IVF) in Sweden: infant outcome after different IVF fertilization methods. Fertil Steril. 2005;84(3):611-7.

167. Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. Fertil Steril. 2012;98(2):368-77.e1-9.

168. Nelson SM, Lawlor DA. Predicting live birth, preterm delivery, and low birth weight in infants born from in vitro fertilisation: a prospective study of 144,018 treatment cycles. PLoS medicine. 2011;8(1):e1000386.

169. Pinborg A, Loft A, Aaris Henningsen AK, Rasmussen S, Andersen AN. Infant outcome of 957 singletons born after frozen embryo replacement: the Danish National Cohort Study 1995-2006. Fertil Steril. 2010;94(4):1320-7.

170. Edi-Osagie E, Hooper L, Seif MW. The impact of assisted hatching on live birth rates and outcomes of assisted conception: a systematic review. Hum Reprod. 2003;18(9):1828-35.

171. Edi-Osagie EC, Hooper L, McGinlay P, Seif MW. Effect(s) of assisted hatching on assisted conception (IVF & ICSI). The Cochrane database of systematic reviews. 2003(4):Cd001894.

172. Mansour RT, Rhodes CA, Aboulghar MA, Serour GI, Kamal A. Transfer of zona-free embryos improves outcome in poor prognosis patients: a prospective randomized controlled study. Hum Reprod. 2000;15(5):1061-4.

173. Zhou H, Zao W, Zhang W, Shi J, Shi W. No adverse effects were identified on the perinatal outcomes after laser-assisted hatching treatment. Reprod Biomed Online. 2014;29(6):692-8.

174. Aston KI, Peterson CM, Carrell DT. Monozygotic twinning associated with assisted reproductive technologies: a review. Reproduction. 2008;136(4):377-86.

175. Hershlag A, Paine T, Cooper GW, Scholl GM, Rawlinson K, Kvapil G. Monozygotic twinning associated with mechanical assisted hatching. Fertil Steril. 1999;71(1):144-6.

176. Schieve LA, Meikle SF, Peterson HB, Jeng G, Burnett NM, Wilcox LS. Does assisted hatching pose a risk for monozygotic twinning in pregnancies conceived through in vitro fertilization? Fertil Steril. 2000;74(2):288-94.

177. Martins WP, Rocha IA, Ferriani RA, Nastri CO. Assisted hatching of human embryos: a systematic review and meta-analysis of randomized controlled trials. Hum Reprod Update. 2011;17(4):438-53.

178. Carney SK, Das S, Blake D, Farquhar C, Seif MM, Nelson L. Assisted hatching on assisted conception (in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI). The Cochrane database of systematic reviews. 2012;12:Cd001894.

179. Elizur SE, Levron J, Shrim A, Sivan E, Dor J, Shulman A. Monozygotic twinning is not associated with zona pellucida micromanipulation procedures but increases with high-order multiple pregnancies. Fertil Steril. 2004;82(2):500-1.

180. Schachter M, Raziel A, Friedler S, Strassburger D, Bern O, Ron-El R. Monozygotic twinning after assisted reproductive techniques: a phenomenon independent of micromanipulation. Hum Reprod. 2001;16(6):1264-9.

181. Sills ES, Moomjy M, Zaninovic N, Veeck LL, McGee M, Palermo GD, et al. Human zona pellucida micromanipulation and monozygotic twinning frequency after IVF. Hum Reprod. 2000;15(4):890-5.

182. Malchau SS, Loft A, Larsen EC, Aaris Henningsen AK, Rasmussen S, Andersen AN, et al. Perinatal outcomes in 375 children born after oocyte donation: a Danish national cohort study. Fertil Steril. 2013;99(6):1637-43.

183. Henne MB, Zhang M, Paroski S, Kelshikar B, Westphal LM. Comparison of obstetric outcomes in recipients of donor oocytes vs. women of advanced maternal age with autologous oocytes. The Journal of reproductive medicine. 2007;52(7):585-90.

184. Krieg SA, Henne MB, Westphal LM. Obstetric outcomes in donor oocyte pregnancies compared with advanced maternal age in in vitro fertilization pregnancies. Fertil Steril. 2008;90(1):65-70.

185. Stoop D, Baumgarten M, Haentjens P, Polyzos NP, De Vos M, Verheyen G, et al. Obstetric outcome in donor oocyte pregnancies: a matched-pair analysis. Reproductive biology and endocrinology : RB&E. 2012;10:42.

186. van der Hoorn ML, Lashley EE, Bianchi DW, Claas FH, Schonkeren CM, Scherjon SA. Clinical and immunologic aspects of egg donation pregnancies: a systematic review. Hum Reprod Update. 2010;16(6):704-12.

187. Mneimneh AS, Boulet SL, Sunderam S, Zhang Y, Jamieson DJ, Crawford S, et al. States Monitoring Assisted Reproductive Technology (SMART) Collaborative: data collection, linkage, dissemination, and use. J Womens Health (Larchmt). 2013;22(7):571-7.

188. Zhang Y, Cohen B, Macaluso M, Zhang Z, Durant T, Nannini A. Probabilistic linkage of assisted reproductive technology information with vital records, Massachusetts 1997-2000. Matern Child Health J. 2012;16(8):1703-8.

189. Ferraretti AP, Goossens V, Kupka M, Bhattacharya S, de Mouzon J, Castilla JA, et al. Assisted reproductive technology in Europe, 2009: results generated from European registers by ESHRE. Hum Reprod. 2013;28(9):2318-31.

190. Sunderam S, Kissin DM, Crawford S, Anderson JE, Folger SG, Jamieson DJ, et al. Assisted reproductive technology surveillance -- United States, 2010. Morbidity and mortality weekly report Surveillance summaries (Washington, DC : 2002). 2013;62(9):1-24.

191. Murray SR, Norman JE. Multiple pregnancies following assisted reproductive technologies - A happy consequence or double trouble? Seminars in fetal & neonatal medicine. 2014.

192. Tepper NK, Farr SL, Cohen BB, Nannini A, Zhang Z, Anderson JE, et al. Singleton preterm birth: risk factors and association with assisted reproductive technology. Matern Child Health J. 2012;16(4):807-13.

193. Sengupta S, Carrion V, Shelton J, Wynn RJ, Ryan RM, Singhal K, et al. Adverse neonatal outcomes associated with early-term birth. JAMA pediatrics. 2013;167(11):1053-9.

194. ACOG. ACOG Committee Opinion No 579: Definition of term pregnancy. Obstet Gynecol. 2013;122(5):1139-40.

195. Szlachter BN, Quagliarello J, Jewelewicz R, Osathanondh R, Spellacy WN, Weiss G. Relaxin in normal and pathogenic pregnancies. Obstet Gynecol. 1982;59(2):167-70.

196. Schieve LA, Cohen B, Nannini A, Ferre C, Reynolds MA, Zhang Z, et al. A population-based study of maternal and perinatal outcomes associated with assisted reproductive technology in Massachusetts. Matern Child Health J. 2007;11(6):517-25.

197. McDonald SD, Han Z, Mulla S, Murphy KE, Beyene J, Ohlsson A. Preterm birth and low birth weight among in vitro fertilization singletons: a systematic review and meta-analyses. Eur J Obstet Gynecol Reprod Biol. 2009;146(2):138-48.

198. Reddy UM, Wapner RJ, Rebar RW, Tasca RJ. Infertility, assisted reproductive technology, and adverse pregnancy outcomes: executive summary of a National Institute of Child Health and Human Development workshop. Obstet Gynecol. 2007;109(4):967-77.

199. Berkowitz GS, Papiernik E. Epidemiology of preterm birth. Epidemiol Rev. 1993;15(2):414-43.

200. Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bulletin of the World Health Organization. 1987;65(5):663-737.

201. Kramer MS. Intrauterine growth and gestational duration determinants. Pediatrics. 1987;80(4):502-11.

202. Kramer MS. The epidemiology of low birthweight. Nestle Nutrition Institute workshop series. 2013;74:1-10.

203. Delbaere I, Vansteelandt S, De Bacquer D, Verstraelen H, Gerris J, De Sutter P, et al. Should we adjust for gestational age when analysing birth weights? The use of z-scores revisited. Hum Reprod. 2007;22(8):2080-3.

204. Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. Obstet Gynecol. 1996;87(2):163-8.

205. Talge NM, Mudd LM, Sikorskii A, Basso O. United States birth weight reference corrected for implausible gestational age estimates. Pediatrics. 2014;133(5):844-53.

206. Basso O, Wilcox A. Mortality risk among preterm babies: immaturity versus underlying pathology. Epidemiology. 2010;21(4):521-7.

207. Schieve LA, Rosenberg D, Handler A, Rankin K, Reynolds MA. Validity of self-reported use of assisted reproductive technology treatment among women participating in the Pregnancy Risk Assessment Monitoring System in five states, 2000. Matern Child Health J. 2006;10(5):427-31.

208. Kuivasaari-Pirinen P, Raatikainen K, Hippelainen M, Heinonen S. Adverse Outcomes of IVF/ICSI Pregnancies Vary Depending on Aetiology of Infertility. ISRN obstetrics and gynecology. 2012;2012:451915.

209. Pandian Z, Bhattacharya S, Templeton A. Review of unexplained infertility and obstetric outcome: a 10 year review. Hum Reprod. 2001;16(12):2593-7.

210. Land JA. How should we report on perinatal outcome? Hum Reprod. 2006;21(10):2638-9.

211. Imudia AN, Awonuga AO, Doyle JO, Kaimal AJ, Wright DL, Toth TL, et al. Peak serum estradiol level during controlled ovarian hyperstimulation is associated with increased risk of small for gestational age and preeclampsia in singleton pregnancies after in vitro fertilization. Fertil Steril. 2012;97(6):1374-9.

212. Pelinck MJ, Keizer MH, Hoek A, Simons AH, Schelling K, Middelburg K, et al. Perinatal outcome in singletons after modified natural cycle IVF and standard IVF with ovarian stimulation. Eur J Obstet Gynecol Reprod Biol. 2010;148(1):56-61.

213. Chung K, Coutifaris C, Chalian R, Lin K, Ratcliffe SJ, Castelbaum AJ, et al. Factors influencing adverse perinatal outcomes in pregnancies achieved through use of in vitro fertilization. Fertil Steril. 2006;86(6):1634-41.

214. Daniel Y, Schreiber L, Geva E, Lessing JB, Bar-Am A, Amit A. Morphologic and histopathologic characteristics of placentas from twin pregnancies spontaneously conceived and from reduced and nonreduced assisted reproductive technologies. The Journal of reproductive medicine. 2001;46(8):735-42.

215. Esh-Broder E, Ariel I, Abas-Bashir N, Bdolah Y, Celnikier DH. Placenta accreta is associated with IVF pregnancies: a retrospective chart review. BJOG. 2011;118(9):1084-9.

216. Healy DL, Breheny S, Halliday J, Jaques A, Rushford D, Garrett C, et al. Prevalence and risk factors for obstetric haemorrhage in 6730 singleton births after assisted reproductive technology in Victoria Australia. Hum Reprod. 2010;25(1):265-74.

217. Prysak M, Lorenz RP, Kisly A. Pregnancy outcome in nulliparous women 35 years and older. Obstet Gynecol. 1995;85(1):65-70.

218. Thomson F, Shanbhag S, Templeton A, Bhattacharya S. Obstetric outcome in women with subfertility. BJOG. 2005;112(5):632-7.

219. Ziadeh SM. Maternal and perinatal outcome in nulliparous women aged 35 and older. Gynecologic and obstetric investigation. 2002;54(1):6-10.

220. Dunietz GL, Holzman C, McKane P, Li C, Boulet SL, Todem D, et al. Assisted reproductive technology and the risk of preterm birth among primiparas. Fertil Steril. 2015.

221. Kissin DM, Kawwass JF, Monsour M, Boulet SL, Session DR, Jamieson DJ. Assisted hatching: trends and pregnancy outcomes, United States, 2000-2010. Fertil Steril. 2014;102(3):795-801.

222. Levron Y, Dviri M, Segol I, Yerushalmi GM, Hourvitz A, Orvieto R, et al. The 'immunologic theory' of preeclampsia revisited: a lesson from donor oocyte gestations. Am J Obstet Gynecol. 2014;211(4):383.e1-5.

223. Soderstrom-Anttila V. Pregnancy and child outcome after oocyte donation. Hum Reprod Update. 2001;7(1):28-32.

224. Wiggins DA, Main E. Outcomes of pregnancies achieved by donor egg in vitro fertilization--a comparison with standard in vitro fertilization pregnancies. Am J Obstet Gynecol. 2005;192(6):2002-6; discussion 6-8.

225. Malchau SS, Loft A, Henningsen AK, Nyboe Andersen A, Pinborg A. Perinatal outcomes in 6,338 singletons born after intrauterine insemination in Denmark, 2007 to 2012: the influence of ovarian stimulation. Fertil Steril. 2014;102(4):1110-6.e2.

226. Gibbons WE, Cedars M, Ness RB. Toward understanding obstetrical outcome in advanced assisted reproduction: varying sperm, oocyte, and uterine source and diagnosis. Fertil Steril. 2011;95(5):1645-9.e1.