Efficacy, pregnancy and birth outcomes following IVF treatments in different age groups and with different time for embryo transfer and freezing in Australia and New Zealand, 2002–2008

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Efficacy, pregnancy and birth outcomes following IVF treatments in different age groups and with different time for embryo transfer and freezing in Australia and New Zealand, 2002–2008

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BMed, Grad Dip (Business), MPH

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Background: Outcomes of assisted reproductive technology (ART) treatment are primarily determined by woman's age, number of embryos transferred and stage of embryo development. However, it is not clear how each additional year of age impacts ART treatment outcomes. Inconsistent results about the relationship between ART treatment outcomes and number of embryos transferred and stage of embryo development still exist in the literature. The objective of this thesis is to inform patients, fertility professionals and the general population regarding fertility preservation, the optimal goal of an ART treatment and the best population-based clinical practice model that optimises the overall efficacy of ART treatment. **Methods:** The thesis includes five coherent studies using population data from the Australian and New Zealand Assisted Reproduction Database. Pregnancy, live delivery, and "healthy baby" (term liveborn singleton of normal birthweight without congenital anomaly) were used to measure the success of ART treatment. **Results:** For patients aged≥35 years, the likelihood of pregnancy and live delivery decreased by each additional year of age. It is appropriate for women aged <43 years to initiate a new fresh ART treatment. Singletons following single embryo transfer (SET) had lower odds of adverse perinatal outcomes than those following double embryo transfer. For fresh cycles in patients aged <35 years, selective transfer of a single blastocyst resulted in a higher rate of "healthy baby" than a single cleavage embryo. For thaw cycles, a higher likelihood of "healthy baby" following transfers of blastocyst cultured from thawed cleavage embryos was observed. Transfers of fresh blastocysts and blastocysts cultured from thawed cleavage embryos reduced the risk of miscarriage. **Conclusions:** This thesis suggests that, from a population perspective, to maximise the births of a "healthy baby", the optimum clinical practice model for younger patients is the selective transfer of a single blastocyst in a fresh cycle and a single blastocyst cultured from thawed cleavage embryos in subsequent thaw cycles. It confirmed the importance of community-based education regarding fertility potential and the benefits of early fertility assessment and ART treatment. It provided evidence that the continuing increase in SET would improve the overall birth outcomes of ART treatment.

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Originality statement

'I hereby declare that this submission is my own work and to the best of my knowledge it contains no materials previously published or written by another person, or substantial proportions of material which have been accepted for the award of any other degree or diploma at UNSW or any other educational institution, except where due acknowledgement is made in the thesis. Any contribution made to the research by others, with whom I have worked at UNSW or elsewhere, is explicitly acknowledged in the thesis. I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the project's design and conception or in style, presentation and linguistic expression is acknowledged.'

Signed …….....

Yueping Alex Wang

Date …….....

Supervisor statement

I confirm that, to the best of my knowledge, the five published studies of this PhD thesis (Chapters 3 to 7) represent the original research work of the candidate. He is the first author and responsible for at least 80% of the work of each study. The research was carried out and the thesis was prepared under my direct supervision. The contribution made to the research by me, by co-supervisor, and by members of advisory team was consistent with normal supervisory and advisory practice. Other external contributions to the research are acknowledged

I confirm that co-authors of the five studies, Professor Michael Chapman, Dr Michael Costello, Professor David Healy, and Professor Gab Kovacs have agreed to the submission of the nominated studies as part of this PhD thesis.

Signed …….....

Elizabeth Anne Sullivan

Date …….....

Declarations

I hereby declare that the coherent five published studies of this PhD thesis (Chapters 3 to 7) represent my own research work. I am the first author and responsible for at least 80% of the work of each study. The contribution of my supervisors and other co-authors to the five studies was consistent with normal supervisory and advisory practice. Before commencing each study, co-authors Professor Michael Chapman, Dr Michael Costello, Professor David Healy, and Professor Gab Kovacs agreed that the study would be included as an individual chapter of this PhD thesis.

I confirm that the following permissions were granted to use published articles as an individual chapter of the PhD thesis.

Oxford University Press for three articles:

Wang YA, Healy D, Black D, Sullivan EA. Age-specific success rate for women undertaking their first assisted reproduction technology treatment using their own oocytes in Australia, 2002-2005. Hum Reprod. 2008 Jul;23(7):1633-8.

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Wang YA, Kovacs G, Sullivan EA. Transfer of a selected single blastocyst optimizes the chance of a healthy term baby: a retrospective population based study in Australia 2004-2007. Hum Reprod. 2010 Aug;25(8):1996-2005.

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Yueping Alex Wang

Date ……

Thesis abstract

Background: The successful or adverse outcomes of assisted reproductive technology (ART) treatment are primarily determined by woman's age and number of embryos transferred. However, it is not clear how each additional year of woman's age impacts the ART treatment outcomes. Inconsistent findings about the relationship between ART treatment outcomes and number of embryos transferred and stage of embryo development still exist in the literature. Differences in the findings relate to what outcome is chosen and how it is measured. The objective of this PhD thesis is to inform infertile patients, fertility professionals and the general population regarding fertility awareness, the optimal goal of an ART treatment and the best population-based clinical practice model. It aims to investigate the age-specific live delivery rate by single year increments, to compare perinatal outcomes of babies following single embryo transfer (SET) with double embryo transfer (DET), to propose a new indicator "healthy baby" as the optimal goal for an ART treatment, and to build a clinical practice model that maximises the likelihood of a "healthy baby". **Materials and methods:** The thesis includes five coherent studies using population data extracted from the Australian and New Zealand Assisted Reproduction Database. Pregnancy, live delivery, and "healthy baby" (term liveborn singleton of normal birthweight without

congenital anomaly) were used to measure the success of an ART treatment. **Results:** For patients aged ≥35 years, the likelihood of pregnancy and live delivery decreased by each additional year of age. It is appropriate for women aged <43 years to initiate a fresh ART treatment. Singletons following SET had lower odds of adverse perinatal outcomes than those following DET. For fresh cycles in patients aged <35 years, selective transfer of a single blastocyst resulted in a higher rate of "healthy baby" than the transfer of a single cleavage embryo. For thaw cycles, a higher likelihood of a "healthy baby" following transfer of a single blastocyst cultured from thawed cleavage embryos was observed. Transfers of fresh blastocysts and blastocysts cultured from thawed cleavage embryos reduced the risk of miscarriage. **Conclusions:** This PhD thesis suggests that, from a population perspective, to minimise adverse outcomes in parallel with maximising births of a "healthy baby", the optimum clinical practice model for younger patients is the selective transfer of a single blastocyst in a fresh cycle and a single blastocyst cultured from thawed cleavage embryos in subsequent thaw cycles. It confirmed the importance of community-based education regarding fertility potential and the benefits of early fertility assessment and ART treatment where clinically indicated. It provided evidence that the continuing increase in SET would improve the overall birth outcomes of ART treatment.

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Thirdly, I acknowledge co-authors of the five studies of this PhD thesis, namely Professor Michael Chapman, Dr Michael Costello, Professor David Healy, and Professor Gab Kovacs. You have provided me clinical and strategic expertise on individual study. Without your advices this PhD thesis would not have been completed timely and comprehensively.

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And finally, thank you to my family members and friends for sharing my achievements, and disappointments too.

Abbreviations

AHR	adjusted hazard ratio
AIHW	Australian Institute of Health and Welfare
ANZARD	Australian and New Zealand Assisted Reproduction Database
AOR	adjusted odds ratio
ARR	adjusted rates ratio
ART	assisted reproductive technology
BESST	Birth emphasizing a successful singleton at term
BMI	body mass index
CDC	Centers for Disease Control and Prevention
CI	confidence intervals
DET	double embryo transfer
FSA	Fertility Society of Australia
FSH	follicle stimulating hormone
ESHRE	European Society of Human Reproduction and Embryology
GIFT	gamete intrafallopian transfer
HFEA	Human Fertilisation and Embryology Authority
HREC	Human Research Ethics Committee
HR	hazard ratio
ICD-9-BPA	International Classification of Diseases, 9th Revision, British Paediatric Association Publication
ICD-10-AM	International Statistical Classification of Diseases and Related Health Problems, 10th Revision, Australian Modification
ICMART	International Committee Monitoring Assisted Reproductive Technologies
ICSI	intracytoplasmic sperm injection
IVF	in vitro fertilisation

NPSU	National Perinatal Statistics Unit
OHSS	ovarian hyperstimulation syndrome
OPU	oocyte pick-up
OR	odds ratio
PGD	preimplantation genetic diagnosis
PRERU	Perinatal & Reproductive Epidemiology Research Unit
RR	rates ratio
RTAC	Reproductive Technology Accreditation Committee
SD	standard deviation
SDET	selective double embryo transfer
SET	single embryo transfer
SLK	statistical linkage key
SSET	selective single embryo transfer
UNSW	The University of New South Wales
USDET	unselective double embryo transfer
USSET	unselective single embryo transfer
WHO	World Health Organization

Chapter 1

Introduction to this PhD thesis

Background

The inability to have a baby affects millions of women and couples worldwide. The failure to achieve a clinical pregnancy after 12 or more months of regular unprotected sexual intercourse is called infertility (Zegers-Hochschild et al., 2009). It is estimated that up to 15% of women worldwide experience infertility (Boivin et al., 2007). An Australian survey shows that approximately one in six couples suffer to some extent primary or secondary infertility during their reproductive years (Labett Research and Marketing, 2006). A recent cohort study reported that almost a quarter of women in their early 30s in Australia had difficulty to conceive (Marino et al., 2011).

Infertility is a condition found among both the female and male population (Cui, 2010). However, infertility is not an absolute or irreversible condition, and it can be overcome through changes in behaviour, alternative methods and medical treatments (Cui, 2010; Norman et al., 2004). About half of couples with infertility will eventually conceive naturally, and less than a quarter need advanced treatment such as tubal surgery, insemination, ovulation induction, or in vitro fertilisation (IVF) (Jones & Toner 1993). IVF is applicable in about 20% of infertile couples (Hull et al., 1985). In July 1978, the IVF treatment of infertility made history, marked by the world's first IVF baby, Louise Brown, born in Manchester, England (Steptoe & Edwards, 1978). In June 1980 the world's third and Australia's first IVF baby was born in Melbourne (Lopata et al., 1980).

IVF is a typical procedure of assisted reproductive technology (ART) treatment in which both human oocytes and sperm or embryos are handled in vitro (outside of body) for the purpose of establishing a pregnancy (Zegers-Hochschild et al., 2009). In 2009, the International Committee Monitoring Assisted Reproductive Technologies (ICMART) and the World Health Organization (WHO) published the updated glossary for various ART treatments and procedures, outcomes and efficacy measures of an ART treatment (Zegers-Hochschild et al., 2009). The complete glossary is attached at Appendix 1.

Since the birth of Louise Brown three decades ago, an estimated 4.3 million babies have been born following ART treatment worldwide (ICMART, 2010). It was estimated that 3.4% of all babies born in Australia in 2008 were a result of ART treatment (Laws et al., 2010; Wang et al., 2010). ART has become a route to overcome most causes of infertility and increasingly been seen as a response to changing community patterns of childbearing.

The aim of an ART treatment is to establish a pregnancy, maintain the pregnancy and have a healthy baby. Clinical pregnancy and live birth have been frequently used to indicate the success of a treatment (Kovacs, 2011). A number of adverse pregnancy and birth outcomes have been identified as being associated with ART treatment (Koivurova et al., 2002; Wang et al., 2005; Hansen et al., 2005). Multiple gestation pregnancy is the most notable adverse outcome following ART treatment and is responsible for the majority of maternal and obstetric complications (Koivurova et al., 2002). Preterm birth,

low birthweight and congenital anomaly are also prevalent amongst babies born following ART treatment (Schieve et al., 2002; Wang et al., 2005; Hansen et al., 2005).

Both successful and adverse outcomes vary by different ART treatment factors and patients' demographic characteristics (Roberts et al., 2010). The association between ART treatment factors and resulting outcomes is not consistent in the literature. The measurement of the success of an ART treatment is mostly limited to clinical pregnancy and live delivery (Nygren at al., 2011). However, the optimal goal of an ART treatment is not pregnancy or live delivery, but a healthy baby. Health indicators for perinatal outcomes such as multiple birth, preterm birth, low birthweight, congenital anomaly and perinatal death need to be summarised in measuring the efficacy of an ART treatment.

Objectives

The overarching objectives of this PhD thesis are to inform infertile patients, fertility professionals and the general population regarding fertility awareness, and to build a population-based clinical practice model that optimises the overall efficacy of ART treatment. Five studies were conducted to meet the overarching objectives:

- Study one aims to provide national evidence based age-specific live delivery rates for women undergoing ART treatment,

- Study two aims to compare the perinatal outcomes of births following (SET) with those following double embryo transfer (DET) to demonstrate the benefits of SET,

- Using the newly proposed "healthy baby" indicator, study three aims to hypothesise a clinical practice model in combining a fresh cycle and subsequent thaw cycles at either cleavage or blastocyst stage,

- Study four aims to investigate the efficacy of selective SET and DET of fresh ART treatment at either cleavage or blastocyst stage using "healthy baby" indicator,

- Using miscarriage rate, study five aims to evaluate the efficacy of the proposed clinical practice model.

Data

The data used in this PhD thesis are a research extract obtained from the Australian and New Zealand Assisted Reproduction Database (ANZARD). Data from ANZARD are supplied to the National Perinatal Statistics Unit (NPSU) by all clinics in Australia and New Zealand on an annual basis. ANZARD was established in 2002 as a joint initiative of the University of New South Wales (UNSW), the NPSU and the Fertility Society of Australia (FSA). ANZARD collects information on patients' demographic characteristics, ART

and donor sperm insemination treatments, and resulting pregnancy and birth outcomes.

Patients' demographic characteristics include the woman's age, cause of infertility, previous pregnancies of ≥20 weeks gestation, previous pregnancies of <20 weeks gestation and number of previous ART treatments. Treatment factors comprise oocyte pick-up (OPU), sperm source, fertilisation procedures, number of embryos transferred, stage of embryo development, assisted hatching, preimplantation genetic diagnosis (PGD), embryo cryopreservation and subsequent uses, oocyte/embryo donation arrangements and surrogacy arrangements. The outcomes include type of clinical pregnancy, number of fetal hearts, method of birth, plurality, birth status, birthweight, gestational age, congenital anomaly and neonatal mortality. The complete ANZARD data item list is found at Appendix 2.

ANZARD is one of the most comprehensive ART registries in the world. All data supplied to NPSU undergo a validation process, with data queries being followed up with fertility centre staff. However, follow-up of pregnancy and birth outcomes is limited because the ongoing care of pregnant patients is often carried out by non-ART practitioners. The information on pregnancy and birth outcomes is not stated in <2.0% of clinical pregnancies annually. For pregnancies in which there is follow-up, data on birthweight, gestation and plurality at birth are reliably defined from the various data sources including hospital records, self-reporting by patients and their obstetricians. However, the

information on congenital anomalies and perinatal mortality is likely to be incomplete. An Australian comparative study of a regional birth defect register relying of practitioner reporting found congenital anomalies were underreported by the Assisted Conception Database (Hanson et al., 2007).

The PhD thesis intended to use the most recent data for each of the five studies. Given the lag of data collection, the thesis included seven years data from 2002 to 2008. Specifically, following treatment year data were used for each study: 2002 to 2005 data for the first study, 2002 to 2006 for the second study, 2002 to 2006 for the third study, 2004 to 2007 for the fourth study, and 2004 to 2008 for the fifth study.

The PhD thesis was proposed to use treatment cycles from both Australia and New Zealand. However, due to different health care systems between the two countries, the first treatment cycle of a patient could be identified in Australia only (Department of Health and Ageing, 2006). Therefore, the first and fourth studies of this PhD thesis only included treatment cycles from Australian clinics.

Ethics and approval

Ethics approval for this PhD thesis was granted by the Human Research Ethics Committee (HREC 08171) of UNSW. Access to ANZARD was approved by the FSA.

Structure

This PhD thesis has eight chapters, including this chapter "Introduction to this PhD thesis" (Chapter 1).

Chapter 2 — "Background and literature review", provides an outline of infertility, history and types of ART treatment, common measures of ART treatment, and summarised literature reviews on the association between successful and adverse outcomes and patients' demographic characteristics and treatment factors.

Chapter 3 — "Age-specific success rate for women undertaking their first assisted reproduction technology treatment using their own oocytes in Australia, 2002-2005", computes the age-specific live delivery rate of autologous cycles. It also models the impact of women aged 35–44 years undergoing their ART treatment one, two or three years earlier and the associated impact on the rate of live delivery.

Chapter 4 — "Perinatal outcomes after assisted reproduction technology treatment in Australia and New Zealand: single- versus double-embryo transfer", compares the rates of preterm birth, low birthweight and perinatal death of babies conceived by SET with those conceived by DET.

Chapter 5 — "Better perinatal outcomes following transfer of fresh blastocysts and blastocysts cultured from thawed cleavage embryos: a population-based

study', develops the "healthy baby" indicator to measure the efficacy of ART treatment. This chapter also proposes that an optimum clinical practice model involves the transfer of a single blastocyst in fresh cycle and a single blastocyst cultured from thawed cleavage embryos in subsequent thaw cycles.

Chapter 6 — "Transfer of a selected single blastocyst optimizes the chance of a healthy term baby: a retrospective population-based study in Australia 2004-2007", compares the efficacy of selective or unselective SET and DET at either cleavage or blastocyst stage by using the "healthy baby" indicator.

Chapter 7 — "Transfers of fresh blastocysts and blastocysts cultured from thawed cleavage embryos are associated with fewer miscarriages", further investigates the efficacy of the proposed practice model from Chapter 5 by using the miscarriage rate.

Chapter 8 — "Conclusions and recommendations", summarises the findings from Chapters 3 to 7. This chapter supports a community education program regarding age-related fertility potential and benefits of early fertility assessment and treatment. It also recommends further research directions.

Appendices — Appendix 1 presents the glossary defined by ICMART/WHO data items in the ANZARD. Appendix 2 lists ANZARD data items.

Format

This PhD thesis is in the format of a series of publications. This format was approved by the Higher Degree Committee of UNSW in November 2010. The thesis includes five coherent published studies (Chapters 3 to 7). As approved by the Higher Degree Committee, references are presented at the end of each chapter in the published format.

The supervisor-signed statement indicating that that all co-authors have agreed to the submission of the nominated papers as part of the PhD thesis is inserted at the beginning of the thesis.

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Chapter 2

Background and literature review

Background

The inability to have a child has significant family, public health and social consequences. For some women, without children, their lives are without hope (Cui, 2010). The biological inability to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse is defined as infertility, a disease classified by the International Committee Monitoring Assisted Reproductive Technologies (ICMART) and the World Health Organization (WHO) (Zegers-Hochschild et al., 2009). Infertility affects up to approximately 15% of women aged 20–44 years worldwide (Boivin et al., 2007). It is estimated that currently more than 72 million women internationally are experiencing difficulty having a baby (Boivin et al., 2007). An Australian survey shows that about one in six couples suffered some extent of primary or secondary infertility during their reproductive years (Labett Research and Marketing, 2006). A recent cohort study reported that almost a quarter of women in their early 30s in Australia had difficulty to conceive (Marino et al., 2011).

Infertility may be due to different reasons and is both female and male-related (Cates et al., 1985). Approximately one in twenty men is affected by male infertility in Australia (McLachlan & de Kretser, 2001). Male factor infertility is mainly related to low semen quality (Hirsh, 2003). In comparison, female factor infertility may be due to ovulation issues, tubal diseases, uterine conditions and advanced age-related factors (Cates et al., 1985). In some cases, both female and

male factors of infertility may be presented. In other cases, infertility is unexplained where neither a female nor male-related cause is diagnosed. Unexplained infertility accounted for 10–20% of all infertility among infertile couples (Cates et al., 1985; Isaksson & Tiitinen, 2004).

However, infertility is not an absolute or irreversible condition. It can be overcome through changes in behaviour, alternative methods and medical treatments (Homan et al., 2007; Hull et al., 1985; Moran et al., 2011; Norman et al., 2004; Paulus et al., 2002). About half of couples with infertility will eventually conceive naturally, and less than a quarter need advanced treatment such as tubal surgery, insemination, ovulation induction, or IVF (Jones & Toner 1993). IVF is applicable in about 20% of infertile couples (Hull et al., 1985).

History of in vitro fertilisation

For hundreds of years, medical professionals have investigated various methods and treatments to overcome human infertility and help couples to have a baby (Cui, 2010; Domar et al., 2000; Moran et al., 2011; Paulus et al., 2002). On 25 July 1978, IVF treatment reached a significant milestone with the birth of the world's first in vitro fertilisation (IVF) baby, Louise Brown, in Manchester, England (Steptoe & Edwards, 1978). In 2010, British physiologist Robert G Edwards, the doctor who was instrumental in the birth of Louise Brown, was awarded the 2010 Nobel Prize in Physiology or Medicine for the development of IVF (Nobel Prize, 2010).

Since the birth of Louise Brown, IVF has become the most effective medical intervention for infertility treatment. In the last three decades, IVF has helped millions women/couples to fulfil their hope of having a baby. The latest estimates show that more than 4.3 million children were born following IVF treatment worldwide (ICMART, 2010). In Australia and New Zealand, 3.4% and 2.0% of all women who gave birth in 2008 in Australia and New Zealand respectively received some form of IVF treatment (Laws et al., 2010; Statistics New Zealand, n.d.).

Both Australia and New Zealand are pioneer countries in IVF treatment. In the late 1960s, a team at Monash University in Melbourne started to investigate methods to fertilise oocytes in laboratories (Cohen et al., 2005; Wood et al., 1971). In 1973, the Monash research team reported the world's first pregnancy following IVF treatment (De Kretzer et al., 1973). Unfortunately, this pregnancy ended in miscarriage. In June 1980, the world's third IVF baby was born in Melbourne's Royal Women's Hospital as a result of the combined efforts of doctors and scientists from Monash University's Department of Obstetrics and Gynaecology (Lopata et al., 1980). IVF was first undertaken in New Zealand in 1983 (Murray et al., 2005). In the following year, the first New Zealand IVF baby was born at Auckland's National Women's Hospital.

The early impetus for IVF was to treat patients with blocked or scarred fallopian tubes where surgical tubal repair was either not successful or not advisable. In the past three decades, IVF has been further developed to meet

other needs of patients, moving from tubal disease to ovulation issues, uterine conditions, advanced maternal age-related factors, male factor-related infertility and unexplained infertility, as well as oocyte donation, surrogacy arrangements, and medical and social reproductive preservation (CDC, 2006; ICMART, 2010).

Type and utilisation of assisted reproductive technology treatment

IVF is a typical procedure of assisted reproductive technology (ART) treatments. ART refers to all treatments or procedures that include the in vitro handling of both human oocytes and sperm or of embryos for the purpose of establishing a pregnancy (Zegers-Hochschild et al., 2009). The term "in vitro" is named to distinguish "in vivo". In vivo fertilisation refers that the oocyte is fertilised by sperm inside the body. Like in a spontaneous pregnancy, an oocyte is fertilised by sperm in the Ampulla of the uterine tube, and the fertilised oocyte (zygote) then travels to the uterus and implants there to form a pregnancy. In vitro fertilisation generally refers to the procedures where oocytes are removed from woman's body, fertilised with sperm in a laboratory, and then the resulted embryos are placed in the woman's uterus to form a pregnancy.

Technically, IVF is the type of fertilisation procedure. IVF, along with other pre- and post-fertilisation procedures such as removing oocytes from a woman's body, and transferring embryos into a woman's uterus form an ART treatment

cycle. A typical ART treatment cycle includes the following treatments and procedures:

- stimulation: controlling the ovulatory process using hormone treatment
- oocyte pick-up (OPU): removing oocytes from the woman's ovaries usually by ultrasound-guided trans-vaginal aspiration
- sperm preparation: obtaining sperm by either ejaculation or surgical procedures
- fertilisation: fertilising oocytes by sperm in a fluid medium in a laboratory
- embryo culture: culturing fertilised oocyte (zygote) for two to three days to form embryos
- embryo transfer: transferring embryos to the patient's uterus with the intention to establish pregnancy.

In the past three decades, along with typical ART treatment cycles, other procedures have been developed to meet various patient needs. These procedures include surgical sperm extraction, intracytoplasmic sperm injection (ICSI), gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer, tubal embryo transfer, PGD, preimplantation genetic screening, assisted hatching, blastocyst transfer, gamete and embryo cryopreservation, oocyte and

embryo donation, and gestational surrogacy (Zegers-Hochschild et al., 2009). Notably, amongst these newly developed technologies and laboratory techniques, ICSI and blastocyst transfers have had significant impact on the outcomes of ART treatment.

In contrast to conventional IVF, ICSI is an ART procedure in which a selected single sperm is injected directly into the oocyte to aid fertilisation. Initially ICSI was developed to overcome male factor infertility. In recent years, the ICSI procedure has been extended for female factor infertility and unexplained infertility. It has become the dominant fertilisation procedure in some countries (Mansour et al., 2011; Nygren et al., 2011). In Australia and New Zealand, the ICSI procedure has accounted for more than 60% of fresh cycles where oocyte fertilisation was performed in 2008 (Wang et al., 2010).

In a typical ART treatment cycle, embryos are transferred at cleavage stage (two or three days after fertilisation). A blastocyst refers to an embryo at blastocyst stage (five or six days after fertilisation), with an inner cell mass, outer layer of trophectoderm and a fluid-filled blastocoele cavity (Zegers-Hochschild et al., 2009). A cleavage embryo usually has 6-8 cells compared to a blastocyst with about 60-100 cells. Blastocyst transfers have become more common in Australia and New Zealand in recent years, accounting for 17.1% of embryo transfer cycles in 2004 and 38.6% in 2008 (Wang et al., 2010).

GIFT is another type of ART procedure. In a GIFT cycle, both gametes (oocytes and spermatozoa) are transferred to a woman's fallopian tube. Technically,

GIFT is not in vitro fertilisation, but in vivo fertilisation where oocytes are fertilised by sperm in a woman's fallopian tube. GIFT has became less common in most countries as it is less effective than IVF and has other complications (ICMART, 2010). In Australia and New Zealand, GIFT cycles accounted for a very small proportion (0.2%) of ART treatment cycles undertaken in 2008 (Wang et al., 2010).

Classification of assisted reproductive technology treatment

Types of ART treatments can be classified according to different criteria. ICMART and WHO have published a glossary including all types of ART treatments and procedures (Zegers-Hochschild et al., 2009). To distinguish the origin of oocytes, an ART treatment cycle can be classified as a non-donor (autologous) cycle, a donation cycle or a recipient cycle. An autologous treatment cycle refers to a cycle where a woman intends to use, or uses her own oocytes or embryos. A donation cycle is where a woman or couple intends to donate, or donates her oocytes or their embryos to others. A recipient cycle is an ART treatment cycle in which a woman receives oocytes or embryos from another woman or couple.

Based on whether embryos were cryopreserved, an ART treatment cycle can be classified as a fresh cycle or a thaw cycle. A fresh cycle is an ART treatment cycle which intends to form embryos. Embryos formed in a fresh cycle can be transferred or cryopreserved for future transfer. In contrast, a thaw cycle begins

at the thawing of cryopreserved embryos with the intention of transfer. A thaw cycle is the subsequent treatment cycle of the initial fresh cycle.

Embryo cryopreservation methods include the traditional slow frozen method and vitrification, the most recently developed method. Vitrification is an ultra-rapid cryopreservation method that prevents ice formation within the suspension which is converted to a glasslike solid (Zegers-Hochschild et al., 2009). The cooling speed in vitrification is about 600 times faster than slow freezing method. Human embryo vitrification was developed by Japanese scientists in the late 1990s (Saito et al., 2000). Vitrification was first introduced in Australia in 2006 and is now widely used for blastocyst cryopreservation (Costigan et al., 2007).

Utilisation of assisted reproductive technology treatment

Choice and utilisation of the different types of ART treatments and procedures vary across clinics, countries and regions. The most recent world report shows that ART was available in 54 countries worldwide in 2003 (Nygren et al., 2011). Approximately 932,400 ART treatment cycles were undertaken by 1,709 clinics in 2003 around the world (Nygren et al., 2011). The ART utilisation rates varied from 5 treatment cycles per million population to 4,916 per million population. Europe had the largest number of OPU cycles, performing 60.9% of the world total. Thaw cycles represented 23.8% of the OPU cycles internationally in 2003. ICSI accounted for 58.1% of fertilisation procedures and varied according to

regions, with 70.5% in Latin America and 96% in the Middle East (Nygren et al., 2011).

Australia and New Zealand are among the countries with the highest utilisation of ART treatment. There were 70 fertility clinics in Australia and 7 in New Zealand in 2008 (Wang et al., 2010). A total of 61,929 ART treatment cycles were reported in Australia and New Zealand in 2008, giving a utilisation rate of 12.6 cycles per 1,000 women of reproductive age (15–44 years) in Australia and 5.5 cycles per 1,000 women of reproductive age in New Zealand (Wang et al., 2010). The number of ART treatment cycles has increased by 70% in Australia and New Zealand from 36,483 in 2002 to 61,929 in 2008. As a result, the number of liveborn babies born as a result of ART treatment has risen from 6,816 in 2002 to 11,528 in 2008 (Bryant et al., 2004; Wang et al., 2010).

Definitions of ART treatment outcomes

Successful outcomes

The success of ART treatment has been evidenced in the last three decades, marked by the birth of millions of babies following ART treatment (Nygren et al., 2011). The term "success" can be defined as a favourable or desired outcome. Success of an ART treatment cycle can be measured according to the outcome. The interim outcome of a treatment cycle is to establish a clinical pregnancy, then to maintain the pregnancy for a live delivery (live birth) of a healthy baby.

There are various terms used in the literature to define the success related to ART treatment. It has been argued that terminology in reproductive medicine related to reproductive success is ambiguous, confusing and misleading (Davies et al., 2005). It is difficult to use a single term serving various purposes related to the risk, safety, success, efficacy, patient satisfaction, community acceptability, and health economics of ART treatment (Davies et al., 2004). In order to guide clinical practice, the ICMART and WHO have defined clinical pregnancy and live birth as successful outcomes for an ART treatment (Zegers-Hochschild et al., 2009).

A "clinical pregnancy" refers to a pregnancy diagnosed by ultrasonographic visualisation of one or more gestational sacs or definitive clinical signs of pregnancy. Ectopic pregnancy is included in the definition and multiple gestational sacs (fetal hearts) are counted as one clinical pregnancy.

A "live birth" (live delivery) is defined as the complete expulsion or extraction from its mother of a product of fertilisation, irrespective of the duration of the pregnancy, which, after such separation, breathes or shows any other evidence of life such as heart beat, umbilical cord pulsation or definite movement of voluntary muscles, irrespective of whether the umbilical cord has been cut or the placenta is attached (WHO, 1992).

Apart from clinical pregnancy and live delivery, take-home baby and the BESST (Birth emphasizing a successful singleton at term) are also used in the literature (Fukuda et al., 2001; Healy, 2004; Min et al., 2004). The parameters of the take-

home baby are similar to live delivery which does not take neonatal outcomes into consideration. The BESST measures singleton birth outcomes, thus differentiating singletons from multiples. The BESST also takes gestation and birth status into consideration. While an evaluation study shows that the BESST is unable to accurately reflect the risk of multiple pregnancy associated with multiple embryo transfer (Davies et al., 2004).

Adverse outcomes

Along with successful outcomes, some pregnancy complications and adverse perinatal outcomes are associated with ART treatment. Multiple gestation/birth is the most notable adverse outcome following ART treatment (Healy, 2004; Min et al., 2004). Multiple gestation/birth is defined as a pregnancy/delivery with more than one fetus/neonate (Zegers-Hochschild et al., 2009). The ICMART and WHO definitions (Zegers-Hochschild et al., 2009) on other adverse pregnancy and perinatal outcomes include:

- Spontaneous abortion/miscarriage: the spontaneous loss of a clinical pregnancy that occurs before 20 completed weeks of gestational age (18 weeks post fertilisation) or, if gestational age is unknown, the loss of an embryo/fetus of less than 400 grams.

- Preterm birth: a live birth or stillbirth that takes place after at least 20 but before 37 completed weeks of gestational age.

- Low birthweight: birthweight less than 2,500 grams.

- Perinatal mortality: fetal or neonatal death occurring during late pregnancy (at 20 completed weeks of gestational age and later), during childbirth and up to seven completed days after birth.

- Neonatal death: death of a liveborn baby within 28 days of birth.

- Congenital anomalies: all structural, functional and genetic anomalies diagnosed in aborted fetuses, at birth or in the neonatal period.

Measurement of the success of ART treatment

The efficacy of an ART treatment can be defined as a rate of the desired outcome. In the literature, clinical pregnancy and live delivery are frequently used to measure the efficacy of an ART treatment, presented as the clinical pregnancy rate or the live delivery rate (Davies et al., 2004; Kovacs, 2011). A rate consists of two components: the numerator and denominator. For ART treatment, the numerator is usually the number of a specified outcome, such as clinical pregnancy and live delivery. The denominator is the number of treatments such as initiated cycles and embryo transfer cycles. The success rate of ART treatment is usually presented as a percentage, for example, the number of clinical pregnancies per 100 initiated cycles.

The adverse pregnancy and perinatal outcomes are measured in a different way to successful outcomes. Miscarriage rate is measured in a percentage by using

number of pregnancies as the denominator (Bahceci & Ulug, 2005). Preterm birth, low birthweight, perinatal death and congenital anomaly rates are measured by using the number of births as denominators and presented as percentages (Hansen et al., 2002; Wang et al., 2010).

Both successful and adverse outcomes vary by type of treatment and procedure. A number of patients' demographic characteristics were identified as being associated with ART success and adverse pregnancy and perinatal outcomes. The following section summarises the literature review on treatment factors and patients' demographic characteristics associated with successful outcomes and adverse pregnancy and perinatal outcomes following ART treatment.

Literature review

To better understand the literature in relation to the patient demographics, ART treatment factors and resulting pregnancy/birth outcomes. A literature review was conducted at the beginning of the PhD research and updated while preparing the thesis.

The primary source for the literature review was online databases. Scopus, PubMed, and Medline were used to search human epidemiological and clinical studies using following keywords/Mesh terms: fertility, infertility, subfertility, assisted reproductive technology, in vitro fertilization, intracytoplasmic sperm injection, gamete intrafallopian transfer, oocyte pick-up, cleavage embryo, blastocyst, day 2-3 embryo, day 5-6 embryo, single embryo transfer, double embryo transfer, fresh embryo, thawed embryo, fresh transfer, frozen transfer, pregnancy, clinical pregnancy, live birth, live delivery, take home baby, miscarriage, early pregnancy loss, still birth, fetal death, neonatal death, perinatal death, perinatal mortality, gestational age, preterm birth, birthweight, low birthweight, congenital anomaly, birth defect. The search was restricted to abstract in English from the past 30 years. Individual website search was conducted for some high impact journals of in maternal and reproductive area such as Human Reproduction Update, Human Reproduction, Fertility and Sterility, Biomedicine online. In addition, references cited in the selected articles were also searched.

The outcomes of ART treatment are associated with various treatment factors and patients' demographic characteristics. Treatment factors such as fertilisation procedures, number of embryos transferred, fresh or thawed embryo transfer and stage of embryo development have a significant impact on the treatment, pregnancy and perinatal outcomes (Blake et al., 2007; Bhattacharya et al., 2001; Hansen et al., 2005; Pandian et al., 2005; Schieve et al., 2002). Patient-inherited demographic characteristics are not only related to infertility but also to the outcomes of ART treatment (Alshami et al., 2011; Menken et al., 1986). A number of patient demographic characteristics, such as the woman's age, previous pregnancies, obesity, cause and duration of infertility, smoking and number of previous ART treatments are independently associated with outcomes of ART treatment (Bai et al., 2002; Baird et al., 2005; ESHRE Capri Workshop Group, 2004; Roberts et al., 2010; Sazonova et al., 2011).

Treatment factors

Fertilisation procedures

Fertilisation procedures include IVF and ICSI. ICSI was originally developed to overcome male factor infertility, and is now frequently performed for all causes of infertility in some countries (ICMART, 2010). Australia and New Zealand are amongst those countries with the highest ICSI utilisation rates, with the ICSI procedure accounting for 63% of fresh cycles where fertilisation was attempted (Wang et al., 2010).

The difference in treatment outcomes for IVF and ICSI is inconsistent in the literature. An early randomised trial concluded that ICSI had a similar pregnancy rate to IVF for treatment of tubal factor infertility with normal semen (Aboulghar et al., 1996). In contrast, a later trial reported that even though there was a higher fertilisation rate (measured by number of oocytes fertilised over number of oocytes inseminated) for ICSI than for IVF, the pregnancy rate was higher following IVF (Bhattacharya et al., 2001). An updated Cochrane review did not find significant difference in the pregnancy rates between IVF and ICSI (van Rumste et al., 2003). The newly published Swedish population study also reported similar pregnancy outcomes for IVF and ICSI (Finnström et al., 2011).

There are also inconsistent results for the relationship between IVF and ICSI procedures and the miscarriage rate in the literature. A higher miscarriage rate following thawed embryo transfers was reported for ICSI than for IVF in an early study (Aytoz et al., 1999). Several other studies found, however, that there was no difference in miscarriage rates between IVF and ICSI (Bahceci & Ulug, 2005; Bonduelle et al., 2002; Salumets et al., 2006). Interestingly, a more recent study has re-stated that ICSI was related to a higher rate of miscarriage compared to IVF (Matias et al., 2007). Unfortunately, there are no randomised data on miscarriage rates comparing IVF and ICSI procedures (van Rumste et al., 2004).

Even though there is some evidence that there are no differences in the rates of multiple birth, preterm birth, low birthweight and perinatal mortality between

IVF and ICSI babies (Bonduelle et al., 2002; Finnström et al., 2011), the body of evidence on the association between congenital anomalies and IVF and ICSI procedures is not well established. Most studies have found that babies conceived by ICSI did not have an increased risk of congenital anomalies compared to those conceived by IVF (Bonduelle et al., 2002; Halliday et al., 2010; Hansen et al., 2002; Yan et al., 2011). Other studies reported a higher rate of congenital anomalies among ICSI babies than traditional IVF babies (Belva et al., 2008; Bonduelle et al., 2005; Ericson & Källén, 2001). It was suggested that the increased risk of congenital anomalies following ICSI compared to IVF is probably due to parental factors causing the infertility which has led to the ICSI procedure in the first place (Ludwig & Katalinic, 2002).

Number of embryos transferred

A consequence of the improvement of ART laboratory technologies has been the reduction in the number of embryos transferred in a treatment cycle during recent years (Nygren et al., 2011). In Europe, the transfer of three or more embryos accounted for 20.6% of all embryo transfer cycles in 2006, reduced from 52.7% in 1997 (de Mouzon et al., 2010; Nygren & Andersen, 2001). Similarly, in Australia and New Zealand the proportion of embryo transfer cycles in which three or more embryos were transferred decreased from 27.2% in 1999 to 0.6% in 2008 (Bryant et al., 2004; Wang et al., 2010).

A debate regarding the balance between the higher rate of live delivery and the higher rate of multiple gestation pregnancy following DET has existed in the

literature for many years (Abdalla, 2010; Pandian et al., 2005). On one hand it was stated that DET resulted in higher rates of clinical pregnancy and live delivery than SET (Abdalla, 2010). Data from the United States show that the live delivery rate following DET was almost double the rate following SET (43.7% and 22.0% respectively) (CDC, 2010). On the other hand, DET resulted in a 17.7 times higher rate of twin delivery than SET (33.6% and 1.9% respectively) (CDC, 2010). A Cochrane review of randomised trials shows that the multiple pregnancy rate was up to 60 times higher for DET than for SET (Pandian et al., 2005).

Multiple gestation pregnancy is the most significant adverse outcome following ART treatment. It is considered a significant preventable public health problem with demonstrated poorer perinatal outcomes (Ombelet et al., 2005), increased risk to mothers (Campbell & Templeton, 2004) and greater social economic burden on the parents and on the health care system (Chambers et al., 2007). Comparative regional registry data show that the multiple delivery rate was 22% in Europe in 2004 (Andersen et al., 2008), 31% in the United States in 2006 (CDC, 2008), and 8.4% in Australia and New Zealand in 2008 (Wang et al., 2010).

The high rate of multiple delivery following ART is largely explained by the number of embryos transferred (Kissin et al., 2005). Reducing the number of embryos per transfer was suggested as the most effective way to minimise the complications of multiple pregnancy (Nygren, 2007). The Nordic countries and

Belgium have successfully implemented a policy of SET, with Sweden reporting 70% of all embryo transfer cycles were SET (Nygren, 2007). In Australia and New Zealand, the Reproductive Technology Accreditation Committee (RTAC) has advocated the use of SET since 2002 and formally implemented a SET policy in 2005 (RTAC, 2005). The latest report details the rise in the proportion of SET from 28.8% in 2002 to 67.8% in 2008 in Australia and New Zealand. As a result, the multiple delivery rate declined from 18.9% in 2002 to 8.4% in 2008 (Bryant et al., 2004; Wang et al., 2010). The experiences of Nordic countries, Belgium and Australasian indicate that the shift in clinical practice to SET provides an acceptable pathway to reducing multiple pregnancies following ART (Criniti et al., 2005).

Some recent clinical trials investigated the effectiveness of the combination of selective transfer of a single embryo in a fresh cycle and a single embryo in a thaw cycles in comparison with DET in a fresh cycle (Moustafa et al., 2008; Pandian et al., 2005; Pandian et al., 2009). The cumulative live delivery rate following DET is not significantly higher than the combination of SET in a fresh cycle and SET in the subsequent thaw cycle (Moustafa et al., 2008; Pandian et al., 2009). When term (≥37 weeks gestation) singleton births per transfer was used to measure the success rate of ART, the likelihood of achieving a term singleton birth following elective SET was almost five times higher than DET (McLernon et al., 2010).

It is not clear whether the number of embryos transferred is related to the risk of miscarriage. The transfer of two or more embryos was related to high rates of twin pregnancy and SET was associated with high rates of singleton pregnancy (Pandian et al., 2005). Some studies reported twin pregnancies had less risk of miscarriage than singleton pregnancies (Lambers et al., 2007; Tummers et al., 2003). However, other studies found that SET did not increase the risk of miscarriage compared to DET (Martikainen et al., 2001; Veleva et al., 2006).

International studies have documented that the higher rate of adverse outcomes among multiple compared to singleton pregnancies following ART is associated with the transfer of two or more embryos (Klemetti et al., 2002; Poikkeus et al., 2007; Westergaard et al., 1999). As a result, babies conceived by SET had better neonatal outcomes compared to those conceived following the transfer of two or more embryos (Poikkeus et al., 2007). Even for singletons, babies born following SET had significantly lower rates of low birthweight, preterm birth and perinatal death than singletons following DET (De Sutter et al., 2006; Poikkeus et al., 2007). Higher numbers of embryos transferred are significantly associated with multiple gestational sacs and fetal hearts, which in turn have been inversely related to birthweight or directly related to low birthweight (Pinborg, 2005; Pinborg et al., 2007; Wang et al., 2005).

Fresh or thawed embryo

Embryos formed in a fresh cycle can be transferred (fresh embryo transfer) or cryopreserved. In a subsequent thaw cycle, cryopreserved embryos can be thawed and transferred (frozen/thawed embryo transfer).

Population reports have shown that fresh embryo transfers result in higher rates of clinical pregnancy and live delivery than frozen/thawed embryo transfers (CDC, 2010; Wang et al., 2010). The Australian and New Zealand 2008 data show that the rates of clinical pregnancy and live delivery were 23.5% and 18.0% respectively for autologous fresh cycles, higher than the rates for autologous thaw cycles (21.9% and 16.5% respectively) (Wang et al., 2010). A recent large study from the United Kingdom presents significantly higher rates of clinical pregnancy and live delivery following the transfer of fresh embryos (20.8% and 19.3% respectively) than following the transfer of thawed embryos (12.9% and 12.1% respectively) (Mohamed et al., 2011).

In general, transfers of thawed embryos are substantially less likely to lead to a live birth compared to transfers of fresh embryos (Roberts et al., 2010). However, controlled ovarian hyperstimulation in a fresh cycle has been shown to advance endometrial maturation and adversely affects implantation in ART in selected patients (Aflatoonian et al., 2010a). A recent trial of selected patients with a good prognosis reported higher clinical pregnancy rates for transfers of thawed embryos (39%) than for transfers of fresh embryos (27.8%) (Aflatoonian et al., 2010a). It was also suggested that ovarian hyperstimulation may have

been associated with increased risk of miscarriage and adverse perinatal outcomes (Kansal Kalra et al., 2011; Shih et al., 2008).

Kansal Kalra and colleagues found a nearly two times the odds of first-trimester (1 to 12 weeks of gestation) miscarriage following transfers of fresh embryos compared to transfers of thawed embryos. This indicated that the supraphysiologic endocrine uterine environment prepared in a fresh treatment may be associated with increased risk of first-trimester miscarriage (Kansal Kalra et al., 2011). However, other studies found inconsistent results regarding the relationship between miscarriage and fresh/thawed embryo transfers (Aflatoonian et al., 2010b; Aytoz et al., 1999; Mocanu et al., 2008). These studies reported a lower miscarriage rate following transfers of fresh embryos compared to transfers of thawed embryos.

In relation to perinatal outcomes, recent evidence suggest that better perinatal outcomes follow the transfer of thawed embryos compared to fresh embryos (Pelkonen et al., 2010; Pinborg et al., 2010; Shih et al., 2008; Wang et al., 2005). Babies conceived by transfer of thawed embryos had significantly lower rates of preterm birth and perinatal mortality than those following the transfer of fresh embryos (Pelkonen et al., 2010; Shih et al., 2008). Liveborn babies were less likely to be low birthweight following the transfer of thawed embryos compared to fresh embryos (Wang et al., 2005). The rate of congenital anomalies for babies born after the transfer of thawed embryos was approximately three times lower than for those born following the transfer of fresh embryos (Li et

al., 2010). However, Swedish and Belgian data did not find any difference in neonatal outcomes between transfers of fresh or thawed embryos (Belva et al., 2008; Finnström et al., 2011).

Differences in the outcomes of thaw cycles have been found for the two methods of embryo cryopreservation, namely slow freezing and vitrification. Studies found that the newly developed vitrification method may increase the embryo survival rate during the freezing and thawing processes compared to the traditional slow freezing method (Rezazadeh Valojerdi et al., 2009; Takahashi et al., 2005). It was further suggested that the transfer of vitrified blastocysts would achieve the same pregnancy rate as the transfer of fresh blastocysts (Takahashi et al., 2005). No excess adverse neonatal outcomes were observed in babies born after the transfer of vitrified embryos compared to those born following the transfer of fresh embryos or slow frozen embryos (Wikland et al., 2010).

Stage of embryo development

Embryos can be transferred at cleavage (comprising 6-8 cells at day 2-3 after fertilisation) or blastocyst (comprising about 100 cells at day 5-6 after fertilisation) stage. The proportion of blastocyst transfers has increased in recent years in Australia and New Zealand, from 17.1% in 2004 to 38.6% in 2008 (Wang et al., 2010). This increase is probably related to the evidence of higher rates of clinical pregnancy and live delivery following blastocyst transfers than cleavage embryo transfers (Butterworth, 2001).

Australia and New Zealand 2008 cohort data show that of autologous fresh cycles, blastocyst transfers resulted in 34.6% clinical pregnancies and 26.6% live deliveries compared to 27.4% clinical pregnancies and 21.1% live deliveries following cleavage embryo transfers. Of autologous thaw cycles, the rates of clinical pregnancy and live delivery were 26.7% and 19.7% respectively for blastocyst transfers, and 22.2% and 17.0% respectively for cleavage embryo transfers (Wang et al., 2010).

Interestingly, randomised controlled trials have reported inconsistent results on the difference in the rates of clinical pregnancy and live delivery between blastocyst transfers and cleavage embryo transfers. Several trials have reported no significant differences in the rates of clinical pregnancies and live deliveries between transfers of cleavage embryos and blastocysts (Coskun et al., 2000; Emiliani et al., 2003; Kolibianakis et al., 2004). In contrast, other trials found that transfers of blastocysts resulted in significantly higher rates of clinical pregnancy and live delivery compared to transfers of cleavage embryos (Papanikolaou et al., 2006; Van der Auwera et al., 2002). Two recent meta-analyses on randomised controlled trials have confirmed higher pregnancy and live delivery rates following blastocyst transfers compared to cleavage embryo transfers when equal numbers of embryos were transferred (Blake et al., 2007; Papanikolaou et al., 2008).

The natural selection theory is used to explain the higher rates of clinical pregnancy and live delivery following blastocyst transfers (Butterworth, 2001).

According to this theory, the two or three additional days in the blastocyst culture allow the reduction in chromosomally abnormal embryos. Hence it is proposed that the best embryos survive through the blastocyst culture process (Butterworth, 2001). Since not all embryos would survive through the blastocyst culture (Barrenetxea et al., 2005; Kolibianakis et al., 2004), the differences in clinical pregnancy and live delivery rates between blastocyst transfers and cleavage embryo transfers may be reduced when initiated cycles rather than transfer cycles are used as the denominator to measure the success rates (Papanikolaou et al., 2008). When limited cleavage embryos are available, extended blastocyst culture would result in either no transfer or the possible transfer of a poorer quality embryo (Barrenetxea et al., 2005). Therefore, it has been suggested that blastocyst transfer is only a good alternative for patients with multiple good quality embryos at cleavage stage (Hreinsson et al., 2004).

The findings on the relationship between miscarriage and transfers of cleavage embryos or blastocysts are not consistent in the literature. A couple of early trials suggested a slightly higher miscarriage rate following transfers of blastocysts compared to cleavage embryos (Bungum et al., 2003; Emiliani et al., 2003). However, a 2006 trial found that the miscarriage rate for pregnancies following transfers of single cleavage embryos was 1.6 times the rate for transfers of single blastocysts (Papanikolaou et al., 2006). Given the natural selection in blastocyst culture (Butterworth, 2001) and asynchronisation between altered endometrium and early exposure to cleavage embryos (Papanikolaou et al., 2006), theoretically the miscarriage rate might be lower for

transfers of blastocysts than for cleavage embryos. However, an updated Cochrane review found no significant difference in miscarriage rates between blastocyst and cleavage embryo transfers (Blake et al., 2007).

New evidence shows that babies born following blastocyst transfers had a higher risk of adverse perinatal outcomes compared to cleavage embryo transfers (Finnström et al., 2011; Källén et al., 2010; Kawachiya et al., 2011). Blastocyst transfer was associated with an increased risk of monozygotic twinning (Kawachiya et al., 2011). Babies born after blastocyst transfers had a higher risk of preterm birth and congenital anomalies than babies born after cleavage embryo transfers (Finnström et al., 2011). Even restricted to singletons, the rates of preterm birth, low birthweight and low Apgar score were significantly higher among singletons born following blastocyst transfers than singletons after cleavage embryo transfers (Källén et al., 2010).

Patients' demographic characteristics

A number of patients' demographic characteristics need to be taken into consideration when evaluating the success of ART treatment. The patient's demographic characteristics influence the selection of the type of ART treatment and procedure. For example, sperm-related severe male factor infertility indicates the need for the ICSI procedure (Lanzendorf et al., 1988), and older woman's age usually indicates the transfer of two or more embryos (RTAC, 2005). On the other hand, patient demographic characteristics are associated with the success of ART treatment and adverse pregnancy and

perinatal outcomes. Younger age, male factor infertility, short period of infertility, previous pregnancy of ≥20 weeks gestation, normal body mass index (BMI) and non-cigarette smoking have been classified as good prognostic factors for the success of ART treatment (Menken et al., 1986; Roberts et al., 2010; Sazonova et al., 2011).

Woman's age

Amongst patients' demographic characteristics, woman's age is identified as the most dominant factor associated with pregnancy and birth outcomes. It is well established that younger women have high rates of both spontaneous and assisted reproduction pregnancy (Heffner, 2004; Joseph et al., 2005; Menken et al., 1986). However, in recent years, particularly in high income countries such as Australia, there has been an increasing trend towards delaying childbirth (Laws et al., 2010).

This trend of delaying childbirth has been associated with increased utilisation of ART treatment and access to the treatment at a late age. The number of ART treatment cycles doubled from 30,119 in 2004 to 61,929 in 2008 in Australia and New Zealand (Bryant et al., 2004; Wang et al., 2010). The proportion of non-donor fresh cycles in women aged ≥40 years rose from 21.7% in 2002 to 26.6% in 2008 (Bryant et al., 2004; Wang et al., 2010). Similar trends have been observed in the United Kingdom, where the proportion of cycles in women aged ≥40 years increased from 9.1% in 1991 to 19.4% in 2008 (HFEA, 2010).

A woman's age is an independent factor for fertility, pregnancy and perinatal outcomes (Heffner, 2004; Joseph et al., 2005). Advancing woman's age not only leads to declining fertility by reducing the quality of oocytes, and deterioration in the condition of the uterus and female hormones (Baird et al., 2005; Tufan et al., 2004), but also other chronic health conditions (such as diabetes and hypertension) that may complicate pregnancy (Alshami et al., 2011; Tufan et al., 2004). With advancing woman's age, the fertility rate in the general population decreases from 400 pregnancies per 1,000 married women aged <30 years to 100 per 1,000 married women aged 45 years or older (Heffner, 2004; Menken et al., 1986). Similarly, the miscarriage rate increases with advancing woman's age, from 13% in women aged in their 20s and early 30s to more than 50% in women aged 40 years or older (Nybo Andersen et al., 2000).

Not surprisingly, advancing woman's age is a strong predictor of pregnancy and live birth following ART treatment (Ciray et al., 2004; Petanovski et al., 2011). A woman's age in an autologous fresh treatment best reflects the impact of a woman's age on the pregnancy and birth outcomes. The summarised statistics from a European regional report show that the pregnancy rate per initiated autologous fresh cycle was 28.2% for women aged <35 years, 22.2% for women aged 35−39 years, and 9.6% for women aged ≥40 years. Similarly, the live delivery rate decreased from 26.6% for women aged <35 years to 20.5% for women aged 35−39 years and 8.6% for women aged ≥40 years (de Mouzon et al., 2010). The trend of a decrease in the rates of pregnancy and live delivery

with advancing woman's age has also been shown in the United States, and in Australian and New Zealand ART registers (CDC, 2010; Wang et al., 2010).

As for spontaneous pregnancies, pregnancy complications and obstetric interventions following ART treatment also increase with advancing woman's age (Salumets et al., 2006; Soares et al., 2005). The 2008 United States data show that the miscarriage rate was <14% for women younger than 35 years, increasing to 30% for women aged 40 years and was 58% among women 45 years or older (CDC, 2010). The latest Australian and New Zealand ART report shows that the caesarean section rate increased with advancing woman's age, from 39.5% of women aged less than 30 years to 80.4% of women aged 45 years or older (Wang et al., 2010).

The lower success rate following ART treatment in older women may be explained by a number of factors. Oocyte quality has been identified as the most important reason (Hourvitz et al., 2009; Navot et al., 1991). It has been suggested that oocyte quality diminishes with advancing woman's age (Baird et al., 2005; Tufan et al., 2004). The quality of oocytes determines the outcome of fertilisation and subsequent pregnancy/birth outcomes (Navot et al., 1991). The lower success rate and higher complication rate in older women is largely explained by the diminished oocyte quality (Hourvitz et al., 2009).

In the general population, advanced woman's age has been also associated with an increased risk of adverse perinatal outcomes, namely preterm birth, intrauterine growth restriction, low birthweight, congenital anomalies and

perinatal mortality (Jacobsson et al., 2004; Salem et al., 2011). Women who conceive with ART treatment are, on average, older than those who have spontaneous pregnancies and this has often been used to explain the excess adverse outcomes observed following ART treatment (Aldous & Edmonson, 1993). Preterm birth and low birthweight babies are more commonly born to older women following ART treatment than their younger counterparts (Wang et al., 2005). Advanced woman's age might contribute to congenital anomalies following ART treatment (Bonduelle et al., 2002).

The strong association between a woman's age and fertility potential has made a woman's age not only the best predictor of ART treatment outcome, but also an indicator for accessing ART treatment and transferring a certain number of embryos. Some countries have age limits for women accessing ART treatment using public funding (Gillett & Peek, 1997; Lindström & Waldau, 2008). In New Zealand public funding has been restricted to infertile women who are unlikely to spontaneously conceive and are aged less than 40 years (Gillett & Peek 1997; Gillett et al., 2006). In Australia, a national recommendation stated that it is not clinically appropriate to initiate a new cycle of ART treatment in women using their own eggs at 44 years and older (Department of Health and Ageing, 2006). Clinics in both Australia and New Zealand recommend SET in women aged <35 years in their first ART cycle (RTAC, 2005).

Cause and length of infertility

Published data show a higher success rate in cycles where the male factor is the only cause of infertility compared to those with female factor infertility (CDC, 2010; Wang et al., 2010). Female factor infertility due to, for example, ovulatory dysfunction, tubal disease, endometriosis or hormonal disorders was associated with a lower occurrence of pregnancy (Govaerts et al., 1998). Even when a pregnancy is achieved, female factor infertility is associated with a higher risk for hypertension and bleeding (Tan et al., 1992), which can in turn reduce the chance of live delivery. In women with normal fertility, once male factors are overcome, there is a better chance of pregnancy and live delivery.

A longer duration of infertility is related to a decrease in the chance of both spontaneous pregnancy and ART-related pregnancy (Dunson et al., 2004; Hunault et al., 2004; Templeton et al., 1996; te Velde et al., 2000). For women who have failed to conceive naturally over a one-year period, the probability of spontaneous pregnancy in the subsequent year is 49%, and it declines to 14% for women who have not had pregnancy over a three-year period (te Velde et al., 2000). Similarly, a significant decrease in the age-adjusted live birth rate with increasing duration of infertility between 1 and 12 years was observed (Templeton et al., 1996).

Number of ART treatment cycles

Interacting with woman's age and length of infertility, the number of previous ART treatment cycles has also been associated with the success of ART treatment. It has been suggested that higher pregnancy and live delivery rates follow the first and second ART treatment cycles (Smith et al., 2011). There is a correlation between the decrease in the success rate and the increase in the number of ART treatment cycles (Martin-Johnston et al., 2009). The most notable decrease in clinical pregnancy rates occurred after the third ART treatment cycle, with the likelihood of a successful outcome declining with each additional ART treatment cycle (Martin-Johnston et al., 2009).

History of previous pregnancies

History of previous pregnancies is another patient demographic characteristic related to the outcomes of ART treatment. Number of previous pregnancies of ≥20 weeks gestation is described by the medical term "parity". Nulliparous (parity = 0) refers to a woman who has never had a previous pregnancy of ≥20 weeks gestation and parous (parity >0) refers to a woman who have had at least one previous pregnancy of ≥20 weeks gestation. A clinical pregnancy that ends before 20 completed weeks of gestational age is defined as spontaneous abortion or miscarriage (Zegers-Hochschild et al., 2009).

Parous is a good prognostic factor for successful outcomes of ART treatment (Roberts et al., 2010). In contrast, a history of miscarriage is a risk factor for

adverse pregnancy outcomes (Sneeringer et al., 2008; Wang et al., 2004). There is general agreement that pregnancy outcomes are more favourable for parous women, with higher rates of clinical pregnancy and live delivery compared to nulliparous women (Bai et al., 2002; Olivennes et al., 2002). Even when a pregnancy was achieved following ART treatment, a history of miscarriage may lead to an early loss of the pregnancy (Wang et al., 2004). A history of more than three previous miscarriages has been also associated with increased risk of preterm birth (Vedmedovska et al., 2010).

Body mass index

Body mass index (BMI) is a simple index of weight-for-height that is commonly used to classify population into standard weight categories. WHO has classified BMI into the following categories: underweight with BMI <18.50, normal weight with BMI 18.50–24.99, overweight with BMI 25.00–29.99, obese with BMI ≥30 (WHO, n.d.).

BMI is considered as an important factor to infertility and treatment outcomes (Bellver et al., 2007; Davies, 2003; Veleva et al., 2008). Either high or low BMI was associated with reduced probability of achieving pregnancy in women receiving ART treatment (Wang et al., 2000). Overweight and obese populations have increased risk of infertility (Davies, 2006; Hassan & Killick, 2004; Norman et al., 2004; Mulders et al., 2003). In comparison with women of normal weight, overweight women have significantly fewer oocytes retrieved at an OPU (Zhang et al., 2010). Obesity has also been observed to detrimentally affect

endometrial development and implantation (Brewer & Balen, 2010). The rates of clinical pregnancy and live delivery are significantly lower in obese women compared to women with normal weight (Bellver et al., 2010; Dessolle et al., 2011; Shah et al., 2011). Veleva and colleagues reported a U-shaped association between BMI and miscarriage which indicates a higher risk of miscarriage in underweight and obese women (Veleva et al., 2008). An Australian study found a positive relationship between BMI and the risk of spontaneous abortion in pregnant women after ART treatment (Wang et al., 2002). Furthermore, obesity also leads to a higher risk of pregnancy complications such as gestational diabetes, preeclampsia, emergency caesarean delivery, and adverse perinatal outcomes (Nohr et al., 2005; Ovesen et al., 2011; Luke et al., 2011).

Cigarette smoking

There is a great deal of evidence demonstrating that cigarette smoking reduces reproductive potential (Baird & Wilcox, 1985; Zenzes, 2000), as well as the probability of clinical pregnancy and live delivery following ART treatment (Ben-Haroush et al., 2011; Dessolle et al., 2011). The miscarriage rate following ART treatment was significantly higher amongst smokers than non-smokers (Lintsen et al., 2005). Cigarette smoking during pregnancy was also associated with increased risk of a number of maternal and obstetric complications (Hayashi et al., 2011). Low birthweight, preterm birth and small for gestational age were more frequent among babies born to mothers who were smokers than those born to mothers who were non-smokers (La Merrill et al., 2011; Sazonova

et al., 2011). A recent systematic review stated that all stages of the reproductive function are targets of cigarette smoke toxicants and the effects of cigarette smoking are dose-dependent (Dechanet et al., 2011).

Demographic characteristics related to male partner

A number of male demographic characteristics were reported to be associated with declined male fertility. Older male age, obesity, and cigarette smoking are all negatively associated with the quantity and quality of sperm (Levitas et al., 2007; Nguyen et al., 2007; Wegner et al., 2010). Sperm motility was found to be inversely related to advancing male age with peak motility of sperm at age <25 years and lowest motility at age ≥55 years (Levitas et al., 2007). There was a negative correlation between semen parameters and being overweight or obese with significantly decreased proportion of normal motile sperm in both overweight and obese men compared to normal weight men (Kort et al., 2006). Cigarette smoking was significantly correlated with reduced sperm count, motility and morphology (Mostafa et al., 2006). Interestingly, advanced male age has little impact on the likelihood of pregnancy, miscarriage and live delivery following ART treatment (Aboulghar et al., 2007; Dain et al., 2011; Duran et al., 2010; Whitcomb et al., 2011). However, both male obesity and smoking are associated with decreased clinical pregnancy rates and live birth outcomes (Bakos et al., 2011; Fuentes et al., 2010).

Summary of literature review

Limitations in measurement of the efficacy of ART treatment

The choice of ART treatment and procedure varies significantly across patients, clinics, countries and regions. Therefore measures used to quantify ART treatment success and monitor the efficacy of ART treatment are limited and inconsistent. Success measures are mostly limited to clinical pregnancy and live delivery, and the efficacy of ART treatment is usually presented as a clinical pregnancy rate or live delivery rate per initiated cycle or embryo transfer cycle. However, the optimum outcome of an ART treatment is not clinical pregnancy or live delivery, but a healthy baby. Perinatal outcomes to indicate a healthy baby are usually measured per birth or live birth, not per initiated cycle or embryo transfer cycle. Singleton live birth rate per cycle commenced was suggested to measure the efficacy of an ART treatment. However, this measure does not include other common health indicators of babies. Absence of adverse perinatal outcomes such as multiple birth, preterm birth, low birthweight, congenital anomalies and neonatal death were not summarised in studies to measure the efficacy of ART treatment per initiated cycle or embryo transfer cycle.

Inconsistent association between outcomes and treatment factors

There are inconsistencies in the literature regarding the association between the successful and adverse pregnancy and perinatal outcomes and different

treatment/procedures. Firstly, there is general consensus that there is no difference in the pregnancy and live delivery rates between IVF and ICSI procedures. In contrast, the association between congenital anomalies and ICSI and IVF procedures remains inconclusive. Secondly, there are inconsistent results for fresh and thaw cycles. Fresh cycles result in higher rates of pregnancy and live delivery than thaw cycles, but babies born following thaw cycles have better perinatal outcomes than those born following fresh cycles. Thirdly, DET results in higher rates of pregnancy and live delivery than SET. However, rates of multiple pregnancy and multiple birth are significantly higher for DET than SET, as are poorer perinatal outcomes which are more frequent among babies born following DET when compared to SET. Finally, for both fresh and thaw cycles, blastocyst transfer is associated with higher rates of clinical pregnancy and live delivery compared to cleavage embryo transfers. Interestingly, in relation to perinatal outcomes, babies born following the transfer of cleavage embryos appear to have lower rates of preterm birth, low birthweight and congenital anomaly.

The inconsistent findings regarding the association between different treatment factors and successful ART treatment outcomes and adverse pregnancy and perinatal outcomes may be related to what measurement was chosen for both the numerator and denominator. Choice of an alternative numerator and denominator may result in different directions of the association. For example, when live deliveries are used as the numerator, DET cycles results in a higher rate of live delivery than SET cycles. However, when term liveborn singletons

are used as the numerator, DET cycles results in lower rate of term liveborn singletons than SET cycles.

As the optimal outcome of an ART treatment is a healthy baby, common perinatal health indicators such as multiple birth, gestational age, birthweight, perinatal mortality and congenital anomalies should be summarised in the numerator and measured per initiated cycle or embryo transfer cycle. Therefore, the inconsistent findings for IVF and ICSI procedures, between fresh and thaw cycles, between SET and DET, and between cleavage embryo and blastocyst may be clarified.

Woman's age – the most predominant demographic characteristic

In the literature, woman's age was identified as the most important demographic characteristic in relation to pregnancy and birth outcomes. Advancing woman's age significantly reduces the likelihood of successful outcomes of ART treatment, and increases the risk of adverse pregnancy and perinatal outcomes. What constitutes advancing woman's age varies from study to study, with the cut-off point usually at 35 or 40 years. Patients are usually categorised into five-year age groups or other age groups dependent upon the conventions and population size. However, this approach lacks precision as the success rates of ART treatment measured by clinical pregnancies and live deliveries per cycle vary by each year of a woman's age. Age-specific success rates using large population cohort data should be provided.

Rationale of the PhD thesis

Study 1 Research question: How does each additional year of a woman's age impact the rate of live delivery?

The age of a woman intending to undergo autologous ART treatment has been identified in the literature as the most significant demographic characteristic associated with the success of ART treatment. It is critical to provide population-based evidence on the impact of each additional year of delay in ART treatment to the success rates. The first study of this PhD thesis (Chapter 3) will calculate age-specific rates of clinical pregnancy and live delivery per initiated cycle by single year increments for women who underwent their first autologous ART treatment. It will estimate the additional live deliveries that could potentially be achieved if the population of patients had had their first ART treatment one, two or three years earlier. This study will provide evidence for benchmark, education and counselling of patients, service providers and the community.

Study 2 Research question: Does implementation of a policy of SET in Australia and New Zealand improve perinatal outcomes of ART babies?

Along with woman's age, the number of embryos transferred was one of most important treatment factors related to pregnancy and birth outcomes. Transfer of two or more embryos significantly increased the risk of multiple gestation pregnancy which is responsible for the majority of adverse pregnancy and birth outcomes. Implementation of a policy of SET has successfully reduced the rates of multiple pregnancy and birth in some countries. The second study of this PhD thesis (Chapter 4) will evaluate the RTAC policy of SET in Australia and New Zealand by comparing adverse perinatal outcomes of babies following SET and DET. This study will demonstrate the benefits of SET in Australia and New Zealand.

Study 3 Research question: Do transfers of fresh blastocysts and blastocysts cultured from thawed cleavage embryos increase the likelihood of the birth of a term liveborn singleton of normal birthweight without congenital anomaly?

The optimum goal of an ART treatment is to have a healthy baby. However, a summarised measure of a "healthy baby" was not being commonly used to measure the efficacy of ART treatment. It has been advocated that the outcome of ART treatment needs to focus on the Birth Emphasizing a Successful Singleton at Term (BESST), but the BESST measure does not take birthweight and congenital anomalies into consideration. The third study of the PhD thesis (Chapter 5) will propose a new indicator "healthy baby" which summarises a composite of perinatal outcomes as the optimum goal for an ART treatment. Using the "healthy baby" rate per embryo transfer cycle, the third study will suggest an optimum clinical practice model to maximise the chance of the birth of a "healthy baby".

Study 4 Research question: Does selective transfer of a single blastocyst optimise the chance of a healthy term baby compared to a single cleavage embryo?

To eliminate the risk and complications due to multiple gestations without compromising the clinical pregnancy rate, elective SET was introduced. However, the efficacy of elective SET is usually measured by the rates of clinical pregnancy and live delivery. Using the newly proposed "healthy baby" indicator from third study of the PhD thesis, the fourth study (Chapter 6) will compare the likelihood of a "healthy baby" between the selective transfer of a single cleavage embryo and a single blastocyst by the number of embryos available for transfer. This study will confirm the high efficacy of selective SET of a fresh blastocyst.

Study 5 Research question: Do transfers of fresh blastocysts and blastocysts cultured from thawed cleavage embryos reduce the hazard of miscarriage?

Miscarriage is one of the common complications following ART treatment. The reporting of an association between miscarriage and ART treatment factors is not consistent in the literature. The relationship between miscarriage and the proposed clinical practice model suggested from the third study of the PhD has not been assessed. The fifth study of the PhD thesis (Chapter 7) will use the miscarriage rate to evaluate the proposed clinical practice model. It will demonstrate that for younger patients, better pregnancy and perinatal outcomes are associated with a practice model which includes transfer of a selected single blastocyst and freezing of cleavage embryos in a fresh cycle and transfer of a selected single blastocyst cultured from these thawed cleavage embryos in subsequent thaw cycles.

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Chapter 3

Study 1:

Age-specific success rate for women undertaking their first assisted reproduction technology treatment using their own oocytes in Australia, 2002-2005

About this chapter

Woman's age is identified as the dominant demographic characteristic for the success of autologous ART treatment. It is important to investigate the impact of each additional year of age on the success of ART treatment and to provide population based evidence on what is the recommended age to initiate an autologous ART treatment. This chapter presents the first study of the PhD thesis "Age-specific success rate for women undertaking their first assisted reproduction technology treatment using their own oocytes in Australia, 2002-2005". This study estimated the livelihood of pregnancy and live delivery for women who underwent their first autologous cycle. It predicted the number of extra live deliveries that could potentially be achieved if patients aged 35–44 years had had their ART treatment one, two or three years early. It addressed the importance of early fertility assessment and early access to ART treatment. This study also provided evidence for policy makers to review and update the recommended age limit to initiate a new fresh ART treatment for women intended to use their own oocytes.

This study was presented at the FSA 2007 annual meeting in Hobart, Australia. The manuscript of this study was published in Human Reproduction in 2008.

List of presentation and publication from this study:

Oral presentation. Age-specific success rates of autologous fresh cycles, Australia 2002-2004. The annual conference of Fertility Sociality of Australia. Hobart, Australia (9-12 Sep 2007).

Wang YA, Healy D, Black D, Sullivan EA. Age-specific success rate for women undertaking their first assisted reproduction technology treatment using their own oocytes in Australia, 2002-2005. Hum Reprod. 2008 Jul;23(7):1633-8. doi: 10.1093/humrep/den135.

Abstract

BACKGROUND: Woman's age is an independent factor determining the success of assisted reproductive technology treatment. This study presents the age-specific success rate of first autologous fresh treatment in Australia during 2002–2005. **METHODS:** This is a retrospective population-based study of 36412 initiated first autologous fresh cycles conducted in Australian clinics during 2002–2005. Pregnancy and live delivery rates per initiated cycle were determined for each age. **RESULTS:** The overall live delivery rate per initiated cycle was 20.4% with the highest success rate in women aged between 22 and 36 years. Male factor only infertility had a higher live delivery rate (22.0%) than female factor only infertility (19.2%). Advancing woman's age was associated with a decline in success rate. For women ≥30 years, each additional 1 year in age was associated with an 11% (99% CI: 10–12%) reduction in the chance of achieving pregnancy and a 13% (99% CI: 12–14%) reduction in the chance of a live delivery. If women aged 35 years or older would have had their first autologous fresh treatment 1 year earlier, 15% extra live deliveries would be expected. **CONCLUSIONS:** This study suggested that women aged 35 years or older should be encouraged to seek early fertility assessment and treatment where clinically indicated.

Introduction

In general, younger women achieve higher rates of pregnancy and live births than their older counterparts (Menken et al., 1986; Cnattingius et al., 1992; Dunson et al., 2004; Heffner, 2004; Joseph et al., 2005). However, in recent years, there has been a trend of women deferring childbirth. In the USA, the number of first births per 1000 women aged 35–39 years increased by 36% between 1991 and 2001, whereas the rate among women aged 40–44 years increased by 70% (Heffner, 2004). In Australia, one in eight (13.3%) first time mothers in 2005 was aged 35 years or older compared to 6.9% in 1995. Similarly, the average age of first time mothers increased by 2.2 years from 25.8 years in 1991 to 28.0 years in 2005 (Laws et al., 2007).

The aging of the general reproduction population has been paralleled by the increasing age of women undergoing assisted reproduction technology (ART) treatment. The average age of women who received In vitro fertilization (IVF) or micromanipulation in United Kingdom increased from 33.8 in 1992 to 34.8 in 2004 (Human Fertilisation and Embryology Authority, 2007). The average age of women who underwent ART treatment in Australia and New Zealand was 35.5 years in 2005, 0.3 years older than in 2002 (Wang et al., 2007).

The literature has shown that women seeking fertility treatment face the same age related issues as women conceiving naturally. It is likely that younger women who undergo ART treatment will have a better chance of getting

pregnant and having a live delivery than older women (Kramer, 1987; Bai et al., 2002; Olivennes et al., 2002; Wang et al., 2007). Even though the success rate of ART has increased in recent years (Ciray et al., 2004; CDC, 2006), this rise in success rates has not been seen in older women. Since 2000, for women aged less than 35 years in the USA, the live birth rate per fresh non-donor cycle has increased from 33 to 37% in 2004, whereas it remains unchanged at about 4% for women aged 43 years or older (CDC, 2006).

Other female conditions, such as cause of infertility, previous pregnancies, obesity and chronic disease also contribute to the success of ART treatment (Tough et al., 2002, 2006; Bellver et al., 2006). On the other hand, these conditions, such as cause of infertility, are highly correlated with advancing age (Menken et al., 1986; Heffner, 2004; Baird et al., 2005). Studies have found that woman's age intersects with other demographic conditions in determining the success of fertility treatment (Heffner, 2004; Tufan et al., 2004; Baird et al., 2005).

What constitutes advancing age varies from study to study (Salihu et al., 2003), with the cut-off point usually at 35 (Prysak and Laros, 1995) or 40 years (Zaideh and Yahaya, 2001). Previously published papers classify patients into 5 year groups or other year groups dependent upon the conventions and population size. However, this lacks precision as success rates of ART treatment measured by clinical pregnancies and live deliveries per cycle vary by each year of age. The aim of this study is to provide comprehensive age-specific rates of pregnancy and live birth to assist couples in planning their reproductive future.

In addition, the impact of cause of infertility on age-specific success rates was investigated. This study uses population data from the Australia and New Zealand ART Database (ANZARD) from 2002 to 2005 to provide the success rates in 1 year increments for women undergoing their first fresh ART treatment using their own oocytes.

Materials and Methods

Data

Data and definitions used in this study are from the ANZARD, which are maintained at the National Perinatal Statistics Unit. ANZARD are collected annually, in de-identified format, from all fertility centers in Australia and New Zealand. ANZARD include information about the ART procedures, use of thawed embryos, blastocyst culture, embryo transfer and donation of gametes or embryos. It also includes information on pregnancy (ectopic pregnancy, spontaneous abortion and termination) and birth outcomes (gestational age, birthweight and perinatal mortality).

Data on women aged 18 years or older who had their first autologous fresh cycles in Australian clinics from 1 January 2002 to 31 December 2005, and subsequent pregnancy and birth outcomes were extracted from the ANZARD. Mixed fresh–thaw cycles, gamete intrafallopian transfer cycles, natural cycles and surrogacy cycles were excluded. A total of 36 412 women who had their first autologous fresh cycles are included in the final analysis.

Main outcome measures

Woman's age is calculated in completed years of age. Cause of infertility is classified as: male factor only infertility (a male factor problem was diagnosed and not any female factor problem), female factor only infertility (tubal disease, endometriosis or another female factor problem was diagnosed and not any male factor problem), combined male–female factor infertility (both male and female factor problems were diagnosed), unexplained infertility (neither a male nor female factor problem was diagnosed) and not stated (where cause of infertility was not reported to ANZARD).

Gestational age is defined as the completed weeks of gestation of the fetus and is calculated by (pregnancy end date − embryo transfer date) + 16 days. A clinical pregnancy was defined as one of the following criteria: evidence by ultrasound of intrauterine sac(s) or fetal heart(s); examination of products of conception reveal chronic villi; an ectopic pregnancy that had been diagnosed laparoscopically or by ultrasound. A delivery is defined as a birth event in which one or more baby was born of ≥20 weeks gestational age or of ≥400 g birthweight. A live delivery is a birth event in which one or more baby is live born of ≥20 weeks gestation or of ≥400 g birthweight.

An initiated cycle is defined as a cycle in which follicle-stimulating hormone was administered. The success was measured by live deliveries per initiated cycle.

Statistical analysis

Success rates were calculated for each woman's age and compared amongst cause of infertility. Because of small numbers, women aged 21 years or younger and women aged 45 years or older were grouped into two age groups. Logistic regression was used to investigate the odds of success in women aged 30 years or older. A further logistic regression model was built to illustrate the likelihood of success in 5 year age groups for women age 30 years or older compared to 25–29 years.

Two methods were tested to predict the number of live deliveries for the women aged 35–44 years if they undergo ART treatment 1, 2 or 3 years earlier. The first method directly applied the live delivery rate per initiated cycle of an age to 1, 2 or 3 years older. The second method built three logistic regression models for women aged 34–43, 33–42 and 32–41 years. The probability of live delivery at each age from the three logistic regression models is then applied to women aged 35–44 years, respectively. After comparing the two methods, the second method was more conservative and was used in the prediction.

Odds ratios (OR), adjusted odds ratios (AOR) (adjusted for cause of infertility) and 99% confidence intervals (99% CI) were calculated. Data were analysed with SPSS software (version 15.0; SPSS Inc, Chicago, IL, USA).

Ethics

Ethics approval for this study was granted by the Human Research Ethics Advisory Panel of the University of New South Wales, Australia.

Results

The mean age of women having their first autologous fresh cycles was 34.4 years (SD 4.9 years), with about half of the women (47.4%) aged 35–44 years. About 12% (4321) of initiated cycles ended before oocyte pick-up, 87.0% (31 692) had oocytes collected and 75.7% (27 561) had an embryo transferred. One in four initiated cycles resulted in a clinical pregnancy, and one in five resulted in a live delivery. Nearly 17% of initiated cycles resulted in a singleton live delivery.

Table I details the outcomes of the first autologous fresh cycles and presents the age-specific rates of pregnancy and live delivery. Women aged 22–36 years achieved a clinical pregnancy rate per initiated cycle of 25% or higher at each age. However, the clinical pregnancy rate per initiated cycle declined with each advancing year of age from 30 years onwards and was most marked in women aged older than 35 years. For women aged 45 years or older, the average pregnancy rate was 1.9%.

Women aged 22–36 years also had a higher rate of live deliveries per initiated cycle. Among this age group, the live delivery rate was above 20% at each age.

The highest live delivery rate per initiated cycle was in women aged 24 years (30.3%). The live delivery rate decreased with increasing woman's age. For women aged 45 years or older, there was only one live delivery of 471 initiated cycles (Table I).

Table I also compares the live delivery rate per initiated cycle by cause of infertility at each age. Overall, couples with male factor only infertility achieved higher live delivery rate (22.0%) than those who had female factor only infertility (19.2%) or combined male–female factor infertility (18.4%).

Table I

Figure 1 illustrates the pregnancy outcomes by woman's age. Over all, with advancing woman's age, the proportion with deliveries decreased and the proportion with spontaneous abortions increased. For women aged in their 20s to early 30s, less than 15% pregnancies ended in a spontaneous abortion and more than 80% resulted in a delivery. However, there is a marked increase in spontaneous abortion and decrease in delivery for women aged 36 years onward. In women aged ≤42 years, the proportion of delivery was higher than the proportion of spontaneous abortion, while from age of 43 onward, proportionally more spontaneous abortions than deliveries were observed (29.5% of the 78 pregnancies resulted in a delivery and 66.7% were spontaneous abortions).

Figure 1

The ectopic pregnancies and terminations were around 1.2% in women aged in their 20s, 1.5% in women aged in their 30s and above 3.8% in women aged 40 years or older.

To assess the impact of each additional 1 year of age on pregnancy and live delivery, a logistic regression model adjusted for cause of infertility was conducted for women aged 30 years or older. For each 1 year increment in woman's age, the chance of clinical pregnancy decreased 0.89 times and the chance of a live delivery decreased 0.87 times (Table II).

A further logistic model was established to investigate the odds of getting pregnant and having a live delivery in women aged 30 years or older compared to those aged 25–29 years. Women aged 30–34 years had similar chance of pregnancy and live delivery compared to women aged 25–29 years. Women aged 35–39 years were 34% less likely to achieve clinical pregnancy and 39% less likely to have a live delivery compared to women aged 25–29 years. While, compared to women aged 25–29 years, women aged 45 years or older were 96% less likely to get pregnant and 99% less likely to have a live delivery (Table II).

Table II

Table III presents the predicted number of live deliveries for women aged 35–44 years if they underwent first autologous fresh treatment 1, 2 or 3 years earlier. If these women had treatment 1 year earlier, a total of 363 estimated extra live

deliveries would be achieved. If they had treatment 2 years earlier or 3 years earlier, 749 or 1099 extra live deliveries would be expected, respectively.

Table III

Figure 2 illustrates the percentage of extra live deliveries predicted at each age if women aged 35–44 years who had first autologous fresh treatment 1, 2 or 3 years earlier. If 35 year old women would have had treatment 1 year earlier, about 15% extra live deliveries would be expected. If they had treatment 2 or 3 years earlier, 22 or 26% extra live deliveries would be expected, respectively. However, if 43 years old women had treatment 1 year earlier, 236% extra live deliveries would be expected. If 44 years old women had treatment 1 year earlier, 375% extra live deliveries would be expected.

Figure 2

Discussion

This large Australian study confirms that in women undergoing fertility treatment advancing woman's age is one of the most important factors in determining the success of the first autologous fresh cycle. The findings reinforce the important fertility preservation message that women and couples with infertility problems need to have them diagnosed early and treated expeditiously if clinically indicated. It also shows that irrespective of the cause of infertility or which partner has the condition, each additional year of

woman's age results in a relative decline in fertility. The rising proportion (from 6.9% in 1995 to 13.3% in 2005) of women having their first baby aged 35 years or older in Australia suggests that a misperception remains in the community of women's ability to conceive spontaneously with advancing age.

Among the first autologous fresh cycles, the highest success rates were in women aged between 22 and 36 years in which the clinical pregnancy rate per initiated cycle was above 25% at each age, and the live delivery rate per initiated cycle was above 20% at each age. However, the success rates declined with the increase in woman's age from 35 years onward. For women aged 45 years or older, the success rate was one live delivery of 471 initiated cycles.

Woman's age is an independent factor for pregnancies and perinatal outcomes (Heffner, 2004; Joseph et al., 2005; Wang et al., 2005). Advancing woman's age itself leads to declining fertility by reducing the quality of oocyte and lowering female hormones (Tufan et al., 2004; Baird et al., 2005). With the increase in woman's age, the fertility rate decreases from 400 pregnancies per 1000 married women aged <30 years to 100 per 1000 married women aged 45 years or older (Menken et al., 1986; Heffner, 2004). In our study, the clinical pregnancy rate for women aged less than 30 years was 321 pregnancies per 1000 initiated cycles, and decreased to 19 pregnancies per 1000 initiated cycle for women aged 45 years or older.

Advancing woman's age not only leads to declining fertility, but also pregnancy complications and spontaneous abortion (Nybo Andersen et al., 2000; Heffner,

2004). Nybo Andersen reported that the spontaneous abortion rate in women aged in their 20s and early 30s was about 13%, but increased to 25% in women aged 35–39 years, 50% in 40–44 years old women, and more than 90% in women aged 45 or older (Nybo Andersen et al., 2000). Consistent with a Centers for Disease Control report (CDC, 2006), this study found that the rate of spontaneous abortion in women aged less than 35 years is similar to Andersen's study, but in women aged 35 years or older, it is lower than in Andersen's study. One explanation is that intensive antenatal care was more common in ART pregnancies compared to natural pregnancies (Koivurova et al., 2002).

This study indicates that couples with any female factor infertility had lower success rates compared to those with male factor only infertility. As other studies have found, the lower occurrence of pregnancy was most likely associated with the pathological characteristics of women with female factor causes of infertility, such as tubal disease, endometriosis or hormonal disorders (Wisanto et al., 1995; Govaerts et al., 1998). Tan and colleague's study also suggested that, even when a pregnancy is achieved, female factor infertility would lead to a higher risk for hypertension and bleeding, which can in turn reduce the chance of live delivery (Tan et al., 1992). In women with normal fertility, once male factors are overcome, a better chance of pregnancy and live delivery can be expected.

The models show the effect on the number of live deliveries when treatment is 1, 2 or 3 years earlier. It reconfirms the need for primary care clinicians to refer

couples for fertility assessment and treatment as soon as possible. If women aged 35 years or older had a first autologous fresh cycle 1 year earlier, the number of expected live deliveries would increase by 15% compared with the observed number. If they had treatment 2 or 3 years earlier, 30 and 44% increases in live deliveries would be expected. However, this study was unable to model the likelihood of spontaneous pregnancy among women who had had their first autologous fresh treatment, and hence the models would overestimate the predicted effects of earlier treatment. Several studies have reported spontaneous pregnancy in subfertile couples (Dunson et al., 2004; Hunault et al., 2004; van der Steeg et al., 2007). Van Der Steeg et al. found that 20% of subfertile couples had a spontaneous pregnancy in the 1 year follow-up with around 18% resulting in a live delivery. Hunault et al. and Dunson et al. found that in subfertile couples, advancing woman's age is independently associated with decreasing spontaneous pregnancy. These studies also indicate that couples should be encouraged to seek fertility assessment earlier.

This study retrospectively investigated age-specific success rates at a population level. Because of the large study population and the national coverage, bias would be less than that experienced at the clinic level. One limitation of this study is that the duration of subfertility and intention to treat are not available in ANZARD. Longer duration of subfertility would independently lead to decreases in rates of spontaneous pregnancy and ART related pregnancy (Templeton et al., 1996; te Velde et al., 2000; Dunson et al., 2004; Hunault et al., 2004). For women who have failed to conceive naturally

over a 1 year period, the probability of spontaneous pregnancy in the subsequent year is 49%, and it declines to 14% for women who have not had pregnancy over a 3 year period (te Velde et al., 2000). Templeton et al. found a significant decrease in age-adjusted live birth rate per treatment cycle with increasing duration of infertility between 1 and 12 years.

Another limitation of this study is the potential variability in case reporting. The follow-up information on pregnancy and birth outcomes was collected in a number of ways including follow-up by the treatment doctors and self-reported by patients to fertility clinics. During 2002–2005, information on pregnancy and birth outcomes was not stated for 0.1% of first autologous fresh cycles.

The effectiveness of first autologous fresh ART treatment declines with advancing woman's age. There has been debate in Australia about whether age restriction should be introduced. The Australian policy recommends that it is not clinically appropriate to initiate a new cycle of in IVF treatment in women using their own oocytes at 44 years and over (Australian Government Department of Health Aging, 2006). The Practice Committee of the American Society for Reproductive Medicine (2006) suggests that evaluation and treatment of infertility should not be delayed in women 35 years and older. This study shows that, when the first autologous fresh treatment is at 43 years of age, 6% of initiated cycles result in a clinical pregnancy, but nearly 60% of those pregnancies end in spontaneous abortions. From the unadjusted age-specific rates in this study, first autologous fresh treatment at 42 years would give an

overall 8% rate of clinical pregnancies per initiated cycle, and proportionally more pregnancies resulting in a delivery rather than a spontaneous abortion. However, woman's age is not the only independent factor in predicting the success of ART treatment. A decision to treat and subsequent outcome also depends on the duration and type of subfertility, the chance of spontaneous pregnancy, and the result of fertility assessment, including post-coital and ovarian reserve testing (Hunault et al., 2004; Tufan et al., 2004; Ludwig et al., 2005).

The results of this study suggest that couples should be encouraged not to delay childbearing, because the chances of becoming pregnant by ART treatment or spontaneously decline every year, especially after age 35 years. Women aged 35 years or older who want to have a baby should be encouraged to seek a fertility assessment as early as possible and treatment where clinically indicated.

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Table I: Pregnancies and live deliveries of initiated first autologous fresh cycles by woman's age and cause of infertility Australia, 2002–2005

Woman's age (years)	All women[a]			Cause of infertility							
				Male factor only infertility		Female factor only infertility		Combined male- female factor infertility		Un-explained infertility	
	N[b]	%[c]	%[d]	n[b]	%[d]	n[b]	%[d]	n[b]	%[d]	n[b]	%[d]
≤ 21	125	24.8	17.6	65	21.5	21	23.8	18	5.6	12	0.0
22	124	28.2	21.8	49	30.6	35	14.3	24	12.5	9	11.1
23	181	35.9	28.2	76	31.6	42	26.2	33	21.2	21	23.8
24	304	35.2	30.3	111	29.7	71	28.2	54	33.3	40	22.5
25	508	28.9	24.8	184	25.0	140	22.1	80	25.0	71	26.8
26	726	30.2	24.8	249	31.7	199	19.1	145	21.4	87	29.9
27	969	33.2	29.2	346	31.8	273	26.4	157	23.6	133	35.3
28	1315	33.8	28.6	447	27.3	370	27.8	230	27.0	201	32.3
29	1713	32.0	26.4	575	26.3	486	27.0	281	25.3	273	27.1
30	2058	32.9	27.0	679	28.9	617	28.4	328	21.3	317	27.1
31	2477	32.7	27.2	787	28.5	742	28.0	374	21.1	433	27.0
32	2738	31.4	26.9	816	26.7	804	25.2	417	24.9	525	28.6
33	2694	31.4	26.0	851	27.4	763	25.2	409	25.4	503	24.1
34	2748	29.0	23.3	814	24.0	820	21.2	379	24.3	571	25.2
35	2587	27.7	23.1	763	24.9	774	21.1	348	21.3	551	24.5
36	2492	26.9	21.3	724	21.4	737	21.3	361	16.6	525	24.8
37	2243	22.2	16.6	640	16.1	672	16.1	331	14.8	458	18.8
38	2163	22.8	16.8	596	16.6	631	15.1	325	13.8	475	18.5
39	2107	19.4	13.8	595	14.1	602	11.1	301	14.3	469	15.6
40	1759	16.7	10.7	424	11.1	516	8.7	261	8.8	424	13.4
41	1469	12.3	7.4	366	6.6	476	7.1	208	6.7	312	8.7
42	1159	7.9	3.9	268	3.4	391	4.6	173	1.7	234	3.8
43	782	6.0	2.0	177	0.6	236	3.0	111	0.9	192	1.6
44	500	4.4	1.2	100	1.0	187	1.1	67	1.5	107	1.9
≥45	471	1.9	0.2	82	0.0	171	0.0	76	0.0	101	0.0
Total	**36412**	**25.7**	**20.4**	**10784**	**22.0**	**10776**	**19.2**	**5491**	**18.4**	**7044**	**20.9**

[a] Include women whose cause of infertility were not stated.

[b] Number of initiated cycles.

[c] Clinical pregnancies per initiated cycle.

[d] Live deliveries per initiated cycle.

Table II: Chance of pregnancy and live delivery of initiated first autologous fresh cycle Australia, 2002–2005

Woman's age (years)	%	OR (99% CI)	AOR[a] (99% CI)
		Clinical pregnancies per initiated cycle	
Age ≥30	—	0.89 (0.88-0.90)	0.89 (0.88-0.90)
25-29 (ref)	*32.1*	*1.00*	*1.00*
30-34	31.4	0.97 (0.88-1.06)	0.96 (0.88-1.05)
35-39	24.0	0.67 (0.61-0.73)	0.66 (0.60-0.73)
40-44	11.2	0.27 (0.23-0.30)	0.26 (0.23-0.30)
≥45	1.9	0.04 (0.02-0.10)	0.04 (0.02-0.10)
		Live deliveries per initiated cycle	
Age ≥30	—	0.87 (0.86-0.88)	0.87 (0.86-0.88)
25-29 (ref)	*27.1*	*1.00*	*1.00*
30-34	26.0	0.95 (0.86-1.04)	0.94 (0.86-1.04)
35-39	18.6	0.62 (0.57-0.68)	0.61 (0.55-0.68)
40-44	6.4	0.18 (0.16-0.22)	0.18 (0.16-0.22)
≥45	0.2	0.01 (0.01-0.07)	0.01 (0.01-0.08)

[a] Adjusted for type of infertility (male factor only infertility, female factor infertility, combined male-female infertility, unexplained and not stated).

Table III: Predicted number of live deliveries of initiated first autologous fresh cycles in women aged 35 to 44 years Australia, 2002–2005

		One year earlier[a]		Two years earlier[b]		Three years earlier[c]	
Woman's age (years)	Observed live deliveries	Predicted live deliveries	Estimated extra live deliveries	Predicted live deliveries	Estimated extra live deliveries	Predicted live deliveries	Estimated extra live deliveries
35	597	686.9	89.9	727.3	130.3	750.9	153.9
36	532	569.7	37.7	618.6	86.6	654.2	122.2
37	373	438.4	65.4	489.1	116.1	530.6	157.6
38	363	359.2	-3.8	412.2	49.2	459.4	96.4
39	290	295.7	5.7	349.4	59.4	400.6	110.6
40	188	207.6	19.6	252.8	64.8	298.4	110.4
41	108	145.2	37.2	182.4	74.4	221.8	113.8
42	45	95.6	50.6	123.9	78.9	155.4	110.4
43	16	53.7	37.7	71.8	55.8	92.9	76.9
44	6	28.5	22.5	39.3	33.3	52.5	46.5
Total	**2518**	**2880.5**	**362.5**	**3266.8**	**748.8**	**3616.7**	**1098.7**

[a] Women aged 35-44 years have had treatment at 34-43 years (one year younger at each age).

[b] Women aged 35-44 years have had treatment at 33-42 years (two years younger at each age).

[c] Women aged 35-44 years have had treatment at 32-41 years (three years younger at each age).

Figure 1: Pregnancy outcome following first autologous fresh cycles by woman's age Australia, 2002–2005

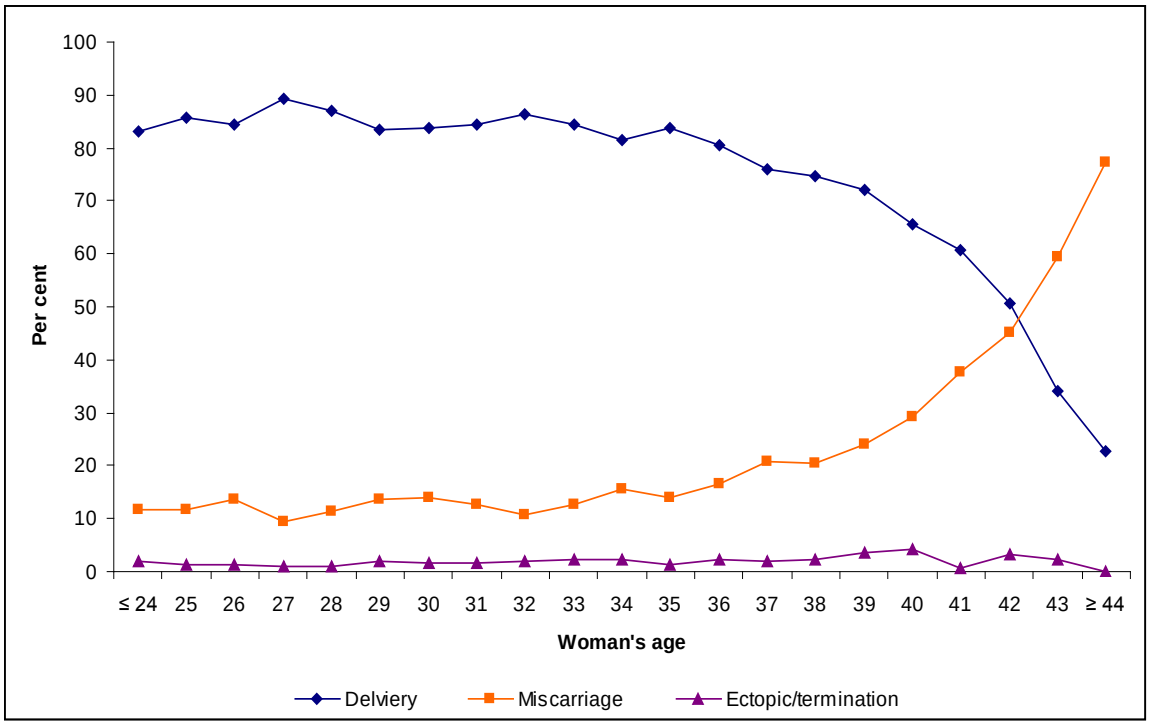
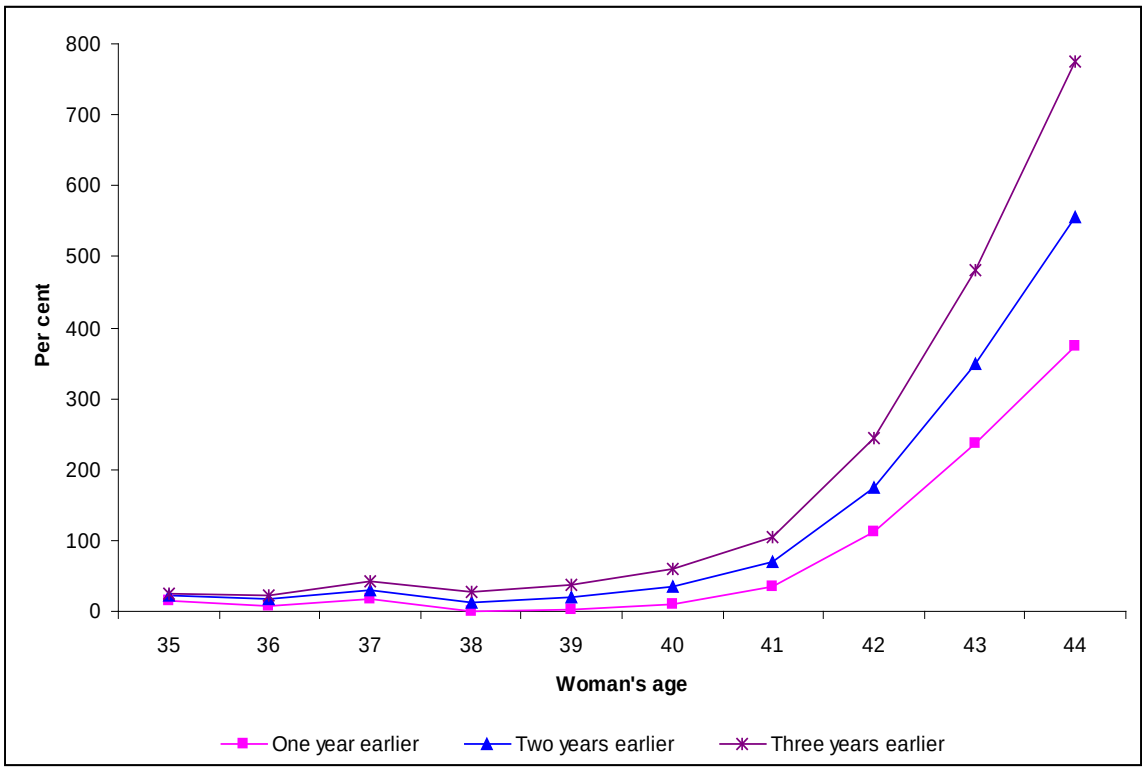


Figure 2: Percentage of extra live deliveries predicted of initiated first autologous fresh cycles in women aged 35 to 44 years Australia, 2002–2005



Chapter 4

Study 2:

Perinatal outcomes after assisted reproduction technology treatment in Australia and New Zealand: single- versus double-embryo transfer

About this chapter

The impact of each additional year of age on pregnancy and live delivery was presented in the previous chapter. Along with woman's age, the number of embryos transferred was one of most important treatment factors related to pregnancy and birth outcomes. Transfer of two or more embryos significantly increased the risk of multiple gestation pregnancy which is responsible for the majority of adverse pregnancy and birth outcomes. Implementation of a policy of SET has successfully reduced the rates of multiple pregnancy and birth in some countries. This chapter presents the second study of this PhD thesis "Perinatal outcomes after assisted reproduction technology treatment in Australia and New Zealand: single- versus double-embryo transfer". This study evaluated the SET policy in Australia and New Zealand by comparing perinatal outcomes of babies following SET and DET. It confirmed that SET not only reduced the multiple pregnancy and birth rates, but also improved the perinatal outcomes for singletons. It suggested that continuously encouraging SET would benefit women and their babies, as well as the community as a whole.

This study was presented at the FSA 2009 annual meeting in Perth, Australia. The manuscript of this study was published in Medical Journal of Australia in 2009.

List of presentation and publication from this study:

Oral presentation. Perinatal outcomes after assisted reproductive technology treatment in Australia and New Zealand: SET versus DET. The annual conference of Fertility Sociality of Australia. Perth, Australia (25-28 Oct 2009).

Wang YA, Sullivan EA, Healy DL, Black DA. Perinatal outcomes after assisted reproductive technology treatment in Australia and New Zealand: single versus double embryo transfer. Med J Aust. 2009 Mar 2;190(5):234-7.

Abstract

Objective: To compare the perinatal outcomes of babies conceived by single embryo transfer (SET) with those conceived by double embryo transfer (DET). **Design, setting and participants:** A retrospective population-based study of embryo transfer cycles in Australia and New Zealand between 2002 and 2006, using data from the Australia and New Zealand Assisted Reproduction Database. **Main outcome measures:** Proportion of SET procedures; comparison of SET and DET procedures with respect to multiple births, low birthweight (LBW), preterm birth and fetal death. **Results:** The proportion of SET procedures has increased from 28.4% in 2002 to 32.0% in 2003, 40.5% in 2004, 48.2% in 2005 and 56.9% in 2006. The multiple birth rate for all babies conceived by SET (4.0%) was 10 times lower than for those conceived by DET (39.1%) (P <0.01). The average birthweight for all liveborn babies conceived by SET (3290 g) was higher than for those conceived by DET (2934 g) (P <0.01). The preterm birth rate of all DET-conceived babies (30.3%) was higher than for SET-conceived babies (12.3%) (adjusted odds ratio [AOR], 3.19 [95% CI, 3.01–3.38]). All babies conceived by DET were more likely to be stillborn than those conceived by SET (AOR, 1.49 [95% CI, 1.21–1.82]). Singletons conceived by DET were more likely to be born preterm than singletons conceived by SET (AOR, 1.13 [95% CI, 1.05–1.22]). Liveborn singletons conceived by DET were 15% more likely to have LBW than liveborn singletons conceived by SET (AOR, 1.15 [95% CI, 1.05–1.26]). There was no significant difference in fetal death rate between

DET- and SET-conceived singletons. **Conclusion:** The increase in proportion of SET procedures has resulted in a lower rate of multiple births and in better perinatal outcomes in Australian and New Zealand assisted reproduction programs.

Introduction

Since 2002, the Reproductive Technology Accreditation Committee in Australia and New Zealand has advocated reducing the number of embryos transferred to women undergoing assisted reproductive technology (ART) treatment, with the aim of minimising the number of multiple births.[1] Studies suggest that the high incidence of multiple births following ART is responsible for most adverse perinatal outcomes (preterm birth, low birthweight [LBW] and perinatal death).[2-4]

The high rate of multiple births following ART is largely explained by the number of embryos transferred. Transferring two embryos (double embryo transfer [DET]) increases the odds of a multiple gestation by more than 60 times[5] compared with single embryo transfer (SET). Recent studies from Finland concluded that babies conceived by SET had better neonatal outcomes than those conceived after transferring two or more embryos.[6] This is the case even among singletons.[7,8] Belgium and the Scandinavian countries have endorsed SET as best practice, with Sweden reporting that SET is used in 70% of its embryo transfer cycles.[9]

The Assisted reproduction technology in Australia and New Zealand 2006 report[10] showed that the proportion of embryo transfer cycles using SET had increased from 28.4% in 2002 to 56.9% in 2006 in Australia and New Zealand. Over the same period, the twin rate declined from 18.8% to 11.3%.

The aim of our study was to compare the perinatal outcomes of babies conceived by SET with those conceived by DET using data from the Australia and New Zealand Assisted Reproduction Database (ANZARD).

Methods

Data source

ANZARD is housed at the Australian Institute of Health and Welfare (AIHW) National Perinatal Statistics Unit. Data from fertility centres in Australia and New Zealand, including information on each treatment cycle commenced, pregnancy and birth outcomes, are validated and entered into the database at the National Perinatal Statistics Unit. Data collection on pregnancy and neonatal outcomes varies between fertility centres but includes follow-up with the patient or clinician and the use of routine data from the relevant health department. Information on pregnancy outcomes and neonatal outcomes is missing for about 2% of clinical pregnancies recorded in ANZARD.

We carried out a retrospective analysis of ANZARD data on embryo transfer cycles and subsequent pregnancy and baby outcomes for the period 2002–2006.

Outcome measures and definition of terms

Maternal age: Maternal age was expressed as the number of completed years at the time of ART treatment.

Type of embryo: Embryos were classified as fresh (never frozen) or thawed (following cryopreservation).

Stage of embryo development: Embryos were classified as cleavage-stage embryos or blastocysts.

Gestational age: Defined as the number of completed weeks of gestation of the fetus, gestational age was calculated from the formula [(pregnancy end date minus embryo transfer date) plus 16 days].

Perinatal outcomes: Outcomes examined were LBW (birthweight <2500 g in liveborn babies), preterm birth (gestational age <37 completed weeks) and fetal death (stillbirth) (number of fetal deaths per 1000 births).

Statistical analysis

The number and proportion of SETs and DETs were stratified by women's age (<35 years, 35–39 years or ≥40 years) and described by year. SET and DET were compared with respect to perinatal outcomes for all babies, with t tests used for continuous variables and $\chi 2$ tests used for categorical variables. Univariate and multivariate logistic regression analyses were used to examine the likelihood of adverse perinatal outcomes. Data were analysed using SPSS software, version 16.0 (SPSS Inc, Chicago, Ill, USA).

Ethics approval

Our study was approved by the Human Research Ethics Committee of the University of New South Wales.

Results

Number of embryos transferred

In Australia and New Zealand over the period 2002–2006, there were 172 190 cycles in which embryos were transferred: 73 563 SET cycles (42.7%), 93 429 DET cycles (54.3%) and 5198 cycles (3.0%) in which three or more embryos were transferred. Women aged 40 years or older tended to have a lower proportion of SETs compared with younger women.

The overall proportion of SETs increased from 28.4% in 2002 to 32.0% in 2003, 40.5% in 2004, 48.2% in 2005 and 56.9% in 2006. Between 2002 and 2006, the proportion of SETs increased from 28.7% to 66.2% for women aged <35 years, from 27.5% to 54.4% for women aged 35–39 years, and from 29.1% to 42.5% for women aged ≥40 years.

Perinatal outcomes

Over the period 2002–2006, 40 483 babies (73.0% singletons, 26.3% twins, 0.7% triplets or higher) were born to women who had either SET or DET cycles in Australia and New Zealand. Of these babies, 14 022 (34.6%) were conceived by

SET and 26 461 (65.4%) by DET. The multiple birth rate for babies born to women who had SET cycles (4.0%) was about 10 times lower than for those born to women who had DET cycles (39.1%) ($P < 0.01$; $\chi 2 = 5768.3$; df = 1) (Box 1).

Box 1

The overall preterm birth rate was 24.1%. The mean birthweight of liveborn babies was 3058 g, with 19.2% of babies having LBW. There were 520 fetal deaths (12.8 fetal deaths per 1000 births) after either SET or DET.

Type of embryo

Liveborn babies conceived after thawed embryo transfer cycles had a significantly lower rate of LBW than those conceived after fresh embryo transfer cycles, regardless of whether they were SET or DET. Similarly, the rate of preterm birth was significantly lower for babies conceived after thawed embryo transfer cycles than after fresh embryo transfer cycles. The fetal death rate was lower after thawed embryo transfer cycles than after fresh embryo transfer cycles for DET babies but not SET babies (Box 2).

Box 2

Stage of embryo development

About 5% of babies conceived by single blastocyst transfer were multiples, which is significantly higher than the 3.4% for babies conceived by single cleavage-stage embryo transfer (P <0.01). The multiple birth rate was 42.1% for babies conceived by double blastocyst transfer compared with 38.7% for babies conceived by double cleavage-stage embryo transfer (P <0.01).

Babies conceived by blastocyst transfer had a significantly lower rate of LBW and preterm birth than those conceived by cleavage-stage embryo transfer. Babies conceived by single blastocyst transfer had slightly higher rates of preterm birth and fetal death than those conceived by single cleavage-stage embryo transfer. The fetal death rate was 15.2 per 1000 births for babies after double blastocyst transfer cycles, compared with 14.3 per 1000 births for babies after double cleavage-stage embryo transfer cycles, but the difference between the two was not significant (Box 3).

Box 3

Birthweight

The mean birthweight for all liveborn babies conceived by SET (3290 g) was significantly higher than the mean for those conceived by DET (2934 g) (P <0.01). Term liveborn singletons conceived by SET had significantly higher mean birthweight (3430 g) than those conceived by DET (3401 g), regardless of sex (P <0.01). There was no significant difference between SET- and DET-

conceived babies in mean birthweight of liveborn singletons born at 32–36 weeks of gestation (Box 4).

Box 4

Liveborn babies conceived by DET were 3.6 times more likely to have LBW than those conceived by SET (Box 5). Liveborn singletons conceived by DET had a significantly lower mean birthweight (3287 g) than singletons conceived by SET (3332 g) (P <0.01). Consistent with this, liveborn singletons conceived by DET were 15% more likely to have LBW than singletons conceived by SET (adjusted odds ratio [AOR], 1.15 [95% CI, 1.05–1.26]) (Box 5).

Box 5

Preterm birth

The preterm birth rate of all babies conceived by DET was 30.3%, compared with a rate of 12.3% for babies conceived by SET. Singletons conceived by DET were 13% more likely to be born preterm than those conceived by SET (Box 5).

Fetal death

Babies conceived by DET were more likely to be stillborn than those conceived by SET (AOR, 1.49 [95% CI, 1.21–1.82]). The fetal death rate for singletons conceived by DET (10.9 deaths/1000 births) was slightly higher than the rate for

singletons conceived by SET (8.6 deaths/1000 births), but the difference was not significant (Box 5).

Perinatal outcomes of multiples

Unlike singletons, multiples conceived by DET had better perinatal outcomes than those conceived by SET (Box 5). However, more than half of liveborn multiples (whether conceived by DET or SET) were LBW and 60% of multiples were born preterm.

Discussion

Consistent with other studies,[6,8,11] we found that babies conceived by SET had better perinatal outcomes than those conceived by DET. This was the case even for singletons. Regardless of maternal age, DET-conceived singletons had 15% greater odds of LBW and 13% greater odds of preterm birth than SET-conceived singletons.

Our analysis showed that the multiple birth rate for babies conceived by DET was nearly 10-fold higher than for babies conceived by SET. Multiple births are strongly associated with poorer perinatal outcomes.[4,12] LBW and preterm birth have been shown to be directly related to fetal and neonatal death, and short- and long-term morbidity and mortality.[13,14]

The rationale for favouring DET over SET, until recently, has been that the transfer of two or more embryos results in a higher clinical pregnancy rate than the transfer of only one embryo, especially in younger women.[15] However, a higher clinical pregnancy rate does not mean better perinatal outcomes for the baby.

Multiple gestations not only increase the risk for babies, but also for mothers.[16,17] Threatened miscarriage, hyperemesis, thromboembolism, hypertension, haemorrhage and maternal mortality are all significantly increased in multiple gestations.[17] There is also a greater risk of depression and marital decline after multiple gestations.[16]

In addition, multiple gestations place a greater economic and social burden on the parents and on the health care system.[18,19] In Australia in 2003, the average combined cost of infant and maternal birth admission following ART treatment was $8053 for singletons compared with $23 214 for twins and $90 742 for higher-order multiples.[20]

One of the findings of our study was that preterm birth and LBW were less likely to occur in babies conceived by thawed embryo transfer than fresh embryo transfer. This is consistent with other studies[3,21] showing that cryopreservation does not adversely affect fetal development or perinatal outcomes, but in fact appears to have a protective effect. The better outcomes of babies conceived from frozen embryos are likely to be related to the more

similar ovarian and uterine conditions in thawed embryo transfer cycles to those for non-ART conceptions.[21]

Twins conceived by SET are considered to be monozygotic twins.[22] In general, monozygotic twins have worse perinatal outcomes than dizygotic twins.[23] Consistent with other studies,[22] our data showed a significantly higher rate of twins following blastocyst transfer (both SET and DET) than cleavage-stage embryo transfer. But overall outcomes were more favourable for babies following blastocyst transfer than cleavage-stage embryo transfer. This may largely be explained by more DET in cleavage-stage embryo transfer cycles than in blastocyst transfer cycles, and hence an overall higher multiple birth rate following cleavage-stage embryo transfer cycles. A significantly lower LBW rate was observed for singletons following blastocyst transfer in our study, which supports the suggestion that the blastocyst culture could have had an impact on higher birthweight.[24]

Strengths of our study were the large study population and the two-nation coverage. One limitation was that elective embryo transfer procedures could not be distinguished from all embryo transfer procedures. (A Belgian study has shown that babies conceived by elective SET have better perinatal outcomes than those conceived by DET.[25]) Another limitation of our study was the potential variability in case reporting. The follow-up information on pregnancy and birth outcomes was collected in a number of ways, including follow-up by the treatment doctors and self-report by patients to fertility clinics. A further

limitation was that data on the 2002–2006 study population were incomplete: information on pregnancy and birth outcomes was not stated for 1.7% of clinical pregnancies.

Our study confirms that the Reproductive Technology Accreditation Committee's policy of shifting towards greater use of SET in ART is working. In 2006, more than half of embryo transfer cycles were SETs, resulting in a fall in the multiple delivery rate to 12%, the lowest rate ever reported in Australia and New Zealand. Given the fewer maternal complications, lower rate of adverse perinatal outcomes and higher cost-effectiveness ratio for SET compared with DET, continuing to encourage SET will benefit women and their babies, as well as society in general.

Acknowledgements

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Box 1: Babies born to women who had SET or DET, Australia and New Zealand, 2002-2006

	SET		DET		Total	
	No.	%	No.	%	No.	%
Singleton	13468	96.0	16100	60.9	29568	73.0
Multiple	554	4.0	10361	39.1	10915	27.0
- Twin	532	3.8	10090	38.1	10622	26.3
- Triplets or higher	22	0.2	271	1.0	293	0.7

DET = double embryo transfer. SET = single embryo transfer.

Box 2: Perinatal outcomes of babies born to women who had SET or DET by type of embryo, Australia and New Zealand, 2002-2006

Number and type of embryo	Total liveborn babies	Low birthweight of liveborn babies		Total babies	Preterm birth of all babies		Foetal death of all babies	
	No.	Per cent	P value*	No.	Per cent	P value*	Per 1000 births	P value*
SET								
Fresh embryo	9121	9.5		9229	12.9		10.1	
			<0.01			<0.01		0.79
Thawed embryo	4736	7.3		4793	11.2		9.6	
DET								
Fresh embryo	18402	27.7		18737	32.5		16.1	
			<0.01			<0.01		<0.01
Thawed embryo	7620	17.7		7724	25.0		10.4	
ALL								
Fresh embryo	27523	21.7		27966	26.0		14.1	
			<0.01			<0.01		<0.01
Thawed embryo	12356	13.7		12517	19.7		10.1	

DET = double embryo transfer. LBW = low birthweight. SET = single embryo transfer. * $\chi 2$ test, df = 1.

Box 3: Perinatal outcomes of babies born to women who had SET or DET by stage of embryo development, Australia and New Zealand, 2002-2006

Number and stage of embryo development	Total liveborn babies	Low birthweight of liveborn babies		Total babies	Preterm birth of all babies		Foetal death of all babies	
	No.	Per cent	P value*	No.	Per cent	P value*	Per 1000 births	P value*
SET								
Cleavage stage	8877	8.6	0.61	8971	12.0	0.08	8.7	0.05
Blastocyst	4980	8.9		5051	13.0		12.1	
DET								
Cleavage stage	22083	24.9	0.55	22447	30.4	0.36	14.3	0.64
Blastocyst	3939	24.3		4014	29.7		15.2	
ALL								
Cleavage stage	30960	20.2	<0.01	31418	25.1	<0.01	12.7	0.55
Blastocyst	8919	15.7		9065	20.4		13.5	

DET = double embryo transfer. LBW = low birthweight. SET = single embryo transfer. * $\chi 2$ test, df = 1.

Box 4: Birthweight of liveborn singletons to women who had SET compared to DET, Australia and New Zealand, 2002-2006

	SET		DET		P value (T-test)
	N	Mean birthweight (SD)	N	Mean birthweight (SD)	
Female singletons					
<32 weeks	98	1483.3 (900.0)	146	1225.0 (757.1)	0.02
32-36 weeks	465	2559.4 (548.3)	614	2518.7 (574.3)	0.24
≥37 weeks	5835	3364.6 (467.7)	7071	3333.1 (475.5)	<0.01
Male singletons					
<32 weeks	115	1367.3 (752.4)	168	1264.5 (703.3)	0.24
32-36 weeks	569	2631.7 (557.1)	721	2616.9 (560.6)	0.64
≥37 weeks	6117	3492.8 (485.3)	7021	3469.3 (493.0)	0.01
All singletons					
<32 weeks	214	1422.4 (822.1)	315	1247.1 (727.0)	0.01
32-36 weeks	1034	2599.2 (554.0)	1337	2571.6 (568.6)	0.24
≥37 weeks	11953	3430.2 (481.1)	14105	3400.8 (489.2)	<0.01

DET = double embryo transfer. SET = single embryo transfer. * ***t*** test.

Box 5: Perinatal outcomes of babies born to women who had SET compared to DET, Australia and New Zealand, 2002-2006

	SET	DET		
Perinatal Outcomes	**(%)**	**(%)**	**OR (95% CI)**	**AOR* (95% CI)**
All babies				
Low birthweight of liveborn babies	8.7	24.8	3.44 (3.23-3.68)	3.55 (3.32-3.80)
Preterm birth of all babies	12.3	30.3	3.10 (2.92-3.28)	3.19 (3.01-3.38)
Foetal death of all babies†	9.9	14.4	1.46 (1.20-1.77)	1.49 (1.21-1.82)
Singletons				
Low birthweight of liveborn singletons	6.6	7.9	1.21 (1.11-1.32)	1.15 (1.05-1.26)
Preterm birth of all singletons	10.1	11.3	1.14 (1.06-1.23)	1.13 (1.05-1.22)
Foetal death of all singletons†	8.6	10.9	1.27 (1.00-1.60)	1.26 (0.98-1.62)
Multiples				
Low birthweight of liveborn multiples	61.4	51.2	0.66 (0.55-0.79)	0.60 (0.50-0.72)
Preterm birth of all multiples	67.1	59.8	0.72 (0.60-0.87)	0.66 (0.55-0.80)
Foetal death of all multiples†	41.5	19.9	0.47 (0.30-0.73)	0.46 (0.29-0.73)

AOR = adjusted odds ratio. DET = double embryo transfer. OR = odds ratio. SET = single embryo transfer. * Adjusted for women's age, cause of infertility, parity, number of previous assisted reproductive technology treatments, type of embryo, and stage of embryo development. † Number of fetal deaths per 1000 births.

Chapter 5

Study 3:

Better perinatal outcomes following transfer of fresh blastocysts and blastocysts cultured from thawed cleavage embryos: a population-based study

About this chapter

The optimum outcome of an ART treatment is a healthy baby. Woman's age, number of embryos transferred and stage of embryo development interactively impact on the likelihood of the birth of a healthy baby. Woman's age and number of embryos transferred were investigated in previous two chapters. This chapter presents the third study of this PhD thesis "Better perinatal outcomes following transfer of fresh blastocysts and blastocysts cultured from thawed cleavage embryos: a population-based study". The third study of this PhD thesis has developed a newly proposed indicator "healthy baby" (term liveborn singleton of normal birthweight without congenital anomaly) as the optimum goal for ART treatment. Using the "healthy baby" indicator, this study assessed the efficacy of embryo transfers at cleavage or blastocyst stage for both fresh and thaw cycles by woman's age and number of embryos transferred. It proposed that for younger patients, an optimum clinical practice model to maximize the likelihood of the birth of a "healthy baby" is the transfer of a single blastocyst and freezing of cleavage embryos in fresh cycles and the subsequent transfer of a single blastocyst cultured from these thawed cleavage embryos.

This study was presented at the ESHRE 2008 annual meeting in Barcelona, Spain. The manuscript of this study was published at Human Reproduction in 2010.

List of presentation and publication from this study:

Oral presentation. What type of transferred embryo gives the best perinatal outcome. The 24th Annual Meeting of ESHRE. Barcelona, Spain (5-9 Sep 2008)

Wang YA, Chapman M, Costello M, Sullivan EA. Better perinatal outcomes following transfer of fresh blastocysts and blastocysts cultured from thawed cleavage embryos: a population-based study. Hum Reprod. 2010 Jun;25(6):1536-42. doi: 10.1093/humrep/deq067.

Abstract

BACKGROUND: Fresh embryo transfer results in higher live birth rates, while thawed embryo transfer appears to result in healthier babies. This study aims to investigate the association between the transfer of fresh or thawed embryos at the cleavage or blastocyst stage and the perinatal outcomes. **METHODS:** This analysis is a retrospective population-based study of 150 376 autologous embryo transfer cycles in Australia during 2002–2006. The rates of pregnancy, live delivery and "healthy baby" delivery (a single baby born live at term, weighing ≥2500 g, surviving for at least 28 days post birth and not having congenital anomalies) were compared after transfer of fresh cleavage embryos, fresh blastocysts, thawed cleavage embryos, blastocysts from thawed cleavage embryos and thawed blastocysts. **RESULTS:** The live delivery rate was significantly higher for transfer of fresh blastocysts (27.9%) than for blastocysts cultured from thawed cleavage embryos (22.0%), fresh cleavage embryos (21.7%), thawed blastocysts (16.3%) and thawed cleavage embryos (15.2%). Compared with the transfer of fresh blastocysts, the likelihood of a "healthy baby" was significantly lower for blastocysts from thawed cleavage embryos [adjusted odds ratios (AOR) 0.73, 95% confidence intervals (CI) 0.65–0.82], fresh cleavage embryos (AOR 0.67, 95% CI 0.64–0.69), thawed blastocysts (AOR 0.57, 95% CI 0.53–0.62) and thawed cleavage embryos (AOR 0.53, 95% CI 0.51–0.56). Of thaw cycles, transfers of thawed blastocysts (AOR 0.79, 95% CI 0.70–0.89) and thawed cleavage embryos (AOR 0.71, 95% CI 0.63–0.79) had significantly

lower odds of “healthy baby” than transfer of blastocysts from thawed cleavage embryos. **CONCLUSIONS:** These data suggest that an optimum practice model to maximize the outcomes of the birth of a “healthy baby” is the transfer of blastocysts and the freezing of cleavage embryos in fresh cycles and subsequent transfer of blastocysts cultured from these thawed cleavage embryos.

Introduction

In the past three decades, assisted reproductive technology (ART) treatment has evolved numerous variations in clinical practice to achieve a pregnancy. In a typical ART cycle, fresh cleavage stage embryo(s) are transferred (Stepoe and Edwards, 1978; Mettler et al., 1984). In recent years, with the merits of cryopreservation and blastocyst culture, fresh blastocyst(s), thawed cleavage stage embryo(s) and thawed blastocyst(s) have also been transferred (Jones et al., 1998; Centers for Disease Control and Prevention, 2008; Nyboe Andersen et al., 2008; Wang et al., 2009).

The outcome of embryo transfers in terms of clinical pregnancy and live delivery are associated with various patient demographic and treatment factors. Studies show that a number of patient demographic characteristics, such as patient age, parity, cause and duration of subfertility are independently associated with pregnancy following ART treatment (Bai et al., 2002; ESHRE Capri Workshop Group, 2004; Baird et al., 2005). The literature also demonstrates that the transfer of more than one embryo results in a higher pregnancy rate compared with the transfer of only one embryo (Giannini et al., 2004; Pandian et al., 2005). Recently published studies also found that, after adjustment for patient demographics and number of embryos transferred, the pregnancy and live delivery rates are higher for the transfer of blastocyst embryos compared with cleavage stage embryos (Papanikolaou et al., 2008) and also higher for the transfer of fresh compared with frozen/thawed embryos

(Human Fertilization and Embryology Authority, 2007; Centers for Disease Control and Prevention, 2008; Wang et al., 2009).

However, in relation to baby outcomes, recent evidence suggests that better perinatal outcomes follow the transfer of frozen/thawed embryos rather than fresh embryos (Wang et al., 2005; Shih et al., 2008). Babies conceived by the transfer of thawed embryos may have significantly lower rates of preterm birth and perinatal mortality than those following the transfer of fresh embryos (Shih et al., 2008). Live born babies are also less likely to be low-birthweight following the transfer of thawed compared with fresh embryos (Wang et al., 2005).

It has been advocated that the outcome of ART treatment needs to focus on 'Birth Emphasizing a Successful Singleton at Term' (Healy, 2004). Our study developed a newly proposed indicator the "healthy baby" as the optimum outcome of ART treatment. A "healthy baby" was defined as a single baby born live at term, weighing ≥2500 g, surviving for at least 28 days post birth and not having congenital anomalies. Our study aims to investigate the association between the rates of live delivery and "healthy baby" and the transfer of fresh or thawed embryos at the cleavage or blastocyst stage, and to suggest a practice model combining fresh and thaw cycles which could result in better perinatal outcomes.

Materials & methods

Data

Data and definitions used in this study are from the Australian and New Zealand Assisted Reproduction Database (ANZARD), located at the Perinatal and Reproductive Epidemiology Research Unit of University of New South Wales (UNSW). Data for ANZARD are collected annually, in a de-identified format, from all fertility centres within Australia and New Zealand. The ANZARD includes information on both the *in vitro* fertilization (IVF) treatment cycle [oocyte pick-up, IVF and intracytoplasmic sperm injection (ICSI) fertilization procedure, use of thawed embryos, blastocyst culture, embryo transfer and donation of gametes or embryos] and the pregnancy and birth outcomes (birth status, gestational age, birthweight and congenital anomalies).

A sub-data set on the embryo transfer cycles undertaken in Australian clinics between 1 January 2002 and 31 December 2006 and resulting pregnancy and birth outcomes were extracted from ANZARD. Mixed fresh–thawed embryo transfer cycles and mixed cleavage/blastocyst embryo transfer cycles were excluded. A total of 150 376 embryo transfer cycles, where women 18 years and older used their own oocytes (autologous cycles), were included in the final analysis.

Study factors

Transferred embryos were categorized as fresh cleavage embryo, fresh blastocyst, thawed cleavage embryo, blastocyst from thawed cleavage embryo and thawed blastocyst. The type of fertilization was classified either as IVF or ICSI. The numbers of transferred embryos were grouped as single embryo (SET), double embryo (DET) and three or more embryos.

The woman's age was calculated in completed years of age at the time of treatment. The cause of infertility was classified as male factor only, female factor only (tubal disease, endometriosis or other female factor problems were diagnosed), combined male–female factor (both male and female factor problems were diagnosed), unexplained (neither male nor female factor problems was diagnosed) or not stated. Previous pregnancy of ≥20 weeks gestation was grouped as yes, no or not stated.

Main outcome measures

Gestational age was defined as the number of completed weeks of gestation and calculated by the formula '(pregnancy end date−embryo transfer date) + 16 days' for cleavage embryo transfer and '(pregnancy end date−embryo transfer date) + 19 days' for blastocyst transfer. A clinical pregnancy was defined by one of the following criteria: evidence by ultrasound of intrauterine sac(s) and/or fetal heart(s); examination of products of conception revealing chorionic villi; an ectopic pregnancy that had been diagnosed laparoscopically or by ultrasound.

A live delivery was defined as a birth event in which one or more babies were born live at ≥20 weeks gestation, or of ≥400 g birthweight.

A newly proposed indicator, the "healthy baby", was utilized to define the best perinatal outcome. A "healthy baby" was defined as single a baby born live at term (≥37 weeks gestational age), weighing ≥2500 g, surviving for at least 28 days post birth and not having known congenital anomalies (major or minor).

Statistical analysis

Patient demographics (woman's age, cause of infertility and previous pregnancy of ≥20 weeks gestation) and treatment factors (type of fertilization and number of transferred embryos) were compared in the various embryo transfer groups, namely fresh or thawed embryos at cleavage or blastocyst stage. Chi-square test was used to test the difference in pregnancy outcomes between IVF and ICSI procedure, and between SET and DET. Pregnancy rate per transfer cycle, live delivery rate per transfer cycle and the rate of "healthy baby" per transfer cycle were calculated and compared for the various embryo transfer groups. The associations between the transfer of fresh or thawed embryos at the cleavage or blastocyst stage and the rates of live delivery and "healthy baby" were investigated in univariate and multivariate logistic regression. Odds ratios (OR), adjusted odds ratios (AOR) (adjusted for woman's age, cause of infertility, previous pregnancy of ≥20 weeks gestation, type of fertilization and number of embryo transferred) and 95% confidence intervals

(CI) were calculated. Data were analysed using SPSS software, version 17.0 (SPSS, Inc., Chicago, IL, USA).

Ethics

Ethics approval for this study was granted by the Human Research Ethics Committee of UNSW, Australia.

Results

Nearly half (46.1%) of the 150 376 autologous cycles involved the transfer of fresh cleavage embryo(s), with 33.0% thawed cleavage embryo(s), 12.9% fresh blastocyst(s), 6.2% thawed blastocyst(s) and 1.8% with transfer of blastocyst(s) from thawed cleavage embryos. Approximately 20% of fresh embryo transfer cycles were in women aged 40 years or older (21.2% for fresh cleavage embryo cycles and 18.9% for fresh blastocyst cycles), compared with <15% of thawed embryo transfer cycles (15.6% for thawed cleavage embryo cycles, 10.9% for thawed blastocyst cycles and 12.0% for cycles with transfer of blastocyst(s) cultured from thawed cleavage embryo(s)). Cycles with transfer of thawed cleavage embryo(s) had the highest proportion of female factor only infertility (37.8%). Three quarters of fresh embryo cycles were in women who never had a pregnancy of 20 weeks or more gestation (75.3% for fresh cleavage embryo cycles and 74.3% for fresh blastocyst cycles) (Table I).

More than half the blastocyst transfer cycles had an SET (55.7% of fresh blastocyst cycles, 66.4% of thawed blastocyst cycles and 60.8% of cycles with transfer of blastocyst from thawed cleavage embryo(s)). SET occurred in 35.5% of fresh cleavage embryo cycles and 43.8% of thawed cleavage embryo cycles (Table I).

Cycles with transfer of blastocyst(s) from thawed cleavage embryo(s) had the lowest proportion of ICSI procedures, followed by cycles with transfer of thawed blastocyst(s) (Table I)

Table I

The pregnancy rate per transfer cycle ranged from 20.1% for transfer of thawed cleavage embryo(s) to 35.9% for transfer of fresh blastocyst(s). Cycles with transfer of fresh blastocyst(s) resulted in the highest live delivery rate (27.9%), followed by blastocyst(s) from thawed cleavage embryo(s) (22.0%), fresh cleavage embryo(s) (21.7%), thawed blastocyst(s) (16.3%) and thawed cleavage embryo(s) (15.2%). More than one in five of the fresh blastocyst cycles resulted in a "healthy baby", compared with 16.2% in cycles with transfer of blastocyst(s) from thawed cleavage embryo(s), 14.8% in fresh cleavage embryo cycles, 13.2% in thawed blastocyst cycles and 11.7% in thawed cleavage embryo cycles (Table II).

Table II

In cycles with transfer of fresh blastocyst(s), the live delivery rate was significantly higher for IVF procedures than for ICSI procedures (29.0 versus 27.0%, *P* <0.01). However, there was no significant difference in the "healthy baby" rate between IVF and ICSI procedures with fresh or thawed embryos at cleavage or blastocyst stage (Table III).

Table III

The live delivery rate was significantly higher for DET than for SET in cycles with transfer of fresh cleavage embryo(s) (24.0 versus 18.6%, *P* <0.01), thawed cleavage embryo(s) (17.4 versus 12.4%, *P* <0.01), blastocyst(s) from thawed cleavage embryo(s) (24.9 versus 20.1%, *P* <0.01) and thawed blastocyst(s) (19.0 versus 15.0%, *P* <0.01). For cycles with transfer of fresh blastocyst(s), DET resulted in significantly lower rate of live delivery than SET (24.8 versus 30.7%, *P* <0.01). The "healthy baby" rate was significantly lower for DET than for SET in cycles with transfer of fresh cleavage embryo(s) (14.8 versus 15.5%, *P* <0.01) and fresh blastocyst(s) (15.2 versus 25.8%, *P* <0.01) (Table IV).

Table IV

Of all fresh embryo transfer cycles, transfer of fresh cleavage embryo(s) had significantly lower odds of live delivery than fresh blastocyst(s) (the group with the highest rates of live delivery and "healthy baby") (AOR 0.67, 95% CI 0.64–0.69). Compared with transfer of fresh blastocyst(s), the likelihood of a live delivery was significantly lower for blastocyst(s) from thawed cleavage

embryo(s) (AOR 0.71, 95% CI 0.64–0.79), for thawed blastocyst(s) (AOR 0.50, 95% CI 0.47–0.54) and for thawed cleavage embryo(s) (AOR 0.46, 95% CI 0.44–0.48) (Table V).

Compared with transfer of fresh blastocyst(s) the chance of achieving a "healthy baby" was significantly lower for blastocyst(s) from thawed cleavage embryo(s) (AOR 0.73, 95% CI 0.65–0.82), fresh cleavage embryo(s) (AOR 0.67, 95% CI 0.64–0.69), thawed blastocyst(s) (AOR 0.57, 95% CI 0.53–0.62) and thawed cleavage embryo(s) (AOR 0.53, 95% CI 0.51–0.56) (Table V).

Thawed cleavage embryo cycles and thawed blastocyst cycles were also less likely to produce a "healthy baby" (AOR 0.80, 95% CI 0.77–0.84; and AOR 0.86, 95% CI 0.81–0.92, respectively) compared with fresh cleavage embryo cycles. There was no significant difference in the odds of "healthy baby" between transfer of fresh cleavage embryo(s) and blastocyst(s) from thawed cleavage embryo(s) (AOR 1.10, 95% CI 0.98–1.22).

Of thawed embryo transfer cycles, both thawed cleavage embryo cycles (AOR 0.71, 95% CI 0.63–0.79) and thawed blastocyst cycles (AOR 0.79, 95% CI 0.70–0.89) had significantly lower odds of "healthy baby" compared with cycles with transfer of blastocyst(s) from thawed cleavage embryo(s) (Table V). Thawed cleavage embryo cycles were also less likely to produce a "healthy baby" compared with thawed blastocyst cycles (AOR 0.89, 95% CI 0.84–0.97).

Table V

Discussion

This population-based study found that the transfer of fresh blastocyst(s) had a significantly better perinatal outcomes measured as a "healthy baby" [single baby born live at term (≥37 weeks gestation), weighing ≥2500 g, surviving for at least 28 days post birth and not having congenital anomalies]. Of thaw cycles, transfer of blastocyst(s) from thawed cleavage embryo(s) had higher rates of live delivery and "healthy baby" than transfer of thawed cleavage embryo(s) and thawed blastocyst(s). This study suggests that transfer of fresh blastocyst(s) in fresh cycles and blastocyst(s) from thawed cleavage embryo(s) in thaw cycles would result in more 'healthy babies'.

The strength of this retrospective cohort study is the large study population and the comprehensive coverage of all embryo transfer cycles performed in Australia from 2002 to 2006. This produces a significant study power and allows us to generalize the results that would not be possible from a clinic-based study. One limitation of this study is the potential variability in reporting of perinatal outcomes. Birthweight, gestation and plurality at birth are reliably defined from the various data sources, including hospital records, self-reporting by patients and their obstetricians. However, the information on pregnancy and birth outcomes was not stated in 1.7% of clinical pregnancies in the study. In addition, the information on congenital anomalies from ANZARD was likely to be incomplete. A comparative study of a regional birth defect register with

practitioner reporting in Australia found congenital anomalies were under reported in the assisted conception database (Hansen et al., 2007).

This study's definition of a "healthy baby" is restricted to singletons. This eliminates the potential confounders and selection bias of multiple pregnancies with their poorer perinatal outcomes (Ombelet et al., 2005), increased risk to mothers (Campbell and Templeton, 2004) and greater economic and social burden on the parents and on the health care system (Chambers et al., 2007). The definition of "healthy baby" was based on term gestation, normal birthweight and without known congenital anomalies including both minor and major congenital anomalies. Preterm birth and low-birthweight are related to the short- and long-term health conditions of a baby (Goldstein, 1981). Most major congenital anomalies require surgery or other medical intervention and are related to early death (Walden et al., 2007). Even minor congenital anomalies are also related to future development of a baby (Sutcliffe et al., 1995).

Consistent with other studies, we found that the live delivery rate per transfer cycle was higher with fresh blastocyst embryo transfer than with fresh cleavage-stage embryo transfer (Butterworth, 2001; Papanikolaou et al., 2008). An explanation of the difference is the natural selection that occurs with the two or three additional days in culture which allows a reduction in the number of chromosomally abnormal embryos. Hence, it is proposed that the best embryos survive through the blastocyst culture process (Butterworth, 2001).

However, this difference in live delivery rates between fresh blastocysts and cleavage embryos is reduced when the denominator used is the initiated cycle rather than the transfer cycle because there is a reduced embryo utilisation rate between blastocysts and cleavage embryos. A recent study found that the rate of embryo transfer cancellation was significantly higher in blastocyst transfer cycles than in cleavage embryo transfer cycles (Papanikolaou et al., 2008). As our study is retrospective, it is not possible to determine the decisions for the stage of transfer. The standard practice in Australian and New Zealand IVF clinics at the time was planned cleavage embryo transfer. However, it is likely that a proportion of cleavage embryo transfers occurred with poor quality of embryos.

There have been a number of changes in clinical practice in Australia and New Zealand in recent years. The proportion of blastocyst transfers has increased from 13.4% in 2003 to 30.6% in 2007. This was accompanied by the increase of SET from 32% in 2003 to 64% in 2007 (Wang et al., 2009). A randomised controlled trial suggested that among women under 36 years of age who are undergoing a first or a second ART treatment cycle, transfer of a single blastocyst significantly increases the probability of live birth when compared with transfer of a single cleavage embryo (Papanikolaou et al., 2006). Our study found that without adjusting for woman's age and number of previous ART treatment cycles, transfer of a single fresh blastocyst resulted in the highest rates of live delivery (30.7%) and “healthy baby” (25.8%). The continued

increase in single fresh blastocyst transfers results in better outcomes following ART treatment.

Another important change in clinical practice is the use of vitrification to cryopreserve embryos. Vitrification was first introduced into Australian ART clinics in 2006 (Costigan et al., 2007), the last year of our 5 year study. Data are not available from ANZARD on vitrification of embryos during the treatment period 2002–2008 and are not included in the study. Population-based monitoring of the safety and quality of vitrification is important as some studies suggest that vitrification may increase the embryo survival rate during both the freezing and thawing processes compared with slow freezing (Takahashi et al., 2005; Rezazadeh Valojerdi et al., 2009). Takahashi et al. (2005) suggests that transfer of thawed blastocysts following vitrification would achieve the same pregnancy and implantation rates similar to transfer of fresh blastocysts. There is limited published information on baby outcomes following vitrification. More population-based evidence of the baby outcomes following vitrification compared with slow freezing is needed to guide the future clinical practice.

Our study found that cycles with transfer of blastocyst(s) from thawed cleavage embryo(s) resulted in significantly better outcomes than thawed cleavage embryo transfer cycles and thawed blastocyst transfer cycles. Interestingly, the rates of live delivery and "healthy baby" following transfer of blastocyst(s) from thawed cleavage embryo(s) were statistically similar to transfer of fresh cleavage(s) despite the physical insults of freezing and thawing. Apart from

natural selection in post-thaw blastocyst culture process as discussed above (Butterworth, 2001), the freezing/thawing process is also considered as another selection process for the improved outcomes following transfer of blastocyst(s) from thawed cleavage embryo(s) (Pinborg et al., 2009). It is also suggested that embryos which are able to survive the freezing and thawing process are likely to be healthier and so result in better outcomes (Pinborg et al., 2009). In addition to the above, the natural uterine environment for a thaw cycle, which is similar to spontaneous pregnancy, may also play a role in improved perinatal outcomes (Shih et al., 2008). There have also been suggestions of a negative effect of fresh cycles where the pre-replacement treatment including controlled FSH ovarian stimulation, anesthesia and surgery for oocyte collection might affect early pregnancy with later effects on baby outcomes (Shih et al., 2008).

This large cycle-based study would suggest that the optimum practice model would be that in the initial fresh cycle where there are adequate numbers of good quality cleavage embryos, a proportion are frozen at the cleavage stage and a small cohort of three or four is cultured onto the blastocyst stage and a single blastocyst is transferred. Subsequently, the frozen embryos would be thawed and cultured onto blastocyst stage for transfer (Fig. 1). This model would result in higher rate of live delivery and more 'healthy babies'. The suggested practice model would be primarily applicable to good prognosis patients who have a number of oocytes fertilized. For those patients, such as younger patients undergoing first ART treatment, there is evidence that the

cancellation rate is not significantly different between the cleavage and blastocyst stages (Blake et al., 2007).

Figure I

The suggested model does not reflect the current practice. In Australia and New Zealand, the vast majority of clinics either transfer and freeze at the cleavage stage or transfer and freeze at the blastocyst stage in fresh cycles. In this study, of the 48 255 fresh cycles in which embryos were transferred and frozen, only 641 cycles (1.3%) had blastocyst(s) transferred and cleavage embryo(s) frozen. Reasons for such a small proportion of cycles with blastocyst(s) transferred and cleavage embryo(s) frozen need further investigation but may suggest that this group is inherently at a higher potential to conceive because of large numbers of cleavage embryos. The efficacy of this model, which is derived from this large, comprehensive epidemiological study, warrants prospective evaluation at a clinical level.

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Table I: Selected women's demographic and treatment factors of embryo transfer cycles, Australia 2002-2006

	Fresh cleavage (n=69308)		Fresh blastocyst (n=19483)		Thawed cleavage (n=49656)		Blastocyst from thawed cleavage (n=2665)		Thawed blastocyst (n=9264)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Women's age (years)										
≤ 24	886	1.3	202	1.0	650	1.3	28	1.1	96	1.0
25-29	7568	10.9	1994	10.2	5826	11.7	410	15.4	1100	11.9
30-34	21765	31.4	6290	32.3	17723	35.7	1001	37.6	3547	38.3
35-39	24410	35.2	7310	37.5	17702	35.6	905	34.0	3511	37.9
40-44	13634	19.7	3570	18.3	7240	14.6	294	11.0	967	10.4
≥45	1045	1.5	117	0.6	515	1.0	27	1.0	43	0.5
Cause of infertility										
Male factor	22095	31.9	5913	30.3	14389	29.0	766	28.7	2582	27.9
Female factor	20859	30.1	5335	27.4	18776	37.8	900	33.8	2349	25.4
Combined male/female factor	11619	16.8	2607	13.4	7353	14.8	374	14.0	1035	11.2
Unexplained	12184	17.6	4104	21.1	7118	14.3	471	17.7	1984	21.4
Not stated	2551	3.7	1524	7.8	2020	4.1	154	5.8	1314	14.2
Previous pregnancy of 20 weeks or more gestation										
No	52162	75.3	14474	74.3	33727	67.9	1628	61.1	6450	69.6
Yes	13312	19.2	3833	19.7	13205	26.6	699	26.2	1710	18.5
Not stated	3834	5.5	1176	6.0	2724	5.5	338	12.7	1104	11.9
Type of fertilization										
IVF	27974	40.4	8773	45.0	21147	42.6	1422	53.4	4675	50.5
ICSI	40161	57.9	10582	54.3	26638	53.6	983	36.9	4085	44.1
Not stated	1173	1.7	128	0.7	1871	3.8	260	9.8	504	5.4
Number of embryos transferred										
1	24571	35.5	10861	55.7	21769	43.8	1609	60.4	6154	66.4
2	42006	60.6	8292	42.6	26772	53.9	1021	38.3	3056	33.0
≥3	2731	3.9	330	1.7	1115	2.2	35	1.3	54	0.6

Table II: Pregnancy, live delivery and the "healthy baby" of embryo transfers cycles, Australia 2002-2006

	Transfer Cycles (No.)	Clinical pregnancy rate (%)	Live delivery rate (%)	"Healthy baby"* rate (%)
Fresh cleavage	69308	28.0	21.7	14.8
Fresh blastocyst	19483	35.9	27.9	21.0
Thawed cleavage	49656	20.1	15.2	11.7
Blastocyst from thawed cleavage	2665	28.8	22.0	16.2
Thawed blastocyst	9264	22.5	16.3	13.2

*A "healthy baby" was defined as a single baby born live at term (≥37 weeks gestation), weighing ≥2500 g, surviving for at least 28 days post birth and not having congenital anomalies.

Table III: Live delivery and the "healthy baby" of embryo transfers cycles by type of fertilization, Australia 2002-2006

	Transfer Cycles (No.)		Live delivery rate (%)			"Healthy baby"* rate (%)		
	IVF	ICSI	IVF	ICSI	P value	IVF	ICSI	P value
Fresh cleavage	27974	40161	21.7	21.5	0.51	14.7	14.8	0.73
Fresh blastocyst	8773	10582	29.0	27.0	<0.01	21.6	20.5	0.07
Thawed cleavage	21147	26638	15.4	14.8	0.10	11.7	11.5	0.40
Blastocyst from thawed cleavage	1422	983	21.7	21.7	1.00	15.8	16.2	0.82
Thawed blastocyst	4675	4085	16.4	16.7	0.75	13.3	13.5	0.78

*A "healthy baby" was defined as a single baby born live at term (≥37 weeks gestation), weighing ≥2500 g, surviving for at least 28 days post birth and not having congenital anomalies.

Table IV: Live delivery and "healthy baby" of embryo transfers cycles by number of embryos transferred, Australia 2002-2006

	Transfer Cycles (No.)		Live delivery rate (%)			"Healthy baby"* rate (%)		
	SET	DET	SET	DET	P value	SET	DET	P value
Fresh cleavage	24571	42006	18.6	24.0	<0.01	15.5	14.8	<0.01
Fresh blastocyst	10861	8292	30.7	24.8	<0.01	25.8	15.2	<0.01
Thawed cleavage	21769	26772	12.4	17.4	<0.01	10.7	12.5	<0.01
Blastocyst from thawed cleavage	1609	1021	20.1	24.9	<0.01	16.9	15.4	0.30
Thawed blastocyst	6154	3056	15.0	19.0	<0.01	13.1	13.5	0.59

SET, single embryo transfer; DET, double embryo transfer.

*A "healthy baby" was defined as a single baby born live at term (≥37 weeks gestation), weighing ≥2500 g, surviving for at least 28 days post birth and not having congenital anomalies.

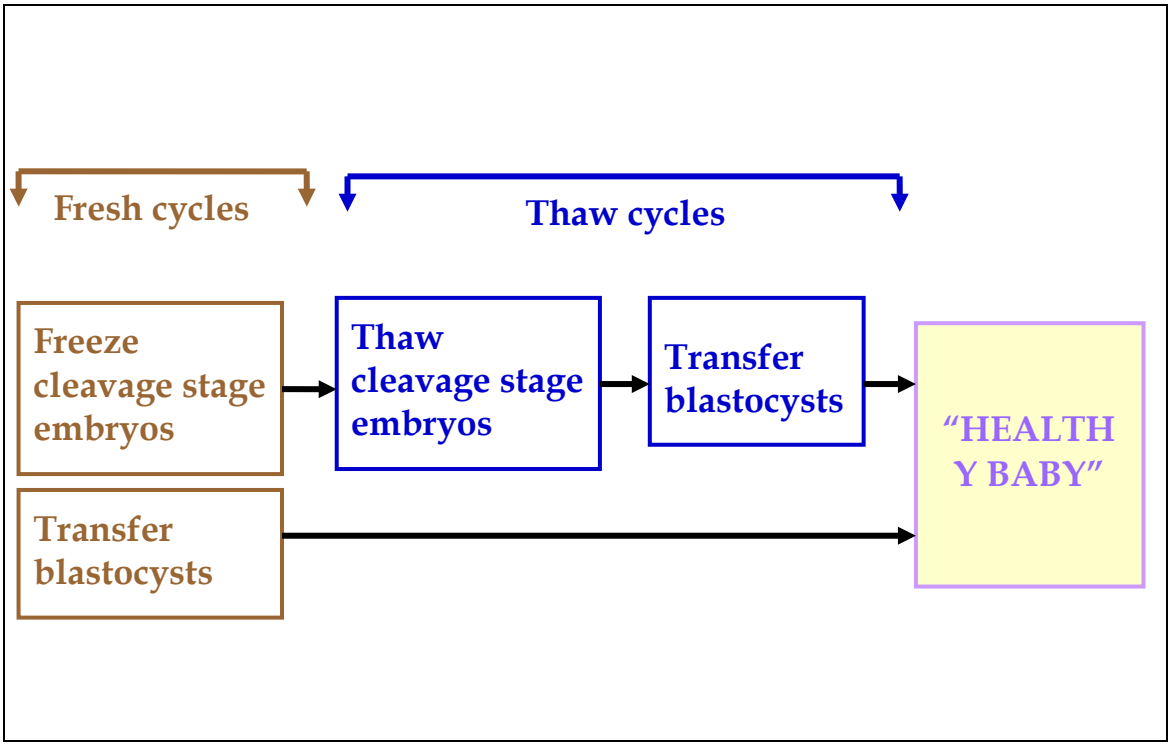
Table V: Odds ratio of live delivery and the "healthy baby" of embryos transfer cycles, Australia 2002-2006

	Live delivery			"Healthy baby"*		
	Rate (%)	OR (95% CI)	AOR** (95% CI)	Rate (%)	OR (95% CI)	AOR** (95% CI)
All embryos transfer cycles						
Fresh cleavage	21.7	0.71 (0.69-0.74)	0.67 (0.64-0.69)	14.8	0.65 (0.63-0.68)	0.67 (0.64-0.69)
Fresh blastocyst	27.9	1.00	1.00	21.0	1.00	1.00
Thawed cleavage	15.2	0.46 (0.44-0.48)	0.46 (0.44-0.48)	11.7	0.50 (0.48-0.52)	0.53 (0.51-0.56)
Blastocyst from thawed cleavage	22.0	0.73 (0.66-0.80)	0.71 (0.64-0.79)	16.2	0.73 (0.66-0.81)	0.73 (0.65-0.82)
Thawed blastocyst	16.3	0.50 (0.47-0.54)	0.50 (0.47-0.54)	13.2	0.57 (0.53-0.61)	0.57 (0.53-0.62)
Thawed embryo transfer cycles						
Thawed cleavage	15.2	0.64 (0.58-0.70)	0.63 (0.57-0.70)	11.7	0.68 (0.61-0.76)	0.71 (0.63-0.79)
Blastocyst from thawed cleavage	22.0	1.00	1.00	16.2	1.00	1.00
Thawed blastocyst	16.3	0.69 (0.62-0.77)	0.71 (0.64-0.79)	13.2	0.78 (0.70-0.88)	0.79 (0.70-0.89)

*A "healthy baby" was defined as a single baby born live at term (≥37 weeks gestation), weighing ≥2500 g, surviving for at least 28 days post birth and not having congenital anomalies.

**ARR was adjusted for women's age, causes of infertility, previous pregnancy of 20 weeks or more gestation, type of fertilization and number of embryos transferred.

Figure I: Suggested clinical practice model



Chapter 6

Study 4:

Transfer of a selected single blastocyst optimizes the chance of a healthy term baby: a retrospective population based study in Australia 2004-2007

About this chapter

Elective SET was introduced to minimise the risk and complications due to multiple births following ART treatment. However, the efficacy of elective SET is usually measured by the rates of clinical pregnancy and live delivery. Using the "healthy baby" indicator developed in previous chapter, the fourth study of this PhD thesis "Transfer of a selected single blastocyst optimizes the chance of a healthy term baby: a retrospective population based study in Australia 2004-2007" compared the likelihood of "healthy baby" between a modelled selective transfer of single cleavage embryo or blastocyst by the number of embryos available for transfer. It concluded that selective single blastocyst transfer is the optimal clinical practice in a fresh ART treatment, especially for patients aged younger than 35 years in their first ART cycle who had four or more embryos available at day two or day three for transfer.

This study was presented at the ESHRE 2010 annual meeting in Rome, Italy. The manuscript of this study was published at Human Reproduction in 2010.

List of presentation and publication from this study:

Oral presentation. Transfer of a selected single blastocyst optimizes the chance of a healthy term baby: a retrospective population based study in Australia 2004-2007. The 26th Annual Meeting of ESHRE. Rome, Italy (27 Jun to 1 Jul 2010)

Wang YA, Kovacs G, Sullivan EA. Transfer of a selected single blastocyst optimizes the chance of a healthy term baby: a retrospective population based study in Australia 2004-2007. Hum Reprod. 2010 Aug;25(8):1996-2005. doi: 10.1093/humrep/deq145.

Abstract

BACKGROUND: The practice of single embryo transfer (SET) is highly accepted by clinicians in Australia. This study investigates whether the SET of blastocysts results in optimal perinatal outcomes. **METHODS**: This retrospective population-based study included 34 035 single or double embryo transfer cycles in women who had their first fresh autologous treatment in Australia during 2004–2007. Pregnancy, live delivery and "healthy baby" (live born term singleton of ≥2500 g birthweight and survived for at least 28 days without a notified/reported congenital anomaly) rates per transfer cycle were compared in four groups: selective single embryo transfer (SSET), unselective single embryo transfer (USSET), selective double embryo transfer (SDET) and unselective double embryo transfer (USDET). Live delivery and "healthy baby" rates per transfer following SSET were further compared by number of embryos available. The analysis was stratified by woman's age and stage of embryo development. **RESULTS:** The highest rates of live delivery and "healthy baby" per transfer cycle (46.2 and 38.0%) were achieved with transfer of a single blastocyst in women aged younger than 35 years. In women aged younger than 40 years, SSET had a significantly higher rate of "healthy baby" per transfer cycle than did SDET regardless of stage of embryo development. In woman aged younger than 35 years who had SSET, there was no significant difference in live delivery and "healthy baby" rates per transfer cycle whether two, three, four or five embryos were available. For all of these women, SSET of a cleavage

embryo had significantly lower rates of live delivery and “healthy baby” per transfer cycle compared with SSET of a blastocyst where only two blastocysts were available. **CONCLUSIONS:** Consultation with the patient with respect to the advantage of extended culture and selective single blastocyst transfer will result in better success rates following assisted reproductive technology treatment in Australia.

Introduction

Multiple gestation is the most common complication following assisted reproductive technology (ART) treatment. Multiple births following ART are associated with increased risks of adverse perinatal outcomes (Kissin et al., 2005; Wang et al., 2006) and maternal complications (Campbell and Templeton, 2004) and higher cost to the health care system (Chambers et al., 2007).

Regional registry data show that the reported multiple delivery rate was 22% in Europe in 2004 (Nyboe Andersen et al., 2008), 31% in USA in 2006 (Centers for Disease Control and Prevention, 2008) and 10% in Australia and New Zealand in 2007 (Wang et al., 2009). The high rate of multiple delivery following ART is largely explained by the number of embryos transferred (Kissin et al., 2005).

There is a worldwide tendency to reduce the number of embryos transferred in order to reduce the complications of multiple pregnancy. The shift in clinical practice to single embryo transfer (SET) is the pathway to decrease multiple pregnancies following ART (Criniti et al., 2005). The Nordic countries and Belgium have successfully implemented a policy of SET, with Sweden having reported that 70% of all embryo transfer cycles being from SET (Nygren, 2007). In Australia and New Zealand, the regulatory group has advocated SET since 2005 with the aim of minimizing the number of multiple births (Reproductive Technology Accreditation Committee, 2005). The latest regional report detailed the rise in the proportion of SET in Australia and New Zealand from 32% in

2003 to 64% in 2007. As a result, the multiple delivery rate has declined from 19% in 2003 to 10% in 2007 (Wang et al., 2009).

The principal reason for double or triple embryo transfer is the perception that the more embryos transferred the better the outcome. This belief by both patients and some clinicians is supported by the literature: the transfer of more than one embryo results in a higher pregnancy and live birth rates (Pandian et al., 2005; Baruffi et al., 2009; Bechoua et al., 2009; Pandian et al., 2009). A recent observational study showed that the pregnancy rate per transfer was significantly higher in the double embryo transfer (DET) group compared with the elective SET group (49.3 versus 32.1% in the DET and eSET groups, respectively) (Bechoua et al., 2009). However, none of these studies have reported resulting perinatal outcomes other than pregnancy and live birth rates.

The outcome of ART treatment needs to focus on perinatal outcomes, not pregnancy or live birth. Some studies have used 'take home baby' as the optimum outcome following ART treatment (Botchan et al., 1993; Giorgetti et al., 2006). We introduced the "healthy baby" concept (a surviving live born term (≥37 weeks gestational age) single neonate of ≥2500 g birthweight and without congenital anomaly) to indicate the best perinatal outcomes following ART treatment. Our study investigates the association between pregnancy, live delivery and "healthy baby" and the number of embryos transferred by the number of embryos available, and by the stage of embryo development (cleavage or blastocyst). Our hypothesis is that when "healthy baby" is used as

the measurement of the outcome of pregnancy and hence ART treatment, transfer of a selected single blastocyst is the best treatment strategy.

Materials and methods

We retrospectively analysed data from the Australian and New Zealand Assisted Reproduction Database (ANZARD), and compared outcomes between SET and DET, according to whether the embryos were the only ones available, or whether they were selected from a cohort, as well as according to the stage of embryo development.

Subjects

ANZARD is housed at the Perinatal and Reproductive Epidemiology Research Unit of the University of New South Wales, and consists of data collected annually from all fertility centers in Australia and New Zealand. ANZARD includes information about the oocyte pick-up and number of oocytes collected, *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) fertilization procedures, the number of embryos created, stage of embryo development at transfer, number of embryos transferred, freezing of embryos and donation of gametes or embryos. It also includes information on pregnancy and birth outcomes (birth status, gestational age, birthweight, congenital anomaly and neonatal mortality).

Procedures

The study data set was extracted from ANZARD for women undergoing their first stimulated fresh cycle using follicle stimulating hormone. Data were limited to women aged 18 years or older who used their own oocytes (autologous cycles) between 1 January 2004 and 31 December 2007 (Figure 1). Mixed cleavage-blastocyst stage embryo transfer cycles were excluded. A total of 34 035 women who had single or DET at their first fresh autologous cycles were included in the denominator to measure the pregnancy and perinatal outcomes.

Study factors

The number of embryos available for transfer is defined as: (for transfer of cleavage embryos) number of cleavage embryos transferred + number cleavage embryos frozen; and (for transfer of blastocysts) number of blastocysts transferred + number blastocysts frozen. Embryo transfer cycles were categorized into four groups based on number of embryos available:

i. selective single embryo transfer (SSET): at least two embryos available and only one embryo transferred;

ii. unselective single embryo transfer (USSET), only one embryo available and one embryo transferred;

iii. selective double embryo transfer (SDET), at least three embryos available and two embryos transferred; and

iv. unselective double embryo transfer (USDET), only two embryos available and two transferred.

The woman's age was calculated in completed years at the time of treatment and classified into 5-year groups: ≤24 years, 25–29 years, 30–34 years, 35–39 years and >40 years. The cause of infertility was classified as male factor infertility (a male factor problem was diagnosed and no female factor problem), female factor infertility (tubal disease, endometriosis or other female factor problem were diagnosed and no male factor problem), combined male–female factor infertility (both male and female factor problems were diagnosed), unexplained infertility (neither male nor female factor problem was diagnosed) and not stated. Previous pregnancy of greater than or equal to 20 weeks or more gestation was grouped as yes, no and not stated.

Type of fertilization was classified either IVF or ICSI. Stage of embryo development was grouped by cleavage embryos (that is Day 2 or 3 after insemination/ICSI) or blastocysts (Day 5 or 6). The number of embryos available for transfer was grouped as 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 or more embryos and further grouped as 1, 2, 3, 4, 5, 6 or more embryos.

Gestational age was defined as the number of completed weeks of gestation and calculated by the formula: (pregnancy end date − embryo transfer date) + 16

days for cleavage embryo transfer and (pregnancy end date − embryo transfer date) + 19 days for blastocyst transfer.

Main outcome measures

A clinical pregnancy was defined as one of the following criteria: evidence by ultrasound of intrauterine sac(s) and/or fetal heart(s); examination of products of conception revealing chronic villi; an ectopic pregnancy that had been diagnosed laparoscopically or by ultrasound. A live delivery was defined as a birth event in which one or more babies were live born of greater than or equal to 20 weeks gestation or of 400 g or more birthweight.

An indicator, the "healthy baby", was developed to demonstrate the best perinatal outcome. The "healthy baby" was defined as live born term (≥37 weeks gestational age) singleton of ≥2500 g birthweight and surviving for at least 28 days and without a notified/reported congenital anomaly.

Statistical analysis

Patient demographics (woman's age, cause of infertility and previous pregnancy of greater than or equal to 20 weeks or more gestation) were compared by SSET, USSET, SDET and USDET. Rates of clinical pregnancy, live delivery and "healthy baby" per transfer cycle were measured and compared by the four groups of embryo transfer cycle. Data were further stratified by woman's age group (<35 year, 35–39 years and ≥40 years) and stage of embryo development (cleavage and blastocyst) for comparison.

Chi-square test was used to measure the association between the outcomes and treatment factors. Univariate Cox regression was used to investigate the chance of live delivery and "healthy baby" following SSET, USSET, SDET and USDET of cleavage embryos and/or blastocysts. Multivariate Cox regression was used to overcome the influence by potential demographics and treatment confounders. Further Cox regression models separated for cleavage embryos or blastocysts were conducted to estimate the likelihood of live delivery and "healthy baby" by number of embryos available for transfer for SSET. Analyses were conducted separately for women aged <35 years, 35–39 years and ≥40 years. Rates ratio (RR) and adjusted rate ratio (ARR) (adjusted for clinics, cause of infertility, previous pregnancy of 20 weeks or more gestation and type of fertilization) and 95% confidence intervals (95% CI) were calculated. Data were analysed with SPSS software (version 17.0; SPSS Inc., Chicago, IL, USA).

Ethics

Ethics approval for this study was granted by the Human Research Ethics Committee of the University of New South Wales, Australia.

Results

Table I details the demographic characteristics by number of embryos transferred. Less than 5% of SSET was in women aged 40 years or older compared with 20.9% of USSET, 16.3% of SDET and 30.5% of USDET. Male factor only infertility was slightly higher in SSET than in other groups of

embryo transfer cycles. SDET had marginally higher proportion of women who never had pregnancy of 20 weeks or more gestation (Table I).

The overall pregnancy rate per transfer cycle was significantly higher for transfer of blastocysts (42.3%) compared with transfer of cleavage embryos (32.4%) (Chi-square test, df = 1, P <0.01). Transfer of blastocysts had a significantly higher rate of live delivery per transfer cycle (34.6%) than did transfer of cleavage embryos (26.0%) (Chi-square test, df = 1, P <0.01). Similarly, the "healthy baby" rate per transfer cycle was significantly higher for transfer of blastocysts (27.4%) than for transfer of cleavage embryos (18.0%) (Chi-square test, df = 1, P <0.01).

Of cycles where cleavage embryos were transferred, SDET had a significantly higher rate of clinical pregnancy per transfer cycle than SSET regardless of woman's age (Chi-square test, df = 1, P <0.01 for cycles in women aged <35 years, P <0.01 for women aged 35–39 years and P = 0.01 for women aged 40 years or older). Similarly, SDET had significantly higher rate of live delivery per transfer cycle than SSET (Chi-square test, df = 1, P <0.01 for cycles in women aged <35 years, P <0.01 for women aged 35–39 years and P = 0.01 for women aged 40 years or older). However, SSET had significantly higher rate of "healthy baby" per transfer cycle than SDET in women aged younger than 40 years (Chi-square test, df = 1, P <0.01 for cycles in women aged <35 years and P = 0.02 for women aged 35–39 years). There was no significant difference in

"healthy baby" rate per transfer cycle between SDET and SSET in women aged 40 years or older (Chi-square test, df = 1, P = 0.32) (Table II).

Of cycles where blastocysts were transferred, SDET had a higher rate of clinical pregnancy per transfer cycle in women aged 40 years or older (Chi-square test, df = 1, P = 0.01). There was no significant difference in clinical pregnancy rate per transfer cycle between SDET and SSET for women aged younger than 40 years (Chi-square test, df = 1, P = 0.65 for cycles in women aged <35 years and P = 0.34 for women aged 35–39 years). There was no significant difference in live delivery rate per transfer cycle between SDET and SSET regardless of woman's age (Chi-square test, df = 1, P = 0.57 for cycles in women aged <35 years, P = 0.22 for women aged 35–39 years and P = 0.47 for women aged 40 years or older). The "healthy baby" rate per transfer cycle was again significantly higher for SSET than SDET in women aged younger than 40 years, but not significantly different in women aged 40 years or older (Chi-square test, df = 1, P <0.01 for cycles in women aged <35 years, P <0.01 for women aged 35–39 years and P = 0.82 for women aged 40 years or older) (Table II).

Transfer of single blastocysts in women aged younger than 35 years achieved the highest rates of live delivery and "healthy baby" per transfer cycle (46.2 and 38.0%) (Table II).

Table III compares the live delivery and "healthy baby" rates per transfer cycle following SSET of a blastocyst against USSET of a blastocyst, SDET of blastocysts, USDET of blastocysts, SSET of a cleavage embryo, USSET of a

cleavage embryo, SDET of cleavage embryos and USDET of cleavage embryos. In woman aged younger <35 years and 35–39 years, SSET of a blastocyst had a significantly higher rate of "healthy baby" per transfer cycle than all other groups of embryo transfer cycles. In woman aged 40 years or older, SSET of a blastocyst had a significantly higher rate of "healthy baby" per transfer cycle than USSET and USDET of blastocysts, and all groups of cleavage embryo transfer cycles. There was no significant difference in "healthy baby" rate per transfer cycle between SSET and SDET of blastocysts in woman aged 40 years or older (Table III).

Table IV demonstrates the rates of live delivery and "healthy baby" per transfer cycle following SSET by number of embryos available. A non-overlapped 95% CI indicates the significantly higher rates of live delivery and "healthy baby" per transfer cycle when compared within cleavage embryos or blastocysts as well as across cleavage embryos and blastocysts (Table IV).

Table V shows the adjusted rate ratio (ARR) of live delivery and "healthy baby" per transfer cycle following SSET cycles. For cycles in woman aged younger than 35 years, when five or less cleavage embryos were available, SSET from two, three, four or five cleavage embryos did not vary the rates of live delivery and "healthy baby" per transfer cycle. However, the live delivery rate and "healthy baby" rate per transfer cycle following SSET where six or more cleavage embryos were available was 26 and 28% higher than SSET where only two cleavage embryos were available. For SSET of a cleavage embryo in women

aged 35–39 years, there was no significant difference in live delivery and "healthy baby" per transfer cycle by number of cleavage embryos available (Table V).

For SSET of a blastocyst in women aged younger than 35 years, there was no significant difference in live delivery and "healthy baby" per transfer cycle according to number of blastocysts available. In woman aged 35–39 years, the rates of live delivery and "healthy baby" per transfer cycle did vary by number of embryos available (Table V).

Of SSET cycles in women aged <35 years, SSET of a cleavage embryo where two, three, four or five cleavage embryos were available had significantly lower rates of live delivery and "healthy baby" per transfer cycle compared with SSET of a blastocyst where only two blastocysts were available. There was no significant difference in live delivery and "healthy baby" rates per transfer cycle between SSET of a cleavage embryo where six or more cleavage embryos were available and SSET of blastocyst where only two blastocysts were available. Of SSET cycles in women aged 35–39 years, there was no significant difference in live delivery and "healthy baby" rates per transfer cycles between SSET of a blastocyst where only two blastocysts were available and SSET of cleavage embryo regardless number of cleavage embryos available (Table VI).

Discussion

This large retrospective population-based study suggests that the optimal strategy to achieve a healthy term singleton is the transfer of a selected single blastocyst. This treatment strategy resulted in the highest rate of births of term live born singletons of normal birthweight and without congenital anomaly. This study also suggests that in clinical practice when two or more embryos are available for transfer, DET would not increase the "healthy baby" rate per transfer cycle. It also confirms that blastocyst transfer results in significantly higher rates of pregnancy than that of cleavage stage embryos.

For SSET of a cleavage embryo in women aged younger than 35 years, there was no significant difference in live delivery and "healthy baby" rates per transfer cycle among cycles where two, three, four or five cleavage embryos were available. In SSET of a blastocyst, the rates of live delivery and "healthy baby" per transfer cycle also did not vary by the number of blastocysts available. This finding suggested that regardless of the number of embryos available, SSET should be the preferred practice.

The strength of this cohort study includes the national coverage and the timeliness of the data with inclusion of embryo transfer cycles performed in Australia from 2004 to 2007. This gives the study significant power and allows generalizability of results that would not be possible from clinic-based studies. One limitation of this study is that we are unable to measure treatment

outcomes from an initiated cycle due to the structure of ANZARD. The measurement of the outcomes in this study was per transfer cycle. Another limitation of this study is the potential variability in reporting of perinatal outcomes. The information on pregnancy and birth outcomes was not stated for 0.8% of clinical pregnancies of the study cohort. Birthweight, gestation and plurality at birth are reliably defined from the various data sources including hospital records, and self-reporting by patients and their obstetricians. However, the information on congenital anomalies and neonatal mortality within ANZARD was likely to be incomplete and this may have led to a marginal overestimate of the healthy baby. It is likely that this bias would be consistent across all treatment strategies. A comparative study of a regional birth defect register with practitioner reporting in Australia found congenital anomalies were underreported by the assisted conception database (Hansen et al., 2007).

In agreement with the latest literature (Veleva et al., 2006; Bechoua et al., 2009; Gelbaya et al., 2009), our study found that the clinical pregnancy and live delivery rates per cleavage embryo transfer cycle were significantly higher for SDET than SSET regardless of woman's age. However, when we analysed blastocyst transfers, we did not find this same association. Among women aged less than 40 years, there was no significant difference in the clinical pregnancy and live delivery rates per blastocyst transfer cycle between SDET and SSET. When the perinatal outcomes were used to measure the success per embryo transfer cycle, our study found that the “healthy baby” rate per transfer cycle

was significantly higher for SSET than SDET among women aged younger than 40 years and no worse for women aged 40 years and older regardless of the stage of embryo development.

It has been suggested that the outcome of ART treatment needs to focus on 'Birth Emphasizing a Successful Singleton at Term' (Healy, 2004). We agree that a "healthy baby" should always be the optimum outcome of ART treatment. Treatment should be focused on eliminating poorer perinatal outcomes (Kissin et al., 2005; Wang et al., 2006), minimizing risk to mothers (Campbell and Templeton, 2004) and limiting the potential burden on the health care system (Chambers et al., 2007) due to multiple births. We advocate extending the definition of a "healthy baby" to a live born term singleton of normal birthweight without a congenital anomaly who survives the neonatal period.

The classification of selective/unselective embryo transfer in our study was based on quantity not quality of available embryos at the time of embryo transfer. The quantity included the sum of the number of embryo transferred and the number of embryo frozen. ANZARD does not have information on the quality of each embryo. The lower rates of live delivery and "healthy baby" per transfer cycle following unselective embryo transfer compared with selective embryo transfer are possibly related to the quality of transferred embryos especially for cleavage embryos (Bergh, 2005). Since there was only one cleavage embryo available for transfer in USSET and two available in USDET, poorer quality embryo(s) may have been transferred (Kearns et al., 2005; Flisser

and Licciardi, 2006). Similarly for blastocysts, when limited cleavage embryos are available, extended blastocyst culture would result in either no transfer or possibly transfer of a poorer quality embryo (Barrenetxea et al., 2005).

Consistent with other studies, our study found that the clinical pregnancy and live delivery rates per transfer cycle were significantly higher after transfer of blastocysts than after transfer of cleavage embryos (Butterworth, 2001; Papanikolaou et al., 2008). Transfer of a selected single cleavage embryo was less likely to result in a live delivery and a "healthy baby" compared with transfer of a selected single blastocyst. The likely explanation of the difference is the natural selection through 2–3 days of extended culture which allows the reduction in abnormal embryos. Hence the best embryos are proposed to survive through the blastocyst culture process (Butterworth, 2001).

However, this difference in outcomes between fresh blastocysts and cleavage embryos is reduced when the denominator used is initiated cycle since there is reduced embryo utilisation rate of blastocysts compared with cleavage embryos (Papanikolaou et al., 2008). Since our study was a retrospective analysis of actual transfers in terms of cleavage embryo or blastocyst transfers, we are unable to measure the pregnancy and birth outcomes by initiated cycle. A recent Australian study found that in fresh cycles where no embryo was frozen, 73.1% of fertilized oocytes reached transfer of cleavage embryos compared with 38.5% of fertilized oocytes reaching transfer of blastocysts (Wang, 2008).

If we make an assumption that approximately half (38.5% of 73.1%) of the cleavage embryos develop to blastocysts, then compare the outcomes SSET where two cleavage embryos were available to USSET of blastocyst, our study found that there was no significant difference in the live delivery rate nor the "healthy baby" rate per transfer cycle. While, based on the same assumption, when comparing SSET of cleavage embryo where four or five cleavage embryos were available with SSET of blastocyst where two blastocysts were available, the live delivery and "healthy baby" rates per transfer cycle in women aged <35 years were significantly lower for the former than the latter.

Since 2005, clinics in Australia have recommended SET for the first ART cycle in women aged younger than 35 years (Reproductive Technology Accreditation Committee, 2005). This study found that about two-thirds of SSET cycles were in women aged younger than 35 years. Of this age group, 68.4% of cycles had four or more cleavage embryos available and 56.8% cycles had four or more blastocysts available. These findings support the 2007 review which found that embryo transfer cancellation was similar between blastocysts transfer cycles and cleavage embryo transfer cycles for good prognosis patients (Blake et al., 2007). Even based on the above assumption that half of the cleavage embryos develop to blastocysts, our study suggests that if patients aged less than 35 years had four or more cleavage embryos in their first autologous ART cycle, further blastocyst culture would ensure that all patients would have had a SSET of a blastocyst, nearly half of them would have had a live delivery and about 40% would have had a "healthy baby".

Our study advocates that the optimum outcome of ART treatment should focus on a “healthy baby” not pregnancy and live birth. Our findings confirm that an optimal practice of fresh ART treatment is selective single blastocyst transfer. This would result in higher rates of live delivery and more ‘healthy babies’, especially for patients aged younger than 35 years in their first ART cycle who have had four or more embryos available at Day 2/3. Evaluation of embryos at the cleavage stage and consultation with the patient with respect to the advantage of extended culture to selective single blastocyst transfer will result in better perinatal outcomes following ART treatment.

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Table I: Selected woman's demographics by group of embryo transfer cycles, Australia 2004-2007

Demographics	SSET (n=14085)		USSET (n=7256)		SDET (n=7125)		USDET (n=5569)		Total (N=34035)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Woman's age (years)										
≤ 24	403	2.9	103	1.4	90	1.3	53	1.0	649	1.9
25-29	2717	19.3	890	12.3	886	12.4	413	7.4	4906	14.4
30-34	6032	42.8	2193	30.2	2371	33.3	1279	23.0	11875	34.9
35-39	4278	30.4	2554	35.2	2617	36.7	2129	38.2	11578	34.0
≥40	655	4.7	1516	20.9	1161	16.3	1695	30.5	5027	14.7
Cause of sub-fertility										
Male factor	4225	30.0	2005	27.6	1982	27.8	1431	25.7	9643	28.3
Female factor	4064	28.9	2041	28.1	2147	30.1	1523	27.3	9775	28.7
Combined male/female factor	1930	13.7	1050	14.5	908	12.7	813	14.6	4701	13.8
Unexplained	3124	22.2	1721	23.7	1822	25.6	1446	26.0	8113	23.8
Not stated	742	5.3	439	6.1	266	3.7	356	6.4	1803	5.3
Previous pregnancy of ≥20 weeks gestation										
None	10830	76.9	5433	74.9	5808	81.5	4384	78.7	26455	77.7
Yes	1856	13.2	1092	15.0	1104	15.5	882	15.8	4934	14.5
Not stated	1399	9.9	731	10.1	213	3.0	303	5.4	2646	7.8

SSET: one fresh embryo transferred and at least one embryo frozen; USSET: one fresh embryo transferred and no embryo frozen; SDET: two fresh embryos transferred and at least one embryo frozen; USDET: two fresh embryos transferred and no embryo frozen.

Table II: Outcomes by group of embryo transfer cycles, woman's age and stage of embryo development, Australia 2004-2007

Embryo transfer cycle	Cleavage embryo				Blastocyst embryo			
	Number of cycles	Clinical pregnancy rate (%)	Live delivery rate (%)	"Healthy baby"* rate (%)	Number of cycles	Clinical pregnancy rate (%)	Live delivery rate (%)	"Healthy baby"* rate (%)
<35 years								
SSET	6101	40.2	33.6	27.0	3051	54.2	46.2	38.0
USSET	2076	25.0	20.6	16.0	1110	37.5	31.2	25.2
SDET	3161	49.1	42.4	21.4	186	55.9	44.1	21.0
USDET	1471	35.1	30.3	18.9	274	37.2	33.2	20.4
35-39 years								
SSET	2681	32.2	24.4	19.7	1597	45.5	37.1	30.8
USSET	1692	18.8	13.2	10.8	862	27.0	21.2	17.9
SDET	2392	38.1	29.8	17.1	225	48.9	41.3	20.9
USDET	1659	27.7	21.1	14.0	470	32.6	25.3	16.6
≥40 years								
SSET	452	16.2	9.3	8.2	203	30.5	22.7	21.2
USSET	1179	7.1	3.8	2.9	337	18.7	8.6	7.7
SDET	1027	21.7	14.0	9.8	134	42.5	26.1	20.1
USDET	1224	14.4	7.8	5.5	471	18.7	13.0	9.3

* Term liveborn singleton of ≥2500 grams birthweight and survived for at least 28 days and without congenital anomaly.

SSET: one fresh embryo transferred and at least one embryo frozen; USSET: one fresh embryo transferred and no embryo frozen; SDET: two fresh embryos transferred and at least one embryo frozen; USDET: two fresh embryos transferred and no embryo frozen.

Table III: Rate ratio of live delivery and "healthy baby" by group of embryo transfer cycles, woman's age and stage of embryo development, Australia 2004-2007

	Live delivery			"Healthy baby"*		
	Rate (%)	RR (95% CI)	ARR** (95% CI)	Rate (%)	RR (95% CI)	ARR** (95% CI)
<35 years						
SSET BL	*46.2*	*1.00*	*1.00*	*38.0*	*1.00*	*1.00*
USSET BL	31.2	0.67 (0.60-0.76)	0.68 (0.60-0.76)	25.2	0.66 (0.58-0.76)	0.67 (0.59-0.76)
SDET BL	44.1	0.95 (0.76-1.19)	0.99 (0.79-1.24)	21.0	0.55 (0.40-0.76)	0.57 (0.41-0.78)
USDET BL	33.2	0.72 (0.58-0.89)	0.72 (0.58-0.89)	20.4	0.54 (0.41-0.70)	0.54 (0.41-0.71)
SSET CL	33.6	0.73 (0.68-0.78)	0.77 (0.70-0.85)	27.0	0.71 (0.66-0.77)	0.79 (0.71-0.87)
USSET CL	20.6	0.44 (0.40-0.50)	0.47 (0.42-0.53)	16.0	0.42 (0.37-0.48)	0.46 (0.40-0.53)
SDET CL	42.4	0.92 (0.85-0.99)	1.00 (0.90-1.11)	21.4	0.56 (0.51-0.62)	0.62 (0.54-0.70)
USDET CL	30.3	0.66 (0.59-0.73)	0.71 (0.62-0.81)	18.9	0.50 (0.44-0.57)	0.54 (0.46-0.63)
35-39 years						
SSET BL	*37.1*	*1.00*	*1.00*	*30.8*	*1.00*	*1.00*
USSET BL	21.2	0.57 (0.49-0.68)	0.57 (0.48-0.67)	17.9	0.58 (0.48-0.69)	0.58 (0.48-0.69)
SDET BL	41.3	1.12 (0.90-1.39)	1.11 (0.89-1.38)	20.9	0.68 (0.50-0.91)	0.66 (0.49-0.89)
USDET BL	25.3	0.68 (0.56-0.83)	0.69 (0.57-0.84)	16.6	0.54 (0.42-0.68)	0.52 (0.41-0.66)
SSET CL	24.4	0.66 (0.59-0.74)	0.67 (0.60-0.75)	19.7	0.64 (0.57-0.72)	0.66 (0.56-0.77)
USSET CL	13.2	0.36 (0.30-0.41)	0.36 (0.31-0.42)	10.8	0.35 (0.30-0.42)	0.36 (0.30-0.44)
SDET CL	29.8	0.80 (0.72-0.90)	0.82 (0.73-0.91)	17.1	0.56 (0.49-0.63)	0.58 (0.49-0.69)
USDET CL	21.1	0.57 (0.50-0.65)	0.58 (0.50-0.66)	14.0	0.46 (0.39-0.53)	0.48 (0.40-0.58)
≥40 years						
SSET BL	*22.7*	*1.00*	*1.00*	*21.2*	*1.00*	*1.00*
USSET BL	8.6	0.38 (0.24-0.60)	0.40 (0.25-0.64)	7.7	0.36 (0.22-0.59)	0.38 (0.23-0.62)
SDET BL	26.1	1.15 (0.74-1.79)	1.09 (0.70-1.70)	20.1	0.95 (0.59-1.54)	0.90 (0.55-1.46)
USDET BL	13.0	0.57 (0.39-0.84)	0.56 (0.38-0.82)	9.3	0.44 (0.29-0.67)	0.42 (0.28-0.64)
SSET CL	9.3	0.41 (0.27-0.62)	0.50 (0.31-0.81)	8.2	0.39 (0.25-0.60)	0.53 (0.32-0.89)
USSET CL	3.8	0.17 (0.11-0.25)	0.20 (0.13-0.30)	2.9	0.14 (0.09-0.21)	0.17 (0.10-0.28)
SDET CL	14.0	0.62 (0.44-0.86)	0.75 (0.50-1.12)	9.8	0.46 (0.32-0.66)	0.62 (0.40-0.97)
USDET CL	7.8	0.34 (0.24-0.49)	0.40 (0.27-0.59)	5.5	0.26 (0.18-0.38)	0.32 (0.21-0.50)

* Term liveborn singleton of ≥2500 grams birthweight and survived for at least 28 days and without congenital anomaly. ** ARR was adjusted for clinics, cause infertility, previous pregnancy of 20 weeks or more gestation and type of fertilisation.

SSET: one fresh embryo transferred and at least one embryo frozen; USSET: one fresh embryo transferred and no embryo frozen; SDET: two fresh embryos transferred and at least one embryo frozen; USDET: two fresh embryos transferred and no embryo frozen.

BL is for blastocyst(s) and CL is for cleavage embryo(s).

Table IV (a): Outcomes of selective single embryo transfer cycles by woman's age, stage of embryo development and number of embryos available, Australia 2004-2007

Number of embryos available	Number of cycles	Live delivery rate and 95% CI (%)	"Healthy baby"* rate and 95% CI (%)
Cleavage embryo			
<35 years			
2	891	30.1 (27.1-31.6)	23.8 (21.0-25.2)
3	1035	31.2 (28.4-32.6)	25.3 (22.7-26.7)
4	909	32.0 (29.0-33.6)	24.4 (21.6-25.8)
5	821	31.5 (28.4-33.2)	25.2 (22.2-26.7)
6	581	36.3 (32.4-38.3)	29.6 (25.9-31.5)
7	474	36.9 (32.6-39.1)	31.6 (27.5-33.8)
8	385	36.9 (32.1-39.3)	29.9 (25.3-32.2)
9	287	36.9 (31.4-39.8)	31.4 (26.0-34.1)
≥10	718	38.2 (34.6-40.0)	30.4 (27.0-32.1)
35-39 years			
2	538	26.0 (22.3-27.9)	21.2 (17.7-23.0)
3	533	22.5 (19.0-24.3)	16.9 (13.7-18.5)
4	408	23.5 (19.4-25.6)	20.1 (16.2-22.1)
5	338	24.6 (20.0-26.9)	20.1 (15.8-22.3)
6	249	23.3 (18.0-26.0)	18.1 (13.3-20.5)
7	171	22.8 (16.5-26.0)	19.3 (13.4-22.3)
8	126	25.4 (17.8-29.3)	18.3 (11.5-21.7)
9	98	27.6 (18.7-32.1)	22.4 (14.2-26.7)
≥10	220	27.3 (21.4-30.3)	23.2 (17.6-26.0)
≥40 years			
2	126	11.9 (6.3-14.8)	11.1 (5.6-13.9)
3	112	8.0 (3.0-10.6)	6.3 (1.8-8.5)
4	77	11.7 (4.5-15.3)	10.4 (3.6-13.9)
5	37	2.7 (-2.5-5.4)	2.7 (-2.5-5.4)
≥6	100	8.0 (2.7-10.7)	7.0 (2.0-9.6)

* Term liveborn singleton of ≥2500 grams birthweight and survived for at least 28 days and without congenital anomaly.

Table IV (b): Outcomes of selective single embryo transfer cycles by woman's age, stage of embryo development and number of embryos available, Australia 2004-2007

Number of embryos available	Number of cycles	Live delivery rate and 95% CI (%)	"Healthy baby"* rate and 95% CI (%)
Blastocyst embryo			
<35 years			
2	668	43.7 (40.0-45.6)	36.2 (32.6-38.1)
3	651	45.6 (41.8-47.6)	37.2 (33.5-39.1)
4	571	44.0 (39.9-46.0)	35.9 (32.0-37.9)
5	371	48.5 (43.4-51.1)	38.3 (33.3-40.8)
6	247	50.2 (44.0-53.4)	41.3 (35.2-44.4)
7	182	47.8 (40.5-51.5)	40.7 (33.5-44.3)
8	117	48.7 (39.7-53.3)	41.9 (32.9-46.4)
9	74	47.3 (35.9-53.1)	39.2 (28.1-44.9)
≥10	170	51.8 (44.3-55.6)	44.1 (36.7-47.9)
35-39 years			
2	460	30.7 (26.4-32.8)	24.8 (20.8-26.8)
3	377	37.4 (32.5-39.9)	30.5 (25.9-32.9)
4	253	37.5 (31.6-40.6)	32.4 (26.6-35.4)
5	179	40.2 (33.0-43.9)	34.6 (27.7-38.2)
6	114	46.5 (37.3-51.2)	39.5 (30.5-44.1)
7	80	36.3 (25.7-41.6)	28.8 (18.8-33.8)
8	46	39.1 (25.0-46.3)	30.4 (17.1-37.2)
9	27	40.7 (22.2-50.2)	40.7 (22.2-50.2)
≥10	61	52.5 (39.9-58.9)	42.6 (30.2-49.0)
≥40 years			
2	81	17.3 (9.0-21.5)	16.0 (8.1-20.1)
3	50	24.0 (12.2-30.0)	22.0 (10.5-27.9)
4	35	20.0 (6.7-26.8)	20.0 (6.7-26.8)
5	20	25.0 (6.0-34.7)	25.0 (6.0-34.7)
≥6	17	47.1 (23.3-59.2)	41.2 (17.8-53.1)

* Term liveborn singleton of ≥2500 grams birthweight and survived for at least 28 days and without congenital anomaly.

Table V: Rate ratio of live delivery and "healthy baby" of selective single embryo transfer cycles by woman's age, stage of embryo development and number of embryos available, Australia 2004-2007

Number of embryos available	Live delivery		"Healthy baby"*	
	Rate (%)	ARR** (95% CI)	Rate (%)	ARR** (95% CI)
		Cleavage embryo		
<35 years				
2 (Ref)	*30.1*	*1.00*	*23.8*	*1.00*
3	31.2	1.05 (0.89-1.23)	25.3	1.08 (0.90-1.29)
4	32.0	1.07 (0.91-1.27)	24.4	1.03 (0.85-1.25)
5	31.5	1.06 (0.89-1.26)	25.2	1.06 (0.88-1.29)
≥6	37.1	1.26 (1.09-1.44)	30.5	1.28 (1.10-1.49)
35-39 years				
2 (Ref)	*26.0*	*1.00*	*21.2*	*1.00*
3	22.5	0.88 (0.69-1.13)	16.9	0.81 (0.61-1.07)
4	23.5	0.91 (0.70-1.19)	20.1	0.94 (0.71-1.26)
5	24.6	0.96 (0.73-1.26)	20.1	0.95 (0.70-1.29)
≥6	25.0	1.00 (0.80-1.24)	20.1	0.97 (0.76-1.24)
		Blastocyst embryo		
<35 years				
2 (Ref)	*43.7*	*1.00*	*36.2*	*1.00*
3	45.6	1.04 (0.88-1.22)	37.2	1.02 (0.85-1.22)
4	44.0	1.01 (0.85-1.20)	35.9	1.01 (0.83-1.21)
5	48.5	1.11 (0.92-1.33)	38.3	1.07 (0.87-1.32)
≥6	49.5	1.14 (0.98-1.33)	41.6	1.17 (0.99-1.39)
35-39 years				
2 (Ref)	*30.7*	*1.00*	*24.8*	*1.00*
3	37.4	1.17 (0.93-1.49)	30.5	1.20 (0.92-1.55)
4	37.5	1.18 (0.91-1.54)	32.4	1.27 (0.95-1.69)
5	40.2	1.33 (1.00-1.77)	34.6	1.42 (1.04-1.93)
≥6	43.6	1.39 (1.10-1.77)	36.3	1.44 (1.10-1.87)

* Term liveborn singleton of ≥2500 grams birthweight and survived for at least 28 days and without congenital anomaly.

** ARR was adjusted for clinics, cause infertility, previous pregnancy of 20 weeks or more gestation, and type of fertilization.

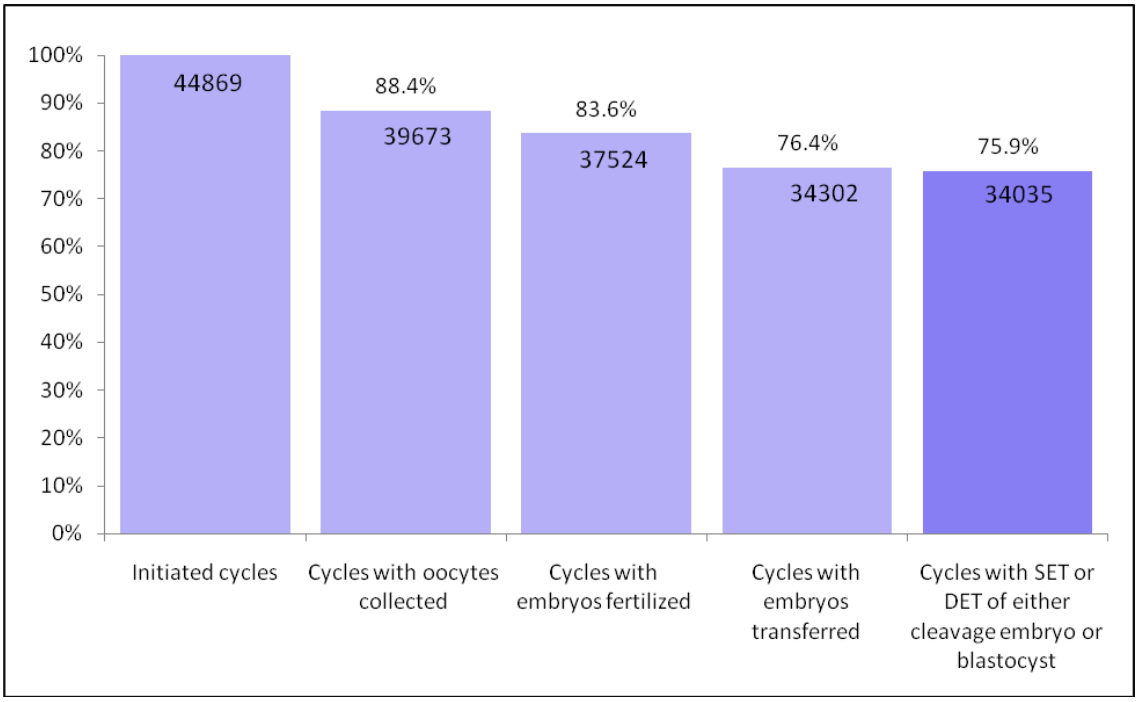
Table VI: Rate ratio of live delivery and "healthy baby" of selective single cleavage embryo transfer compared to selective blastocyst transfer cycles by woman's age, stage of embryo development and number of embryos available, Australia 2004-2007

Number of embryos available	Live delivery		"Healthy baby"*	
	Rate (%)	ARR** (95% CI)	Rate (%)	ARR** (95% CI)
<35 years				
2 blastocysts (Ref)	43.7	*1.00*	36.2	*1.00*
2 cleavage embryos	30.1	0.73 (0.59-0.91)	23.8	0.73 (0.57-0.93)
3 cleavage embryos	31.2	0.77 (0.62-0.95)	25.3	0.79 (0.62-0.99)
4 cleavage embryos	32.0	0.79 (0.64-0.98)	24.4	0.75 (0.59-0.96)
5 cleavage embryos	31.5	0.78 (0.63-0.97)	25.2	0.77 (0.61-0.99)
6 or more cleavage embryos	37.1	0.92 (0.76-1.12)	30.5	0.93 (0.75-1.16)
35-39 years				
2 blastocysts (Ref)	30.7	*1.00*	24.8	*1.00*
2 cleavage embryos	26.0	0.94 (0.69-1.28)	21.2	0.96 (0.68-1.36)
3 cleavage embryos	22.5	0.82 (0.60-1.14)	16.9	0.78 (0.54-1.11)
4 cleavage embryos	23.5	0.86 (0.62-1.21)	20.1	0.91 (0.63-1.32)
5 cleavage embryos	24.6	0.90 (0.64-1.26)	20.1	0.91 (0.63-1.33)
6 or more cleavage embryos	25.0	0.94 (0.69-1.27)	20.1	0.93 (0.66-1.30)

* Term liveborn singleton of ≥2500 grams birthweight and survived for at least 28 days and without congenital anomaly.

** ARR was adjusted for clinics, cause infertility, previous pregnancy of 20 weeks or more gestation, and type of fertilization.

Figure I: Progress of initiated cycles for women who had their first fresh autologous ART treatment, Australia 2004-2007



Chapter 7

Study 5:

Transfers of fresh blastocysts and blastocysts cultured from thawed cleavage embryos are associated with fewer miscarriages

About this chapter

Miscarriage is one of the common complications following ART treatment. Evidence on the association between miscarriage and the combination of fresh or thawed embryo transfers at cleavage or blastocyst stages is limited in the literature. The fifth study of this PhD thesis used the miscarriage rate to evaluate the proposed clinical practice model from Chapter 5. The chapter presents the fifth study of the PhD thesis "Transfers of fresh blastocysts and blastocysts cultured from thawed cleavage embryos are associated with fewer miscarriages". This study found lower miscarriage rates following transfer of single blastocyst and blastocyst cultured from thawed cleavage embryos. These findings confirmed that better pregnancy and perinatal outcomes are associated with a practice model which includes transfer of a single blastocyst and freezing of cleavage embryos in fresh cycles; and in subsequent thaw cycles transfer of a single blastocyst cultured from these thawed cleavage embryos.

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Abstract

The literature shows an inconsistent relationship between miscarriage and assisted reproduction treatment factors. This study assessed the association between miscarriage and transfer of fresh or thawed embryos at cleavage/blastocyst stages. A population study included 52,874 pregnancies following autologous cycles. The miscarriage rate was compared by groups of transferred embryos (fresh cleavage embryo, fresh blastocyst, thawed cleavage embryo, blastocyst from thawed cleavage embryo, thawed blastocyst), IVF/ICSI procedures, number of embryos transferred and woman's demographics. The overall miscarriage rate was 18.7%. Women aged 35–39 years and ≥40 years had a 51% and 177% increased hazard of miscarriage, respectively, compared with women <35 years. Women with history of miscarriage had 1.22 times hazard of miscarriage compared with those without previous miscarriage. Singleton pregnancies following fresh double-embryo transfer had 1.43 times higher rate of miscarriage than fresh single-embryo transfer. Fresh blastocyst transfer was associated with 8% less hazard of miscarriage than fresh cleavage-embryo transfer. Compared with pregnancies following thawed cleavage-embryo transfers, thawed blastocyst transfers were at 14% higher hazard of miscarriage. This study suggests that a practice model that includes transferring blastocysts and freezing cleavage embryos in fresh cycles would result in better outcomes.

Introduction

Miscarriage is a distressing experience for both patients and their clinicians. By the definition, miscarriage refers to the loss of the fetus prior to 20 weeks of gestation (Stephenson and Kutteh, 2007). Miscarriage is a common pregnancy complication, with estimated 15% of clinical pregnancies ending in miscarriage (Wilcox et al., 1988) and about one in four women experience such pregnancy failure during their lifetime (Chen and Creinin, 2007).

The literature shows that miscarriage is associated with several maternal demographic characteristics: maternal age, subfertility, history of miscarriage, obesity and chronic disease such as polycystic ovary syndrome (Bellver et al., 2007; Hakim et al., 1995; Heffner, 2004; Lashen et al., 2004; Nybo Andersen et al., 2000; Tough et al., 2006; Winter et al., 2002). Woman's age is an independent risk factor, with miscarriage rates of 90% in women aged 45 or older, 50% in women aged 40–44 years, 25% in women aged 35–39 years and about 13% among women aged younger than 34 years (Nybo Andersen et al., 2000). Women with subfertility were at 2.6 times increased risk of miscarriage than those without fertility problems (Hakim et al., 1995). Obese women had significantly higher rate of miscarriage than their normal weight controls (Bellver et al., 2007; Lashen et al., 2004).

Miscarriage was also found to be a common complication following assisted reproduction treatment (Tummers et al., 2003). Pregnancies conceived by

assisted reproduction treatment have had the same inherent demographic risks of miscarriage as those conceived spontaneously. Advanced maternal age, the independent risk factor for miscarriage, was well documented among pregnant women following assisted reproduction treatment (Wang et al., 2008). Almost all assisted reproduction patients have had some kind of female/male factor or unexplained subfertility, one of the identified risk factors for miscarriage. Obesity was common among pregnant women following assisted reproduction treatment and was associated with increased risk of miscarriage (Veleva et al., 2008).

Several factors related to assisted reproduction treatment have been suggested as being associated with miscarriage, but the literature about association is inconsistent. Double embryo transfer (DET) was related to high rates of twin pregnancy and single embryo transfer (SET) was associated with high rates of singleton pregnancy (Pandian et al., 2005). Twin pregnancies were shown to have less risk of miscarriage than singleton pregnancies (Lambers et al., 2006; Tummers et al., 2003). However, other studies found that SET did not increase the risk of miscarriage compared with DET (Martikainen et al., 2001; Veleva et al., 2006). An early study showed that transfer of thawed embryos through intracytoplasmic sperm injection (ICSI) resulted in higher miscarriage rate than thawed embryos through typical IVF (Aytoz et al., 1999). Other studies have found that there is no difference in miscarriage rates between IVF and ICSI (Bonduelle et al., 2002; Salumets et al., 2006). A more recent study stated that ICSI was related to higher rate of miscarriage compared with IVF (Matias et al.,

2007). There are also conflicting results on miscarriage rate by fresh embryo transfers versus thawed embryo transfers (Aytoz et al., 1999; Kansal Kalra et al., 2011).

In general, the association between miscarriage and factors related to assisted reproduction treatment is not consistent, and the combination of fresh or thawed embryo transfers at cleavage or blastocyst stages towards miscarriage has not been studied. This study aims to assess the relationship between miscarriage and treatment factor using Australian and New Zealand census data on assisted reproduction treatment from 2004 to 2008 and to investigate the association between miscarriage and fresh or thawed embryos at cleavage or blastocyst stages.

Materials and methods

Data and definitions used in this study are from the Australian and New Zealand Assisted Reproduction Database (ANZARD), located at the Perinatal and Reproductive Epidemiology Research Unit of the University of New South Wales. Data for ANZARD are collected annually, in a de-identified format, from all fertility centres within Australia and New Zealand. The ANZARD consists all assisted cycles with information on oocyte retrieval, number of oocytes collected, IVF and ICSI procedures, number of embryos created, stage of embryo development, number of embryos transferred, freezing of embryos and donation of gametes or embryos. It also includes information on pregnancy

and birth outcomes (birth status, gestational age, birthweight, congenital anomaly and neonatal mortality).

A subdataset of 55,478 clinical pregnancies following autologous (not involving donor spermatozoa, oocytes or embryos) embryo transfer cycles undertaken in Australian and New Zealand between 1 January 2004 and 31 December 2008 was extracted from ANZARD. Of these pregnancies, 1014 (1.8%) had outcomes not stated and so were excluded from analysis as well as 470 pregnancies following mixed fresh/thawed embryo transfer cycles or mixed cleavage/blastocyst embryo transfer cycles. Ectopic pregnancies (766) and fetal reduction/abortions (354) were also excluded. A total of 52,874 pregnancies following transfer of autologous fresh or thawed embryos at cleavage or blastocyst stage were included in the final analysis.

Study factors

Transferred embryos were categorized into five groups: (i) fresh cleavage embryo (day 2 or 3); (ii) fresh blastocyst (day 5 or 6); (iii) thawed cleavage embryo; (iv) blastocyst from thawed cleavage embryo; and (v) thawed blastocyst. Fertilization procedures were by either IVF or ICSI. The number of embryos transferred was grouped as SET, DET and three or more embryos.

For fresh single- and double-embryo transfers, the indicator 'selective/unselective embryo transfer' was adopted (Wang et al., 2010a). This indicator included four categories based on number of embryos available

(number of embryos transferred + number of embryos frozen): (i) selective SET, at least two embryos available and only one embryo transferred; (ii) unselective SET, only one embryo available and one embryo transferred; (iii) selective DET, at least three embryos available and two embryos transferred; and (iv) unselective DET, only two embryos available and two transferred.

Woman's age was calculated in completed years at the time of treatment and classified into three groups: (i) <35 years; (ii) 35–39 years; and (iii) ≥40 years. The cause of infertility was classified as male factor infertility (a male factor problem was diagnosed and no female factor problem), female factor infertility (tubal disease, endometriosis or other female factor problem were diagnosed and no male factor problem), combined male/female factor infertility (both male and female factor problems were diagnosed), unexplained infertility (neither male nor female factor problem was diagnosed) and not stated. Previous pregnancy of ≥20 weeks of gestation was grouped as yes, no and not stated. Previous pregnancy of <20 weeks of gestation (history of miscarriage) was categorized as yes, no and not stated.

Gestational age was defined as the number of completed weeks of gestation and calculated by the following formulae: for cleavage-embryo transfer, (pregnancy end date – embryo transfer date) + 16 days; for blastocyst transfer, (pregnancy end date – embryo transfer date) + 19 days.

Main outcome measures

The ANZARD definition of clinical pregnancy (evidence by ultrasound of intrauterine sac(s) and/or fetal heart(s) or examination of products of conception revealing chorionic villi) was used. This study was unable to identify or date pregnancies with sac only or chorionic villi only. Singleton pregnancy was defined as pregnancy with one fetal heart detected. Twin pregnancy was defined as pregnancy with two fetal hearts detected. Miscarriage was defined as a clinical pregnancy which has spontaneously ended before 20 complete weeks of gestation. For pregnancies with multiple fetal hearts, miscarriage was defined as loss of all gestations before 20 complete weeks.

Statistical analysis

As described elsewhere (Baker et al., 2010; Winter et al., 2002), pregnancy was treated as the unit of analysis in this study. Demographics (woman's age, cause of infertility and previous pregnancy of ≥20 weeks of gestation, previous pregnancy of <20 weeks of gestation) and treatment factors (type of fertilization and number of transferred embryos) were compared by fresh or thawed embryos at cleavage or blastocyst stage. Chi-squared test was used to test the miscarriage rates by patient demographics and treatment factors. Stratification by woman's age, number of embryos transferred and number of fetal hearts was used to overcome the potential confounders and effect modification. Cox regressions were used to estimate the risk of miscarriage (Madsen et al., 2007).

Gestational age was the time variable in the Cox regression models. Potential confounders identified in the literature and significant factors identified in the initial univariate analysis were included (enter method) in the multivariate Cox regression. Hazard ratio (HR) and adjusted hazard ratio (AHR) and 95% confidence intervals (CI) were calculated. Data were analysed using the Statistical Package for Social Sciences version 18.0 (SPSS, Chicago, IL, USA).

Ethics

Ethics approval for this study was granted by the Human Research Ethics Committee of the University of New South Wales, Sydney Australia.

Results

Almost half (45.7%) of the 52,874 clinical pregnancies were following fresh cleavage-embryo transfers, 22.0% following thawed cleavage-embryo transfers, 21.1% following fresh blastocyst transfers, 9.2% following thawed blastocyst transfers and 2.0% following transfer of blastocysts from thawed cleavage embryos.

A slightly higher proportion of pregnancies following transfer of blastocysts from thawed cleavage embryos were in women aged <35 years (56.8%). A little over half (52.5%) of pregnancies following transfer of blastocysts from thawed cleavage embryos were in nulliparous women compared with 74.1% of those following fresh cleavage-embryo transfers. A lower proportion of pregnancies

following transfers of blastocysts from thawed cleavage embryos (51.9%) were in women without history of miscarriage.

The proportion of SET was 53.8% for pregnancies following fresh embryo transfers compared with 57.7% following thawed embryo transfers. SET accounted for less than 50% of pregnancies following transfers of fresh or thawed cleavage embryos, 72.0% of pregnancies by transfers of fresh blastocysts, 65.1% of pregnancies by transfers of blastocysts from thawed cleavage embryos and 76.1% of pregnancies by transfers of thawed blastocysts.

The majority (78.6%) of the 52,874 pregnancies were singleton pregnancies, 10.7% were twin pregnancies, 0.3% had three or more fetal hearts, 6.6% had no fetal hearts and 3.8% did not have the number of fetal hearts stated. Of the 41,545 singleton pregnancies, 61.7% were following SET and 37.5% were following DET. Of the 5663 twin pregnancies, 10.4% were following SET, and 88.2% were following DET.

Table 1

The overall rate of miscarriage was 18.7%. The lowest miscarriage rate was of pregnancies conceived from fresh blastocyst transfers (17.2%), this increased to 18.2% of pregnancies from fresh cleavage-embryo transfers, 19.4% of pregnancies from transfer of blastocysts from thawed cleavage embryos, 20.0% of pregnancies from thawed cleavage-embryo transfers and 22.1% of pregnancies from thawed blastocyst transfers (Figure 1).

Figure 1

Singleton pregnancies following fresh blastocyst transfer had lower rate of miscarriage than those following fresh cleavage embryos (9.8% and 11.0%, respectively; P <0.01, Chi-squared test). Twin pregnancies following fresh blastocyst transfer had higher rate of miscarriage than those following fresh cleavage embryos (6.4% and 4.4%, respectively; P = 0.02, Chi-squared test).

There is a significant difference in miscarriage rates for twin pregnancies amongst thawed cleavage-embryo transfers (2.8%), transfer of blastocysts from thawed cleavage embryos (4.8%) and thawed blastocyst transfers (5.9%; P = 0.03, Chi-squared test). The difference in miscarriage rates of singleton pregnancies was not significant among the three groups of thawed embryo transfers (Chi-squared test).

Pregnancies in women aged 35–39 years and in women aged ≥40 years had 1.52 and 2.85 times higher rate of miscarriage, respectively, than those in woman aged <35 years. Male factor-only infertility was associated with a lower rate of miscarriage than female factor infertility. History of miscarriage was associated with a 36% increased hazard of miscarriage (Table 2).

The miscarriage rate was significantly higher for pregnancies following DET (19.3%) than SET (18.0%; HR 1.07, 95% CI 1.03–1.12). Pregnancies following thawed embryo transfer had 19% higher rate of miscarriage compared with those following fresh embryo transfers (HR 1.19, 95% CI 1.14–1.24; Table 2).

Table 2

For pregnancies following fresh SET (Table 3), the miscarriage rate was significantly higher for twin pregnancies (17.5%) than singleton pregnancies (9.0%; HR 1.96, 95% CI 1.51–2.53), while for pregnancies following fresh DET, the miscarriage rate was significantly lower for twin pregnancies (3.7%) than singleton pregnancies (12.7%; HR 0.27, 95% CI 0.23–0.33). There is no significant difference in miscarriage rates between singleton and twin pregnancies following thawed SET. The miscarriage rate was significantly lower for twin pregnancies (2.9%) than singleton pregnancies (12.0%) following thawed DET (HR 0.23, 95% CI 0.17–0.32).

Table 3

Table 4 details the association between miscarriage and selective/unselective embryo transfers in fresh treatment. For all pregnancies and singleton pregnancies, those following unselective SET, selective DET and unselective DET were related to higher rates of miscarriage compared with pregnancies following selective SET. While for twin pregnancies, selective DET and unselective DET were associated with lower rates of miscarriage than selective SET. Overall, singleton pregnancies following DET had 1.43 times higher rate of miscarriage than those following SET (HR 1.43, 95% CI 1.33–1.54). Twin pregnancies following DET had 0.20 times lower rate of miscarriage than those following SET (HR 0.2, 95% CI 0.14–0.26).

Table 4

After age stratification, the miscarriage rate was significantly lower for pregnancies following single fresh blastocyst transfers than those following single fresh cleavage-embryo transfers in women aged <35 years (HR 1.12, 95% CI 1.01–1.24) and 35–39 years (HR 1.13, 95% CI 1.01–1.26). The miscarriage rate was not statistically significantly lower for single fresh blastocyst transfers than single fresh cleavage-embryo transfers in women aged ≥40 years. There is no significant difference in miscarriage rates amongst SET of blastocyst from thawed cleavage embryo, thawed cleavage embryo and thawed blastocyst as well as those following fresh or thawed DET at cleavage or blastocyst stage (Table 5).

Table 5

Table 6 presents remaining significant factors associated with miscarriage in multivariate Cox regression. The most significant demographic factors associated with miscarriage were advanced maternal age and history of miscarriage. Pregnancies in women aged 35–39 years and ≥40 years were associated with 51% (AHR 1.51, 95% CI 1.45–1.58) and 177% (AHR 2.77, 95% CI 2.61–2.93) increased hazard of miscarriage than those in women aged <35 years. Pregnancies in women had history of miscarriage had 1.22 times hazard of miscarriage compared with those without previous miscarriage (AHR 1.22, 95% CI 1.17–1.28). Pregnancies following fresh blastocyst transfers had 8% lower hazard of miscarriage than those following fresh cleavage-embryo transfers

(AHR 0.92, 95% CI 0.87–0.98). Compared with pregnancies following thawed cleavage-embryo transfers, those following thawed blastocyst transfers had 14% higher hazard of miscarriage (AHR 1.14, 95% CI 1.05–1.23).

Even though a lower rate of miscarriage for pregnancies following transfer of blastocysts from thawed cleavage embryos compared with those following thawed cleavage-embryo and thawed blastocyst transfers was identified, the multivariate analysis did not show a significant difference because of the insufficient number of pregnancies following transfer of blastocysts from thawed cleavage embryos.

Table 6

Table 7 shows the subanalysis of pregnancies following selective or unselective fresh SET cycles. The miscarriage rate for pregnancies following selective transfer of single cleavage embryos was 15.8%, significantly higher than the rate (14.7%) for those following selective transfer of single blastocysts (AHR 1.13, 95% CI 1.04–1.24). Similarly, pregnancies following unselective transfer of single cleavage embryos had 1.15 times hazard of miscarriage compared with unselective transfer of single blastocysts (AHR 1.15, 95% CI 1.02–1.31; Table 7).

Table 7

Discussion

This large population study found pregnancies following fresh blastocyst transfers had significantly lower rate of miscarriage than those following fresh cleavage-embryo transfers. Four factors were associated with a lower rate of miscarriage: fresh blastocyst transfers (as opposed to fresh cleavage-stage transfers), lower number of embryos transferred, younger woman's age and lack of previous miscarriage. Of pregnancies following thawed embryo transfers, the miscarriage rate was lower for thawed cleavage-embryo transfers than thawed blastocyst transfers.

The strengths of this cohort study are the bi-national coverage (Australia and New Zealand) and the inclusion of the most recent pregnancy data (2004–2008). This provides significant study power and generalizability that is difficult to achieve with clinic-based studies. One limitation of this study is the potential variability in reporting of pregnancy outcomes. The information on pregnancy outcomes was not stated for 1.8% of clinical pregnancies of the study cohort. However, the effect on the results of missing data is likely to be minimal as it represents a very small percentage of the total data. It is likely that the missing outcomes would be non-differentially distributed across the groups of embryo transfers. A second limitation of this study is that women may have appeared more than once in the 5-year period, e.g. with two or three pregnancies. Ideally, a generalized estimating equation (GEE) model would overcome the multiple appearances of one patient in the study population. However, due to lack of

unique patient identity in the dataset, this study was unable to apply a GEE model to confirm the independence amongst pregnancies of one patient.

As used in other studies (Baker et al., 2010; Winter et al., 2002), pregnancy was considered as the unit of analysis. The analysis was undertaken with the assumption that each pregnancy was independent to each other, even for pregnancies of same patient/couple. The analysis has two major strengths. First, the most significant risk factors of miscarriage in the literature, namely the advanced maternal age and history of miscarriage, are specific to each pregnancy, not the patient. Second, following assisted reproduction treatment, even pregnancies of the same patient/couple varied by number of embryos transferred, stage of embryo development and fresh/thaw status. It is acknowledged that there are some patient-inherited factors such as genetic factor that are possibly associated with miscarriage. However, the inclusion of maternal age, previous pregnancy of ≥20 weeks of gestation and history of miscarriage in the multivariate analysis has limited the bias due to patient-inherited factors.

In agreement with Papanikolaou et al. (2006), the current study found that fresh blastocyst transfers were associated with a lower rate of miscarriage than fresh cleavage-embryo transfers in singleton pregnancies (Figure 1) and all pregnancies (Table 6). Papanikolaou and colleagues found that the miscarriage rate for pregnancies following transfer of a single day-3 embryo was 1.6 times the rate for transfer of a single day-5 embryo (Papanikolaou et al., 2006). The

reason for this difference between fresh cleavage and blastocyst transfers may have been related to the natural selection for chromosomally competent embryos by growing through to blastocyst (Butterworth, 2001). Thus, pregnancies following fresh blastocyst transfer may have a better prospect for ongoing pregnancy than those following fresh cleavage-embryo transfers. In addition, the asynchronization between the altered endometrium and early exposure of cleavage-stage embryos might relate to the difference in miscarriage rates between fresh transfers at cleavage and blastocyst stages (Papanikolaou et al., 2006). Whilst, the current multivariate analysis controlled for many important potential confounders such as woman's age, cause of infertility, previous pregnancy of ≥20 weeks of gestation, previous pregnancy of <20 weeks of gestation, type of fertilization and number of embryo transferred, it was unable to control other potential confounders such as body mass index and length of infertility. This may limit the clinical validity of the finding.

Interestingly, this study found that pregnancies following thawed cleavage-embryo transfers had a significantly lower rate of miscarriage than those following thawed blastocyst transfers. A possible explanation for this may be that patients who had frozen blastocysts may have had more eggs retrieved at the time of egg collection and there is evidence linking increased egg numbers to increased chromosomal aneuploidy in the subsequent embryos (Verberg et al., 2009). Chromosomal aneuploidy is an identified major underlying cause of miscarriage (Spandorfer et al., 2004). Salumets et al. (2003) suggested that late

freezing (day 3) may have led to more embryo damage in the freezing–thawing process than early freezing (day 2) and more damage of embryos was associated with miscarriage. The same mechanism may have also applied to thawed blastocysts (late freezing) compared with thawed cleavage embryos (early freezing). Hence the higher miscarriage rate for thawed blastocyst transfers may have been caused by more damage of blastocysts in the freezing–thawing procedures.

This study confirms the finding by Aytoz et al. (1999), that miscarriage rate following fresh embryo transfers was lower than thawed embryo transfers. However, a recently published study found that pregnancies following fresh embryo transfers were more likely to end in first-trimester loss with clinical pregnancy loss of 27/193 (14.0%) compared with 12/112 (10.7%) for pregnancies following thawed embryo transfers (Kansal Kalra et al., 2011). The clinical pregnancy loss in the first trimester in the current study was 16.1% for pregnancies following fresh embryo transfer and 19.2% for thawed embryo transfer. Suleena Kansal and colleagues explained that the higher first-trimester loss following fresh transfer may relate to multiple pregnancies. However, in the current study, miscarriage was still lower following fresh embryo transfers (8.9%) than thawed embryo transfers (9.9%) even after stratifying the data by single fetal heart. The conflicting results imply the bias in study sizes and population source, with Suleena Kansal's study including 340 pregnancies from single university centre and the current study including 52,874 pregnancies from all clinics in Australia and New Zealand.

The reason for higher miscarriage rate following thawed embryo transfers than fresh embryo transfer is not clear. A higher pregnancy rate combined with a low rate of miscarriage for fresh embryo transfers when compared with thawed embryo transfers may be related to selection bias. This selection bias relates to the elective embryo transfers in fresh cycles, i.e. the best-quality embryos are elected for transfer and the other relatively poorer-quality embryos are for cryopreservation, according to embryo quality as determined by embryo scoring/grading systems including morphological appearance and growth rate of the embryo (Fisch et al., 2007; Terriou et al., 2007). The embryo damage during the freezing–thawing process may have also been associated with the difference in miscarriage rates between fresh and thawed embryo transfers (Salumets et al., 2006).

It was stated that quality of embryo was associated with miscarriage (Winter et al., 2002). The quality of transferred embryos in terms of embryo score/grade information was not available in the current dataset. A previous published indicator, the selective/unselective embryo transfer was used in the current study to specify the quality of embryo and quantity of embryos available for transfer (Wang et al., 2010a). The subanalysis of pregnancies following fresh SET/DET found that the miscarriage rate for unselective SET was 20.0%, significantly higher than the rate of 15.3% for selective SET. Similarly, the miscarriage rate of 21.7% for unselective DET was significantly higher than the rate of 16.8% for selective DET. The higher rates of miscarriage following unselective embryo transfer are possibly related to the quality of transferred

embryos especially for cleavage embryos (Winter et al., 2002). Since, there was only one cleavage embryo available for transfer in unselective SET and two available in unselective DET, poorer-quality embryo(s) may have been transferred (Flisser and Licciardi, 2006). Similarly for blastocysts, when limited cleavage embryos are available, extended blastocyst culture would result in either no transfer or possibly transfer of a poorer-quality embryo (Barrenetxea et al., 2005).

The current study adds weight and greater clarity to the equivocal body of evidence about the relationship between miscarriage and IVF/ICSI procedures, e.g. higher miscarriage rate following fresh ICSI embryo transfers compared with fresh IVF embryo transfers (Matias et al., 2007), and the lack of difference in miscarriage rates between thawed ICSI embryo transfers and thawed IVF embryo transfers (Salumets et al., 2006). To make more relevant comparisons, the current data were stratified for pregnancies following fresh and thawed embryo transfers. Of pregnancies following fresh transfers, the miscarriage rate of 17.6% for ICSI was slightly lower than the 18.4% for IVF, but not statistically significant. Similarly, of pregnancies following thawed embryo transfers, the miscarriage rate of 20.1% for ICSI was not significantly different from 21.1% for IVF. These results from the multivariate analysis support Salumets's findings, but disagree with Matias' findings. Although, there are no randomised data on miscarriage rates comparing IVF and ICSI procedures (van Rumste et al., 2004), the similar pregnancy and delivery rates between IVF and ICSI suggest that it is likely there is no significant difference in miscarriage rates between the two

procedures (Bahceci and Ulug, 2005; Kozinszky et al., 2003; Tummers et al., 2003).

It was suggested that the success of an assisted reproduction treatment should focus on a healthy singleton considering prevalent adverse maternal and perinatal outcomes of multiple pregnancies (Campbell and Templeton, 2004; Healy, 2004; Wang et al., 2009, 2010b). To achieve this focus, Australia and New Zealand have shifted the clinical practice to SET (RTAC, 2008). The latest Australia and New Zealand report shows that the proportion of SET increased from 40.5% in 2004 to 67.8% in 2008 (Wang et al., 2010c). As a result, the rate of singleton pregnancy in the current study increased from 74.4% in 2004 to 81.7% in 2008. However, twin pregnancies were reported to be less likely to have miscarriages than singleton pregnancies (Tummers et al., 2003): Tummers et al. analysed 1597 clinical IVF/ICSI pregnancies all following DET and reported a lower rate of miscarriage in twin pregnancies (defined by two gestational sacs) than singleton pregnancies (defined by one gestational sac). The current study further compared the miscarriage rates between singleton and twin pregnancies defined by number of fetal hearts as the information on number of gestational sacs was not available. Consistent with Tummers' study, this study found that the rate of miscarriage of twin pregnancies (defined by two fetal hearts) following fresh DET was 3.7%, significantly lower than the rate of 12.7% of singleton pregnancies (defined by one fetal heart) following fresh DET. Also, the rate of miscarriage of twin pregnancies following fresh SET was 17.5%, which was significantly higher than the rate of 9.0% of singleton pregnancies

following fresh SET. The difference may have been related to monozygotic twin pregnancy following SET. It was suggested twin pregnancy following SET is considered as monozygotic twin pregnancy (Vitthala et al., 2009) and that, in general, monozygotic twin pregnancies are at increased risk of miscarriage (Sebire et al., 1997). These results suggest that when comparing the miscarriage rates between twin pregnancies and singleton pregnancies following assisted reproduction treatment, the number of embryos transferred should be taken into consideration.

Of twin pregnancies following fresh embryo transfers (Figure 1), blastocyst transfers were associated with increased hazard of miscarriage than cleavage-embryo transfers. This may have been related to a disproportionate number of unselective DET in double blastocyst transfers (52.8%) compared with 20.2% for unselective DET in double cleavage-embryo transfers. This may have led to a higher proportion of poorer-quality blastocysts being transferred in unselective DET of blastocysts (Barrenetxea et al., 2005). This may have also been associated with a higher rate of monozygotic twin pregnancies following blastocyst transfers than cleavage transfers (Vitthala et al., 2009) and a higher miscarriage rate of monozygotic twin pregnancies than dichorionic twin pregnancies (Sebire et al., 1997).

Supported by the literature, the current study found that the most significant factor intersecting the relationship between miscarriage and assisted reproduction treatment factors is woman's age (Nybo Andersen et al., 2000;

Salumets et al., 2006; Wang et al., 2009). The miscarriage was significantly higher in older women following both fresh and thawed embryo transfers and for both singleton and twin pregnancies. Even in the multivariate analysis, older age remained an independent risk for miscarriage. Woman's age at the time of treatment in fresh cycles was more relevant to miscarriage, as the physiological age of oocyte was considered being highly associated with miscarriage (Salumets et al., 2006) and was suggested as an indicator for SET (RTAC, 2008). When pregnancies were limited to fresh SET, the current study found that the miscarriage rate was 12.7% for women aged <35 years, 20.6% for women aged 35–39 years and 36.5% for women aged ≥40 years. In contrast, woman's age in thaw cycles did not reflect the physiological age of oocyte, but there is still significant difference in miscarriage rates among the three age groups (17.3% for women aged <35 years, 21.9% for women aged 35–39 years and 32.6% for women aged ≥40 years). The evidence of miscarriage rates shown in fresh and thawed embryo transfers among different age groups suggests that access to assisted reproduction treatment at a younger age would result in better pregnancy outcomes.

With the improved clinical and laboratory technologies, more women are able to undertake treatment incorporating fresh embryo transfer and subsequent thawed embryo transfers. A previous study (Wang et al., 2010b) suggested that, for younger patients to achieve a live-born term singleton with normal birthweight, the optimum practice model is the transfer of a single blastocyst and freezing of cleavage embryos in fresh cycles and the subsequent transfer of

a single blastocyst cultured from these thawed cleavage embryos. This current study further confirms the practice model showing a lower rate of miscarriage. It would be interesting to assess whether this proposed practice model would hold true if one restricted the data analysis to comparing the miscarriage rate between the various groups of embryo transfers for selective SET. Unfortunately, the data are unable to be stratified for selective and unselective embryo transfers in thaw cycles (Table 7).

In conclusion, the evidence from this retrospective observational research has generated a hypothesis that better perinatal outcomes are associated with a practice model which includes transfer of a single blastocyst and freezing of cleavage embryos in fresh cycles; and in subsequent thaw cycles, transfer of a single blastocyst cultured from these thawed cleavage embryos. It is important to note that this study may be subject to selection and measurement bias and that there is a need to perform a prospective randomised controlled trial to confirm these findings.

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Table I: Selected women's demographics and treatment factors of embryo transfer cycles, Australia and New Zealand 2004-2008

	Pregnancies following fresh cycles				Pregnancies following thaw cycles					
	Fresh cleavage (n=24163)		Fresh blastocyst (n=11151)		Thawed cleavage (n=11628)		Blastocyst from thawed cleavage (n=1081)		Thawed blastocyst (n=4851)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Women's age (years)										
<35	12432	51.5	5914	53.0	6002	51.6	614	56.8	2510	51.7
35-39	9138	37.8	4110	36.9	4400	37.8	378	35.0	1889	38.9
≥40	2593	10.7	1127	10.1	1226	10.5	89	8.2	452	9.3
Cause of infertility										
Male factor	7786	32.2	3239	29.0	3679	31.6	324	30.0	1300	26.8
Female factor	6823	28.2	3379	30.3	4195	36.1	339	31.4	1362	28.1
Combined male/female factor	3499	14.5	1705	15.3	1563	13.4	155	14.3	619	12.8
Unexplained	5202	21.5	2195	19.7	1848	15.9	216	20.0	1146	23.6
Not stated	853	3.5	633	5.7	343	2.9	47	4.3	424	8.7
Previous pregnancy of ≥20 weeks gestation										
No	17896	74.1	7110	63.8	7501	64.5	567	52.5	3230	66.6
Yes	4845	20.1	2507	22.5	3630	31.2	278	25.7	1120	23.1
Not stated	1422	5.9	1534	13.8	497	4.3	236	21.8	501	10.3
Previous pregnancy of <20 weeks gestation										
No	17126	70.9	6480	58.1	7961	68.5	561	51.9	3006	62.0
Yes	5616	23.2	3137	28.1	3170	27.3	284	26.3	1342	27.7
Not stated	1421	5.9	1534	13.8	497	4.3	236	21.8	503	10.4
Type of fertilization										
IVF	9459	39.1	4789	42.9	4936	42.4	499	46.2	2304	47.5
ICSI	14314	59.2	6250	56.0	6307	54.2	382	35.3	2280	47.0
Not stated	390	1.6	112	1.0	385	3.3	200	18.5	267	5.5
Number of embryos transferred										
1	10961	45.4	8026	72.0	5732	49.3	704	65.1	3691	76.1
2	12837	53.1	3088	27.7	5772	49.6	368	34.0	1151	23.7
≥3	365	1.5	37	0.3	124	1.1	9	0.8	9	0.2

Table II: Miscarriage by women's demographic and treatment factor, Australia and New Zealand 2004-2008

	Number of pregnancies	Number of Miscarriages	Miscarriage rate (%)	HR (95% CI)
Women's age (years)				
<35	27472	3828	13.9	1.00
35-39	19915	4116	20.7	1.52 (1.46-1.59)
≥40	5487	1967	35.8	2.85 (2.70-3.01)
Cause of infertility				
Male factor	16328	2882	17.7	1.00
Female factor	16098	3192	19.8	1.14 (1.08-1.20)
Combined male/female factor	7541	1481	19.6	1.12 (1.06-1.20)
Unexplained	10607	1938	18.3	1.04 (0.98-1.10)
Not stated	2300	418	18.2	1.04 (0.94-1.15)
Previous pregnancy of ≥20 weeks gestation				
No	36304	6700	18.5	1.00
Yes	12380	2397	19.4	1.05 (1.00-1.10)
Not stated	4190	814	19.4	1.05 (0.97-1.13)
Previous pregnancy of <20 weeks gestation				
No	35134	6026	17.2	1.00
Yes	13549	3071	22.7	1.36 (1.30-1.42)
Not stated	4191	814	19.4	1.13 (1.05-1.22)
Type of fertilization				
IVF	21987	4247	19.3	1.00
ICSI	29533	5417	18.3	0.95 (0.91-0.98)
Not stated	1354	247	18.2	0.94 (0.82-1.06)
Number of embryos transferred				
1	29114	5252	18.0	1.00
2	23216	4474	19.3	1.07 (1.03-1.12)
≥3	544	185	34.0	2.04 (1.77-2.37)
Fresh/thaw status				
Fresh embryos	35314	6300	17.8	1.00
Thawed embryos	17560	3611	20.6	1.19 (1.14-1.24)

HR: Hazard ratio by univariate Cox regression.

Table III: Miscarriage by number of fetal hearts and number of embryo transferred, Australia and New Zealand 2004-2008

	Fresh embryo transfers			*Thawed embryo transfers*		
	Number of pregnancies	**Miscarriage rate (%)**	**HR (95% CI)**	**Number of pregnancies**	**Miscarriage rate (%)**	**HR (95% CI)**
Following single embryo transfer						
Singleton pregnancy	16950	9.0	1.00	8672	10.8	1.00
Twin pregnancy	349	17.5	1.96 (1.51-2.53)	238	7.1	0.65 (0.40-1.04)
Following double embryo transfer						
Singleton pregnancy	10466	12.7	1.00	5113	12.0	1.00
Twin pregnancy	3722	3.7	0.27 (0.23-0.33)	1273	2.9	0.23 (0.17-0.32)

HR: Hazard ratio by univariate Cox regression.

Table IV: Miscarriage following fresh embryo transfer by number of fetal hearts and number of embryo transferred, Australia and New Zealand 2004-2008

	Number of pregnancies	Number of Miscarriages	Miscarriage rate (%)	HR (95% CI)
All pregnancy[a]				
Selective SET	13793	2116	15.3	1.00
Unselective SET	5194	1040	20.0	1.34 (1.25-1.45)
Selective DET	9376	1578	16.8	1.11 (1.04-1.18)
Unselective DET	6549	1420	21.7	1.46 (1.37-1.57)
All SET	*18987*	*3156*	*16.6*	*1.00*
All DET	*15925*	*2998*	*18.8*	*1.15 (1.09-1.21)*
Singleton pregnancy[b]				
Selective SET	12421	1053	8.5	1.00
Unselective SET	4529	480	10.6	1.26 (1.14-1.41)
Selective DET	5869	701	11.9	1.43 (1.30-1.58)
Unselective DET	4597	632	13.7	1.67 (1.51-1.84)
All SET	*16950*	*1533*	*9.0*	*1.00*
All DET	*10466*	*1333*	*12.7*	*1.43 (1.33-1.54)*
Twin pregnancy[c]				
Selective SET	266	52	19.5	1.00
Unselective SET	83	9	10.8	0.54 (0.27-1.10)
Selective DET	2594	85	3.3	0.16 (0.11-0.22)
Unselective DET	1128	51	4.5	0.22 (0.15-0.32)
All SET	*349*	*61*	*17.5*	*1.00*
All DET	*3722*	*136*	*3.7*	*0.20 (0.14-0.26)*

DET = double-embryo transfer; HR = hazard ratio by univariate Cox regression; SET = single embryo-transfer.

[a]Including pregnancies with or without fetal heart and where number of fetal hearts was not stated.

[b]Pregnancies with one fetal heart.

[c]Pregnancies with two fetal hearts.

Table V: Miscarriage by embryo transfer cycles and number of embryos transferred and woman's age, Australia and New Zealand 2004-2008

	<35 years		35-39 years		≥40 years	
	Miscarriage rate (%)	HR (95% CI)	Miscarriage rate (%)	HR (95% CI)	Miscarriage rate (%)	HR (95% CI)
Pregnancies following SET						
Fresh blastocyst	11.9	1.00	19.4	1.00	33.6	1.00
Fresh cleavage	13.2	1.12 (1.01-1.24)	21.6	1.13 (1.01-1.26)	38.5	1.18 (0.96-1.45)
Blastocyst from thawed cleavage	16.5	1.00	24.2	1.00	38.1	1.00
Thawed cleavage	16.7	1.00 (0.78-1.29)	20.9	0.85 (0.64-1.12)	32.7	0.87 (0.52-1.46)
Thawed blastocyst	19.9	1.22 (0.95-1.58)	24.2	1.01 (0.76-1.33)	29.7	0.77 (0.46-1.31)
Pregnancies following DET						
Fresh blastocyst	13.6	1.00	20.2	1.00	34.3	1.00
Fresh cleavage	11.0	0.81 (0.67-0.97)	19.2	0.94 (0.82-1.07)	38.3	1.15 (0.99-1.33)
Blastocyst from thawed cleavage	14.8	1.00	20.7	1.00	22.0	1.00
Thawed cleavage	16.6	1.13 (0.77-1.66)	20.7	1.01 (0.69-1.45)	34.3	1.71 (0.88-3.33)
Thawed blastocyst	16.0	1.09 (0.71-1.68)	24.3	1.22 (0.82-1.83)	30.6	1.47 (0.71-3.07)

DET = double-embryo transfer; HR = hazard ratio by univariate Cox regression; SET = single embryo-transfer.

Table VI: Miscarriage by embryo transfer cycles, Australia and New Zealand 2004-2008

	Miscarriage rate (%)	HR (95% CI)	AHR (95% CI)
Group of embryo			
Fresh cleavage	18.2	1.00	1.00
Fresh blastocyst	17.2	0.94 (0.89-0.99)	0.92 (0.87-0.98)
Thawed cleavage	20.0	1.13 (1.07-1.19)	1.12 (1.07-1.18)
Blastocyst from thawed cleavage	19.4	1.09 (0.95-1.25)	1.11 (0.96-1.28)
Thawed blastocyst	22.1	1.26 (1.18-1.35)	1.25 (1.17-1.34)
Women's age (years)			
<35	13.9	1.00	1.00
35-39	20.7	1.52 (1.46-1.59)	1.51 (1.45-1.58)
≥40	35.8	2.85 (2.70-3.01)	2.77 (2.61-2.93)
Cause of infertility			
Male factor	17.7	1.00	1.00
Female factor	19.8	1.14 (1.08-1.20)	1.06 (1.00-1.13)
Combined male/female factor	19.6	1.12 (1.06-1.20)	1.11 (1.04-1.18)
Unexplained	18.3	1.04 (0.98-1.10)	0.97 (0.92-1.03)
Not stated	18.2	1.04 (0.94-1.15)	0.96 (0.86-1.07)
Previous pregnancy of ≥20 weeks gestation			
No	18.5	1.00	1.00
Yes	19.4	1.05 (1.00-1.10)	0.92 (0.88-0.96)
Not stated	19.4	1.05 (0.97-1.13)	0.95 (0.32-2.82)
Previous pregnancy of <20 weeks gestation			
No	17.2	1.00	1.00
Yes	22.7	1.36 (1.30-1.42)	1.22 (1.17-1.28)
Not stated	19.4	1.13 (1.05-1.22)	1.20 (0.41-3.56)
Type of fertilization			
IVF	19.3	1.00	1.00
ICSI	18.3	0.95 (0.91-0.98)	1.00 (0.95-1.05)
Not stated	18.2	0.94 (0.82-1.06)	0.92 (0.79-1.06)
Number of embryos transferred			
1	18.0	1.00	1.00
2	19.3	1.07 (1.03-1.12)	0.96 (0.92-1.00)
≥3	34.0	2.04 (1.77-2.37)	1.27 (1.09-1.47)

The Entre method for embryo group, woman's age, cause of infertility, previous pregnancy of 20 weeks or more gestation, previous pregnancy of less than 20 weeks of gestation, type of fertilization and number of embryos transferred was used in Cox regression variables.

AHR: Adjusted hazard ratio by multivariate Cox regression.

HR: Hazard ratio by univariate Cox regression.

Table VII: Miscarriage following fresh single embryo transfers by embryo transfer cycles, Australia and New Zealand 2004-2008

	Number of pregnancies	Number of Miscarriages	Miscarriage rate (%)	HR (95% CI)	AHR (95% CI)
Selective SET					
Fresh blastocyst	5945	874	14.7	1.00	1.00
Fresh cleavage	7848	1242	15.8	1.08 (0.99-1.18)	1.13 (1.04-1.24)
Unselective SET					
Fresh blastocyst	2081	382	18.4	1.00	1.00
Fresh cleavage	3113	658	21.1	1.17 (1.04-1.33)	1.15 (1.02-1.31)

The Entre method for embryo group, woman's age, cause of infertility, previous pregnancy of 20 weeks or more gestation, previous pregnancy of less than 20 weeks of gestation, and type of fertilization was used in Cox regression variables.

AHR: Adjusted hazard ratio by multivariate Cox regression.

HR: Hazard ratio by univariate Cox regression.

DET = double-embryo transfer; SET = single embryo-transfer.

Chapter 8

Conclusions and recommendations

Main conclusions

The coherent five related studies of this PhD thesis added weight to the published body of evidence on the association between ART treatment factors and pregnancy and birth outcomes. This PhD thesis provides age-specific rates of pregnancy and live delivery which is essential to educate patients regarding their fertility potential and the benefits of early fertility assessment and ART treatment where clinically indicated. It recommended that an age of <43 years is appropriate to initiate a new fresh ART treatment and thus implied the need to review the current national recommendation on the age limit of <44 to initiate a new ART treatment.

The PhD thesis evaluated the RTAC policy on SET in Australia and New Zealand by comparing the perinatal outcomes between SET births and DET births. The lower rates of preterm birth, low birthweight and perinatal mortality for singletons following SET compared to singletons following DET provided evidence that SET not only reduced the multiple pregnancy rate, but also improved overall perinatal outcomes for singletons. It indicated that continuing the increase in the proportion of SET would improve the overall birth outcomes of ART treatment.

The PhD thesis proposed a new indicator "healthy baby" which summarises a composite of perinatal outcomes as the optimum goal for an ART treatment. Using the "healthy baby" indicator, it clarified the inconsistent results in the

literature on the relationship between ART treatment factors and the optimal perinatal outcome. It suggested that selective single blastocyst transfer is the optimal clinical practice in a fresh ART treatment, especially for patients aged younger than 35 years in their first ART cycle who had four or more embryos available at day two or day three for transfer.

Using the "healthy baby" rate and miscarriage rate, the PhD thesis assessed the efficacy of embryo transfer at either cleavage or blastocyst stage for both fresh and thaw cycles. The findings of the PhD thesis hypothesised that for younger patients, better pregnancy and perinatal outcomes are associated with a practice model which includes transfer of a selected single blastocyst and freezing of cleavage embryos in a fresh cycle and transfer of a selected single blastocyst cultured from these thawed cleavage embryos in subsequent thaw cycles.

Fertility potential and early access to ART treatment

The rising proportion (from 6.9% in 1995 to 14.5% in 2008) of women having their first baby aged 35 years or older in Australia (Laws et al., 2007; Laws et al., 2010) suggests that a misperception remains in the community about a woman's ability to conceive spontaneously with advancing age. Fertility potential decreases with advancing woman's age (Menken et al., 1986). ART is not a guarantee of pregnancy for older women (Sullivan et al., 2008). Even when a pregnancy is achieved, the higher risks of miscarriage and other pregnancy complications in older women decrease the livelihood of the birth of

a liveborn baby (Nybo Andersen et al., 2000; Heffner, 2004; Alshami et al., 2011).

The PhD thesis modelled the benefits of early access to ART treatment and estimated the number of extra live deliveries when treatment was one, two or three years earlier. If women aged 35 years or older had a first autologous fresh cycle one year earlier, the number of expected live deliveries would increase by 15% compared with the observed number. If they had treatment two or three years earlier, an increase of 30% and 44% respectively in live deliveries would be expected. Given the fact that younger women who underwent ART treatment would have higher likelihood of live birth spontaneously than older women, the PhD thesis reconfirms the need for primary care clinicians to refer couples for fertility assessment as soon as possible for assessment and treatment where clinically indicated.

Age to initiate new ART treatment

This PhD thesis suggested, using populating data, that it is reasonable to initiate a new autologous ART treatment cycle to the age of 42 complete years. This suggested age is one year less than the national recommendation of <44 years (Department of Health and Ageing, 2006). The first study of the thesis found that when the first autologous fresh treatment was at 43 years of age, 6% of initiated cycles resulted in a clinical pregnancy, but nearly 60% of those pregnancies ended in miscarriage. When the first autologous fresh treatment was at 42 years, it produced an overall clinical pregnancy rate of 8%, and

proportionally more pregnancies resulting in a delivery rather than a miscarriage. A recently published study confirmed that success rates are significantly higher for women aged <43 compared to those aged ≥43 years (Serour et al., 2010). The combined evidence from the PhD thesis and the newly published papers suggest that it is reasonable that the ART regulatory body is considering the review and amendment of the national recommendation of the appropriate age to initiate a new ART treatment for women who intend to use their own oocytes.

Continued increase in the proportion of SET

The PhD thesis also suggested that regardless of a woman's age, babies following DET had worse perinatal outcomes than those following SET. The multiple birth rate for babies following DET was nearly ten folds higher than babies following SET. More than half of liveborn multiples were low birthweight and more than 70% were preterm. Even for singletons, those following DET had 20% and 15% higher odds of preterm birth and fetal death respectively than singletons following SET. Liveborn DET singletons were 1.2 times more likely to be of low birthweight than SET singletons.

These findings confirmed that the RTAC policy of SET has been adopted successfully in Australia and New Zealand. The latest ART report in Australia and New Zealand shows that the proportion of SET continued to increase after the period 2002−2005 of the second study of the PhD thesis, from 56.9% in 2006 to 67.8% in 2008 (Wang et al., 2010). As a result, the multiple delivery rate has

continued to fall from 12.0% in 2006 to 8.2% in 2008 (Wang et al., 2010). Given that multiple gestational pregnancy is associated with more maternal complications and higher rates of adverse perinatal outcomes, and is less cost-effective than singleton pregnancy (Campbell & Templeton, 2004; Chambers et al., 2007; Ombelet et al., 2005), the continued uptake of SET is of benefit to women and their babies, as well as the community as a whole.

"Healthy baby" indicator to measure the efficacy of ART treatment

Runciman and colleagues have suggested that medical treatment needs to maximise the likelihood of desired health outcomes; it should reduce the risk of unnecessary harm associated with the treatment to an acceptable minimum; and it should avoid any type of impairment of body structure or function, activity limitation and/or restriction of participation in society (Runciman et al., 2009). Healy and others have advocated that ART treatment should focus on a live birth of a term singleton and absence of any adverse outcomes (Healy, 2004; Min et al., 2004). The absence of adverse perinatal outcomes such as multiple birth, preterm birth, low birthweight and congenital anomalies should be summarised in measuring the efficacy of ART treatment.

This PhD thesis used a new indicator, "healthy baby", to measure the efficacy of ART treatment. The "healthy baby" indicator is defined as a single baby born live at term (≥37 weeks gestational age), weighing ≥2500 grams, surviving for at least 28 days post birth and not having known congenital anomalies (major or minor). The definition of "healthy baby" is restricted to singleton only

considering multiple pregnancies and births with poorer perinatal outcomes (Ombelet et al., 2005), increased risk to mothers (Campbell & Templeton, 2004) and greater economic and social burden on the parents and on the health care system (Chambers et al., 2007). Preterm birth and low birthweight are common measures of birth and are related to the short-term and long-term health conditions of a baby (Goldstein, 1981). Most major congenital anomalies require surgery or other medical intervention and are related to early death (Walden et al., 2007). Even minor congenital anomalies also have an impact on the long-term development of a baby (Sutcliffe et al., 1995).

It is important to use the optimal outcome when comparing the efficacy of ART treatment across countries and regions. A recently published paper compared the outcomes of ART treatment across the United States, the United Kingdom, and Australia and New Zealand (Abdalla, 2010). The significantly higher rate of live delivery for the United States (28.6%) and the United Kingdom (23.1%) when compared to Australia and New Zealand (19.6%) was explained by the low proportion of SET and higher rate of multiple pregnancy in the United States and the United Kingdom (Abdalla, 2010). When the measure is limited to singleton live birth by number of embryos transferred, the result reverses with Australia and New Zealand achieving the highest rate of live singleton birth per embryo transferred (16.0%), significantly higher than that achieved by the United Kingdom (10.3%) and the United States (9.6%) (Abdalla, 2010).

The inconsistent relationship between ART treatment factors and the measure of success in the literature is largely due to the use of different indicators to measure efficacy. This PhD thesis recommended the use of the "healthy baby" indicator to measure the efficacy of ART treatment and to make international comparison. The "healthy baby" indicator summarises the optimum outcomes of an ART treatment. The "healthy baby" indicator was first published in 2010 and already gained international recognition. Several studies have already cited and used this indicator to measure the efficacy of ART treatment (Dessolle et al., 2011; Norré & Wischmann, 2011; Mesut et al., 2011).

Proposed clinical practice model to improve ART treatment outcomes

With the improved clinical and laboratory technologies, more women are able to undertake treatment incorporating fresh embryo transfers and subsequent thawed embryo transfers (Moustafa et al., 2008; Pandian et al., 2009). The combined evidence from retrospective studies in this PhD thesis generated a hypothesis: better perinatal outcomes are associated with a proposed practice model which includes freezing cleavage embryos and transferring a selective single blastocyst in a fresh cycle, and transferring a single blastocyst cultured from thawed cleavage embryos in a subsequent thaw cycle. The proposed clinical practice model is supported by published evidence which suggests a higher live delivery rate for blastocyst transfer cycles than for cleavage embryo transfer cycles (Papanikolaou et al., 2006; Blake et al., 2007); a higher embryo

freezing rate at cleavage stage than at blastocyst stage (Blake et al., 2007); and less embryo damage for early freezing than late freezing (Salumets et al., 2003).

The proposed practice model is detailed in Figure 1. It is a seven-step model: (1) evaluate embryos at cleavage stage of an initiated fresh cycle; (2) have a mixed approach for transfer and freezing, that is when ≥6 good quality embryos are available at cleavage stage, select three or four embryos to be cultured to blastocysts; (3) freeze the rest of embryos at cleavage stage; (4) select the "best" blastocyst to transfer after culture; (5) in the subsequent thaw cycle, thaw three or four embryos frozen at cleavage stage in the initiated fresh cycle; (6) culture these embryos to blastocysts; and (7) select the "best" blastocyst to transfer.

It is important to clarify that the difference in live delivery rates between blastocysts and cleavage embryos was reduced when the efficacy was measured by initiated cycle rather than embryo transfer cycle. This reduction is related to a higher embryo transfer cancellation rate for blastocysts compared with cleavage embryos (Papanikolaou et al., 2008). Blastocyst culture could potentially reduce the number of embryos cryopreserved (Butterworth, 2001). Further evidence from randomised controlled trials is needed to investigate the cumulative live delivery rate of blastocyst transfer following fresh and subsequent thaw cycles. However, for good prognosis patients such as those aged <35 years, the embryo transfer cancellation rate was similar for blastocyst transfer cycles as for cleavage embryo transfer cycles (Blake et al., 2007). The PhD thesis found that more than 70% of patients aged <35 years had four or

more cleavage embryos available, the recommended number of embryos for blastocyst culture (Racowsky et al., 2000; Mangalraj et al., 2009). This suggests that the proposed clinical practice model will have high utility for younger patients.

For patients aged <35 years, the proposed clinical practice model would result, at population level over time, in accumulatively fewer miscarriages and more term liveborn singletons of normal birthweight without congenital anomalies ("healthy baby"). However, the efficacy of this model, which is derived from large cycle-based population cohort studies, warrants patient-based observational evaluation at a clinical level. It is also important to note that these observational population studies may be subjected to selection bias, measurement errors and heterogeneity issues. A more robust multi-centre randomised controlled trial is needed to make this proposed clinical practice model enforceable at clinical level.

Recommendations

Community education on fertility

Assisted reproduction is not a guarantee of parentage especially for old women (Sullivan et al., 2008). The increasing percentage of women having their first baby at age 35 years or older in Australia suggests that a misperception remains in the community of women's ability to conceive either spontaneously or by fertility treatment with advancing age (Laws et al., 2010). Better community education about fertility potential and the impact of advancing maternal age on both natural and assisted conception is needed (Dick et al., 2003; Sullivan, et al., 2008). Women should be encouraged not to delay childbearing, because the chances of becoming pregnant spontaneously or following ART treatment decline by each additional year of age, especially after the age of 35 years. They should be informed that the ideal decade of childbirth is between 25 and 35 years of age because during this period, a woman's education is usually complete, she have gained some experience in her professional area, and most importantly, pregnancy is at its safest (Heffner, 2004). Women aged 35 years or older who want to have a baby should be encouraged to seek a fertility assessment as early as possible and refer to fertility treatment where clinically indicated.

Enhancement of national ART data collection

ANZARD is one of the most comprehensive ART registries in the world. It continues to play an important role in monitoring the safety and quality of ART treatment, in informing clinical practice, and in providing evidence-based information for policy development. It is feasible, and ANZARD is in a position to, conduct population-based research with its detailed three components: patients' demographic characteristics, treatment factors and pregnancy/birth outcomes. However, with ongoing advances in ART treatments and clinical practice, some important data items need to be included in ANZARD.

Patient unique identifier

One critical limitation of ANZARD is a lack of patient unique identifier (PRERU, 2010). ANZARD data used in this PhD thesis were configured as a cycle-based data collection, meaning that treatment cycles cannot be aligned to a particular patient. Therefore, it is impossible to measure the cumulative success of ART treatment over a number of treatment cycles or over a certain period of treatment. Given that it is common for a patient to have several treatment cycles, cumulative success rates are particularly important measures for guiding clinical practice and counselling patients (Kovacs, 2011).

As a joint initiative of the Fertility Society of Australia and the Perinatal & Reproductive Epidemiology Research Unit at the University of New South Wales, the cycle-based ANZARD was successfully upgraded into a woman-

based data collection (ANZARD2.0) in 2011 (PRERU, 2010). The upgrade to a woman-based data collection was achieved by introducing a statistical linkage key (SLK), a proxy patient unique identifier which comprises the first two letters of a patient's first name, the first two letters of her surname and her date of birth. With the SLK, more advanced research methods such as the General Linear Model of Repeated Measures and Generalised Estimated Equation analysis can be used to overcome the dependence of several treatment cycles of a patient.

The ANZARD2.0 also expanded data items by including information of some newly developed technology such as oocyte/embryo vitrification and oocyte freezing/thawing process. However, there are several important patient demographic characteristics and treatment outcome factors that were not collected by ANZARD2.0.

BMI and smoking

High BMI is one of the independent patients' demographic characteristics associated with the infertility and ART treatment outcomes (Veleva et al., 2008; Bellver et al., 2010). But this important data item is not collected in ANZARD2.0. BMI could be a potential confounder in the relationship between other patients' demographic characteristics and the outcomes of ART treatment. The absence of data on the association of BMI with infertility and ART treatment outcomes was discussed in the relevant chapters.

Cigarette smoking is another important patient demographic characteristic that is not collected in ANZARD. Cigarette smoking is associated with a 60% increase in the risk of infertility (Augood et al., 1998). It also reduces the chance of oocyte fertilisation by altering its competence (Gruber et al., 2008). The body of evidence regarding cigarette smoking and poor pregnancy perinatal outcomes is well established (Dessolle et al., 2011; Lintsen et al., 2005; Sazonova et al., 2011).

Both BMI and cigarette smoking are demographic characteristics that can be modified through change in behaviour. Weight loss and cessation of smoking are recommended as pre-ART treatment behaviour interventions to improve ART treatment outcomes (Klonoff-Cohen et al., 2001; Lintsen et al., 2005). Furthermore, BMI and cigarette smoking are included as criteria for access ART treatment using public funding in some countries (Gillett & Peek, 1997; Lindström & Waldau, 2008). In New Zealand since 1999, the public funding has been restricted to infertile women with BMI <32 kg/m^2 and who do not smoke cigarettes (Gillett & Peek, 1997; Gillett et al., 2006). There are no BMI and smoking restrictions to access public funding for ART treatment in Australia. Given the significant impact of high BMI and cigarette smoking on treatment, pregnancy and birth outcomes, data items on BMI and cigarette smoking status need to be included in ANZARD2.0 for monitoring their impacts on ART treatment outcomes and informing public funding policy using evidence-based reports and research.

Gestational sacs

Gestational sac is early evidence of a clinical pregnancy and used to calculate the implantation rate (number of gestations sacs per 100 transferred embryos) and vanishing twin rate (number of single fetus pregnancies or singleton deliveries per 100 double gestational sac pregnancies) (Fauque et al., 2010; Mansour et al., 2010). Both the implantation rate and vanishing twin rate are important in evaluating SET and DET (De Sutter, 2006). The implantation rate measures the chance of implantation of each transferred embryo rather than an embryo transfer cycle. The vanishing twin rate measures the potential of each implanted embryo's progress towards late pregnancy and delivery. Studies have shown that singletons from vanishing twins (two initial gestational sacs) have poor perinatal outcomes than those from only one initial gestational sac (Pinborg et al., 2005; Pinborg et al., 2007; Shebl et al., 2008).

Given the increase in SET in Australia and New Zealand, it has become critical to collect the number of gestational sacs in ANZARD2.0. This would assist in evaluating the efficacy of SET, especially elective SET as compared to DET allowing the use of the implantation rate and vanishing twin rate as intermediate outcome measures. This would also provide evidence to educate patients and the community to reduce the number of vanishing twins by adopting the SET plan in ART treatment.

Standardised congenital anomaly code

Congenital anomaly is an important outcome of birth. Historically, Australia has not had a standardised national minimum dataset of congenital anomalies. This makes collection of such data in ANZARD impossible. In addition, not all congenital anomalies are diagnosed at birth.

Studies have found inconsistent relationship between congenital anomalies and ART treatment factors (Belva et al., 2008; Bonduelle et al., 2005; Halliday et al., 2010; Hansen et al., 2002). The lack of standardised coding of congenital anomaly in ANZARD makes it impossible to investigate the relationship between specified congenital anomaly and particular ART treatment factor. The under-reported congenital anomaly in ARZARD would lead misclassification of the optimal outcome and bias in the relationship between the outcomes and treatment factors.

In Australia and New Zealand, either the International Classification of Diseases, 9th Revision, British Paediatric Association Publication (ICD-9-BPA) or the International Statistical Classification of Diseases and Related Health Problems, 10th Revision, Australian Modification (ICD-10-AM) was used to code the congenital anomaly (Macaldowie & Hilder, 2011; New Zealand Birth Defects Registry, 2011). To improve the quality and completeness of congenital anomaly in ANZARD, the free text item of congenital anomaly in ANZARD needs to be standardised according to a nationally agreed approach.

Directions of further research

Cohort study using upgraded ANZARD

This PhD thesis proposed that an optimal clinical practice model involves the transfer of a selective single blastocyst, freezing cleavage embryos in a fresh cycle and transfer of a blastocyst cultured from thawed cleavage embryos in subsequent thaw cycles. However, this proposed practice model was hypothesised using each treatment cycle as the unit of analysis. It needs further testing by using each individual patient as the unit of analysis. The 2011 upgrade of ANZARD2.0 from a cycle-based collection into a patient-based collection made it feasible to conduct a cohort study on each individual patient by linking several treatment cycles of a patient through SLK.

In the patient-based cohort study, good prognosis female patients (patients aged <35 years at their first ART treatment using their own oocytes) will be identified from ANZARD2.0 and included in. Patients who had embryos frozen and embryos transferred at their initiated fresh cycle will be categorised into three groups: (a) frozen cleavage embryos and transferred cleavage embryos; (b) frozen cleavage embryos and transfer of blastocysts; and (c) frozen blastocysts and transferred blastocysts. Initiated fresh cycles of the three groups of patients will be followed up until achieving a delivery. If no delivery is achieved from the initiated fresh cycle, the first subsequent thaw cycle will be followed up until achieving a delivery or failing to achieving a delivery.

The primary outcome will be term liveborn singleton of normal birthweight without a congenital anomaly ("healthy baby") and the secondary outcome is a live delivery. Cumulative "healthy baby" and live delivery rates per patient will be calculated and compared across the three groups. The efficacy of transfer blastocysts cultured from thawed cleavage embryos will be evaluated for patients of groups (a) and (b).

The success of this patient-based cohort study will reinforce the proposed clinical practice model and inform the community of the best treatment model to achieve a "healthy baby". However, ANZARD2.0 only records treatments and procedures that actually happened, not intended treatments and procedures. In a patient-based cohort study it is not possible to conduct intention to treatment analysis. The patient-based cohort study can only include patients that have had embryos transferred, not patients at the start of a fresh cycle. Patients who cancelled before the transfer due to inadequate oocytes or embryos are unable to be included. A more robust multi-centre randomised controlled trial will be needed to overcome the above limitations.

Randomised controlled trial

A randomised controlled trial is the gold standard design of epidemiological studies. A multi-centre design will overcome the variability of patients, doctors and scientists from different clinics. As of October 2011, there were 80 fertility clinics in Australia and New Zealand (RTAC, 2011). Two or three clinics with >1000 annual treatment cycles in New South Wales, Queensland, Victoria,

South Australia, West Australia and New Zealand will be invited to join this trial.

Good prognosis patients of participating clinics at their initial ART treatment will be invited to participate. Paticipants will be randomised into three arms:

- ARM I: intended to freeze cleavage embryos and transfer a single cleavage embryo in the fresh cycle and transfer a single thawed cleavage embryo in the subsequent thaw cycles.
- ARM II: intended to freeze cleavage embryos and transfer a single blastocyst in the fresh cycle and transfer a single blastocyst cultured from thawed cleavage embryos in the subsequent thaw cycles.
- ARM III: intended to freeze blastocysts and transfer a single blastocyst in the fresh cycle and transfer a single thawed blastocyst in the subsequent thaw cycles.

Initiated fresh cycles of the three groups of patients will be followed up until achieving a delivery. If no delivery is achieved from the initiated fresh cycle, the first subsequent thaw cycle will be followed up until achieving a delivery or failing to achieve a delivery. The primary outcome will be a term liveborn singleton of normal birthweight without a congenital anomaly ("healthy baby") and the secondary outcome is live delivery. Cumulative "healthy baby" and live delivery rates per patient will be calculated and compared across the three arms. Intention to treatment analysis will be conducted to evaluate the efficacy of the three arms.

Given only a small proportion of fresh cycles involved in freezing cleavage embryos and transfer of blastocysts in Australia and New Zealand, the feasibility of this randomised controlled trial including patient's likely acceptance of randomisation needs to be investigated prior to initiation of the trial. If the multi-centres randomised controlled trial successfully undergoes and confirms the proposed clinical practice model (ARM II), it will provide evidence to clinics to change their practice to achieve more "healthy babies". The success of this multi-centre randomised controlled trial would also inform ART regulatory bodies to amend policy and ART treatment guidelines. Australia's and New Zealand's experience of the proposed clinical practice model would lead other countries and regions to adopt this model to improve the overall outcomes of ART treatment.

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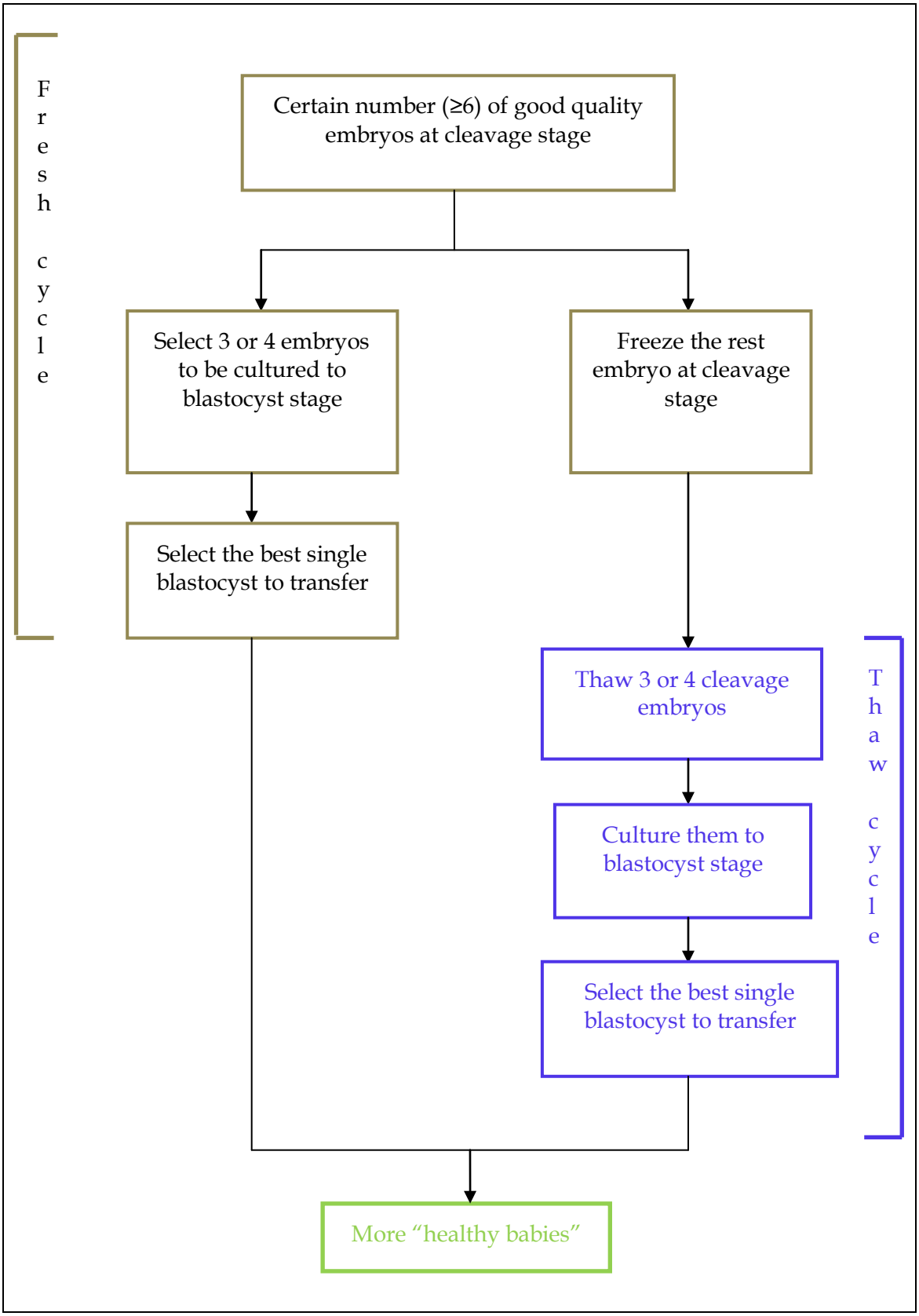
Figure 1: Proposed clinical practice model



Appendix 1

Glossary defined by International Committee Monitoring Assisted Reproductive Technologies and the World Health Organization

Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, Sullivan E, van der Poel S; International Committee Monitoring Assisted Reproductive Technologies; World Health Organization. The International Committee Monitoring Assisted Reproductive Technologies (ICMART) and the World Health Organization (WHO) Revised Glossary on ART Terminology, 2009. Hum Reprod. 2009 Nov;24(11):2683-7.

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Assisted hatching: an in vitro procedure in which the zona pellucid of an embryo is either thinned or perforated by chemical, mechanical or laser methods to assist separation of the blastocyst.

Assisted reproductive technology (ART): all treatments or procedures that include the in vitro handling of both human oocytes and sperm, or embryos, for the purpose of establishing a pregnancy. This includes, but is not limited to, in vitro fertilization and embryo transfer, gamete intrafallopian transfer, zygote intrafallopian transfer, tubal embryo transfer, gamete and embryo cryopreservation, oocyte and embryo donation, and gestational surrogacy. ART does not include assisted insemination (artificial insemination) using sperm from either a woman's partner or a sperm donor.

Biochemical pregnancy (preclinical spontaneous abortion/miscarriage): a pregnancy diagnosed only by the detection of HCG in serum or urine and that does not develop into a clinical pregnancy.

Blastocyst: an embryo, five or six days after fertilization, with an inner cell mass, outer layer of trophectoderm and a fluid-filled blastocoele cavity.

Cancelled cycle: an ART cycle in which ovarian stimulation or monitoring has been carried out with the intention to treat, but did not proceed to follicular aspiration or, in the case of a thawed embryo, to embryo transfer.

Clinical pregnancy: a pregnancy diagnosed by ultrasonographic visualization of one or more gestational sacs or definitive clinical signs of pregnancy. It includes ectopic pregnancy. Note: Multiple gestational sacs are counted as one clinical pregnancy.

Clinical pregnancy rate: the number of clinical pregnancies expressed per 100 initiated cycles, aspiration cycles or embryo transfer cycles. Note: When clinical pregnancy rates are given, the denominator (initiated, aspirated or embryo transfer cycles) must be specified.

Clinical pregnancy with fetal heart beat: pregnancy diagnosed by ultrasonographic or clinical documentation of at least one fetus with heart beat. It includes ectopic pregnancy.

Congenital anomalies: all structural, functional, and genetic anomalies diagnosed in aborted fetuses, at birth or in the neonatal period.

Controlled ovarian stimulation (COS) for ART: pharmacological treatment in which women are stimulated to induce the development of multiple ovarian follicles to obtain multiple oocytes at follicular aspiration.

Controlled ovarian stimulation (COS) for non-ART cycles: pharmacological treatment for women in which the ovaries are stimulated to ovulate more than one oocyte.

Cryopreservation: the freezing or vitrification and storage of gametes, zygotes, embryos or gonadal tissue.

Cumulative delivery rate with at least one live born baby: the estimated number of deliveries with at least one live born baby resulting from one initiated or aspirated ART cycle including the cycle when fresh embryos are transferred, and subsequent frozen/thawed ART cycles. This rate is used when less than the total number of embryos fresh and/or frozen/thawed has been utilized from one ART cycles. Note: The delivery of a singleton, twin or other multiple pregnancy is registered as one delivery.

Delivery: the expulsion or extraction of one or more fetuses from the mother after 20 completed weeks of gestational age.

Delivery rate after ART treatment per patient: the number of deliveries with at least one live born baby per patient following a specified number of ART treatments.

Delivery rate: the number of deliveries expressed per 100 initiated cycles, aspiration cycles or embryo transfer cycles. When delivery rates are given, the denominator (initiated, aspirated or embryo transfer cycles) must be specified. It includes deliveries that resulted in the birth of one or more live babies and/or stillborn babies. Note: The delivery of a singleton, twin or other multiple pregnancy is registered as one delivery.

Early neonatal death: death of a live born baby within 7 days of birth.

Ectopic pregnancy: a pregnancy in which implantation takes place outside the uterine cavity.

Elective embryo transfer: the transfer of one or more embryos, selected from a larger cohort of available embryos.

Embryo: the product of the division of the zygote to the end of the embryonic stage, eight weeks after fertilization. (This definition does not include either parthenotes – generated through parthenogenesis – nor products of somatic cell nuclear transfer.)

Embryo donation: the transfer of an embryo resulting from gametes (spermatozoa and oocytes) that did not originate from the recipient and her partner.

Embryo recipient cycle: an ART cycle in which a woman receives zygote(s) or embryo(s) from donor(s).

Embryo/fetus reduction: a procedure to reduce the number of viable embryos or fetuses in a multiple pregnancy.

Embryo transfer (ET): the procedure in which one or more embryos are placed in the uterus or Fallopian tube.

Embryo transfer cycle: an ART cycle in which one or more embryos are

transferred into the uterus or Fallopian tube.

Extremely low birth weight: birth weight less than 1,000 grams.

Extremely preterm birth: a live birth or stillbirth that takes place after at least 20 but less than 28 completed weeks of gestational age.

Fertilization: the penetration of the ovum by the spermatozoon and combination of their genetic material resulting in the formation of a zygote.

Fetal death (stillbirth): death prior to the complete expulsion or extraction from its mother of a product of fertilization, at or after 20 completed weeks of gestational age. The death is indicated by the fact that, after such separation, the fetus does not breathe or show any other evidence of life such as heart beat, umbilical cord pulsation, or definite movement of voluntary muscles.

Fetus: the product of fertilization from completion of embryonic development, at eight completed weeks after fertilization, until abortion or birth.

Frozen/thawed embryo transfer cycle (FET): an ART procedure in which cycle monitoring is carried out with the intention of transferring a frozen/thawed embryo or frozen/thawed embryos. Note: A FET cycle is initiated when specific medication is provided or cycle monitoring is started with the intention to treat.

Frozen/thawed oocyte cycle: an ART procedure in which cycle monitoring is carried out with the intention of fertilizing thawed oocytes and performing

embryo transfer.

Full-term birth: a live birth or stillbirth that takes place between 37 completed and 42 completed weeks of gestational age.

Gamete intrafallopian transfer (GIFT): an ART procedure in which both gametes (oocytes and spermatozoa) are transferred to the Fallopian tubes.

Gestational age: age of an embryo or fetus calculated by adding2 weeks (14 days) to the number of completed weeks since fertilization. Note: For frozen/thawed embryo transfers, an estimated date of fertilization is computed by subtracting the embryo age at freezing from the transfer date of the FET cycle.

Gestational carrier (surrogate): a woman who carries a pregnancy with an agreement that she will give the offspring to the intended parent(s). Gametes can originate from the intended parent(s) and/or a third party (or parties).

Gestational sac: a fluid-filled structure associated with early pregnancy, which may be located inside or outside the uterus (in case of an ectopic pregnancy).

Hatching: the process by which an embryo at the blastocyst stage separates from the zona pellucida.

High-order multiple: a pregnancy or delivery with three or more fetuses or neonates.

Implantation: the attachment and subsequent penetration by the zona-free blastocyst (usually in the endometrium) that starts five to seven days after fertilization.

Implantation rate: the number of gestational sacs observed, divided by the number of embryos transferred.

In vitro fertilization (IVF): an ART procedure that involves extra-corporeal fertilization.

Induced abortion: the termination of a clinical pregnancy, by deliberate interference that takes place before 20 completed weeks of gestational age (18 weeks post fertilization) or, if gestational age is unknown, of an embryo/fetus of less than 400 grams.

Infertility (clinical definition): a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse.

Initiated cycle: an ART cycle in which the woman receives specific medication for ovarian stimulation, or monitoring in the case of natural cycles, with the intention to treat, irrespective of whether or not follicular aspiration is attempted.

IntraCytoplasmic Sperm Injection (ICSI): a procedure in which a single spermatozoon is injected into the oocyte cytoplasm.

Live birth: the complete expulsion or extraction from its mother of a product of fertilization, irrespective of the duration of the pregnancy, which, after such separation, breathes or shows any other evidence of life such as heart beat, umbilical cord pulsation, or definite movement of voluntary muscles, irrespective of whether the umbilical cord has been cut or the placenta is attached.

Live birth delivery rate: the number of deliveries that resulted in at least one live born baby expressed per 100 initiated cycles, aspiration cycles or embryo transfer cycles. When delivery rates are given, the denominator (initiated, aspirated, or embryo transfer cycles) must be specified.

Low birth weight: Birth weight less than 2,500 grams.

Medically Assisted Reproduction (MAR): reproduction brought about through ovulation induction, controlled ovarian stimulation, ovulation triggering, ART procedures, and intrauterine, intracervical, and intravaginal insemination with semen of husband/partner or donor.

MESA: Microsurgical Epididymal Sperm Aspiration.

MESE: Microsurgical Epididymal Sperm Extraction.

Micromanipulation: a technology that allows micro-operative procedures to be performed on the spermatozoon, oocyte, zygote or embryo.

MicroTESE: Microsurgical Testicular Sperm Extraction.

Mild ovarian stimulation for IVF: a procedure in which the ovaries are stimulated with either gonadotropins and/or other compounds, with the intent to limit the number of oocytes obtained for IVF to fewer than seven.

Missed abortion: a clinical abortion where the embryo(s) or fetus(es) is/are non-viable and is/are not expelled spontaneously from the uterus.

Modified natural cycle: an IVF procedure in which one or more oocytes are collected from the ovaries during a spontaneous menstrual cycle. Drugs are administered with the sole purpose of blocking the spontaneous LH surge and/or inducing final oocyte maturation.

Multiple gestation/birth: a pregnancy/delivery with more than one fetus/neonate.

Natural cycle IVF: an IVF procedure in which one or more oocytes are collected from the ovaries during a spontaneous menstrual cycle without any drug use.

Neonatal death: death of a live born baby within 28 days of birth.

Neonatal period: the time interval that commences at birth and ends 28 completed days after birth.

Oocyte donation cycle: a cycle in which oocytes are collected from a donor for clinical application or research.

Oocyte recipient cycle: an ART cycle in which a woman receives oocytes from a donor.

Ovarian Hyper Stimulation Syndrome (OHSS): an exaggerated systemic response to ovarian stimulation characterized by a wide spectrum of clinical and laboratory manifestations. It is classified as mild, moderate or severe according to the degree of abdominal distension, ovarian enlargement and respiratory, haemodynamic and metabolic complications.

Ovarian torsion: the partial or complete rotation of the ovarian vascular pedicle that causes obstruction to ovarian blood flow, potentially leading to necrosis of ovarian tissue.

Ovulation Induction (OI): pharmacological treatment of women with an ovulation or oligo-ovulation with the intention of inducing normal ovulatory cycles.

Perinatal mortality: fetal or neonatal death occurring during late pregnancy (at 20 completed weeks of gestational age and later), during childbirth and up to 7 completed days after birth.

PESA: Percutaneous Epididymal Sperm Aspiration.

Post-term birth: a live birth or stillbirth that takes place after 42 completed weeks of gestational age.

Preimplantation Genetic Diagnosis (PGD): analysis of polar bodies, blastomeres or trophectoderm from oocytes, zygotes or embryos for the detection of specific genetic, structural and/or chromosomal alterations.

Preimplantation Genetic Screening (PGS): analysis of polar bodies, blastomeres or trophectoderm from oocytes, zygotes or embryos for the detection of aneuploidy, mutation and/or DNA rearrangement.

Preterm birth: a live birth or stillbirth that takes place after at least 20 but before 37 completed weeks of gestational age.

Recurrent spontaneous abortion/miscarriage: the spontaneous loss of two or more clinical pregnancies.

Reproductive surgery: surgical procedures performed to diagnose, conserve, correct and/or improve reproductive function.

Severe Ovarian Hyper Stimulation Syndrome: severe OHSS is defined to occur when hospitalization is indicated. (see definition of Ovarian Hyper Stimulation Syndrome)

Small for gestational age: birth weight less than2 standard deviations below the mean or less than the 10^{th} centile according to local intrauterine growth charts.

Spontaneous abortion/miscarriage: the spontaneous loss of a clinical

pregnancy that occurs before 20 completed weeks of gestational age (18 weeks post fertilization) or, if gestational age is unknown, the loss of an embryo/fetus of less than 400 grams.

TESA: Testicular Sperm Aspiration.

TESE: Testicular Sperm Extraction.

Total delivery rate with at least one live birth: the estimated total number of deliveries with at least one live born baby resulting from one initiated or aspirated ART cycle including all fresh cycles and all frozen/thawed ART cycles. This rate is used when all of the embryos fresh and/or frozen/thawed have been utilized from one ART cycles. Note: The delivery of a singleton, twin or other multiple pregnancy is registered as one delivery.

Vanishing sac(s) or embryo(s): spontaneous disappearance of one or more gestational sacs or embryos in an ongoing pregnancy, documented by ultrasound.

Very low birth weight: Birth weight less than 1,500 grams.

Very preterm birth: a live birth or stillbirth that takes place after at least 20 but less than 32 completed weeks of gestational age.

Vitrification: an ultra-rapid cryopreservation method that prevents ice formation within the suspension which is converted to a glass-like solid.

Zygote: a diploid cell resulting from the fertilization of an oocyte by a spermatozoon, which subsequently divides to form an embryo.

Zygote Intra-Fallopian Transfer (ZIFT): a procedure in which zygote(s) is/are transferred into the Fallopian tube.

Appendix 2

Australian & New Zealand Assisted Reproduction Technology Database (ANZARD) data items

Wang YA, Chambers GM, Sullivan EA. Assisted reproductive technology in Australia and New Zealand 2008. Assisted reproduction technology series no. 14. Cat. no. PER 49. Canberra: AIHW, 2010.

Variable	Data domain
Unit identifier	3-digit code for clinics provided by NPSU.
Site of main treatment	For centres with multiple sites, this identifies location of most significant part of the treatment.
Patient ID/medical record number	Unique ID for patient.
Woman's date of birth	Day/month/year.
Husband/male partner DOB	Day/month/year.
Oocyte/embryo donor's age	Completed years at time of donation.
Previous Medicare item 13200s	The number of billed Australian Medicare item 13200. New Zealand units leave this field blank.
Cause of infertility: tubal disease	Yes—in the opinion of the treating clinician or clinic there is significant tubal disease present.
	No—other.
Cause of infertility: endometriosis	Yes—in the opinion of the treating clinician or clinic there is significant endometriosis contributing to this couple's subfertility.

Variable	Data domain
	No—other.
Cause of infertility: male factor	Yes—in the opinion of the treating clinician or clinic there is a significant male factor problem.
	No—other.
Cause of infertility: other factors	Yes—in the opinion of the treating clinician or clinic there is subfertility due to any other factors apart from female age, tubal disease, male factor or endometriosis. Possible examples are fibroids, ovulation disorders or premature ovarian failure. There is no clinical subfertility (e.g. egg donor, preimplantation genetic diagnosis or other non-fertility reason for ART).
	No—other.
Cause of infertility: idiopathic	Yes—in the opinion of the treating clinician or clinic there is clinical subfertility without any apparent explanation.
	No—other, including case of PGD for genetic disease.
Previous pregnancies <20 weeks	Number of known pregnancies less than 20 weeks in the female partner regardless of whether by ART or by a different partner.
Previous pregnancies ≥20 weeks	Number of known pregnancies reaching 20 weeks or more in the female partner regardless of whether by ART or by a different partner.
Cycle ID	Unique cycle identifier.
Cycle date	For treatment cycles this is according to the Medicare definition and is the date of LMP for unstimulated cycles or, where FSH is used, the first day of FSH administration. For cycles where the only process is movement or disposal of embryos, this is the date of embryo movement. This date defines the year in which a cycle is reported to NPSU.
Surrogacy	Yes—the procedure is part of a surrogate arrangement.
	No—the procedure is not part of a surrogate arrangement.
Injectable FSH stimulation given	Yes—FSH administered. Does not include clomiphene or hCG alone unless FSH was also given.
	No—other.
DI date	Date of first insemination with donor sperm.
OPU date	Date of oocyte retrieval.

Variable	Data domain
Number of eggs retrieved	Number of eggs retrieved at OPU. Include any immature oocytes that are identified.
Number of eggs donated	Number of eggs donated to someone else.
Number of eggs received	Number of eggs received from someone else.
Number of eggs GIFT	Number of eggs replaced in a GIFT procedure.
Number of eggs IVF	Number of eggs treated with IVF.
Number of eggs ICSI	Number of eggs treated with ICSI.
Site of sperm used	Site of sperm extraction: ejaculated, epididymal (whether by open biopsy or by PESA), testicular or other.
Person from which sperm derives	Husband/partner (h), known donor (k), anonymous donor (a), embryo received or embryo transferred is a donated embryo (e).
Number of eggs fertilised normally	Number of eggs fertilised normally.
Preimplantation genetic diagnosis	Yes—preimplantation genetic diagnosis in any form (including aneuploidy screening or sex selection) has been performed on any of the embryos (transferred or not).
	No—PGD not performed.
Assisted hatching	Yes—where assisted hatching in any form has been performed on any of the embryos (transferred or not).
	No—assisted hatching not performed.
Number of embryos received from someone else or imported into the unit	To minimise the number of required fields in the data collection, this field serves two purposes: 1. Records the number of embryos to be received from donation (recipient cycle); or 2. Records the number of embryos to be imported into the current unit from another unit.
Number of cleavage embryos thawed	Number of zygotes or cleavage stage embryos (up to 4 days) thawed with intention of performing an embryo transfer if they survive.
Number of blastocysts thawed	Number of blastocysts (i.e. greater than 4 days culture from fertilisation) thawed with intention of performing an embryo transfer if they survive.
ET date	Embryo transfer date.
Number of early	Number of zygote or cleavage stage embryos (i.e. up to

Variable	Data domain
embryos transferred	4 days since fertilisation) transferred.
Number of blastocysts transferred	Number of blastocyst embryos (i.e. >4 days since fertilisation) transferred.
Any embryos ICSI?	Yes—any embryos transferred were fertilised by ICSI.
	No—no transferred embryos were fertilised by ICSI.
Number of zygotes/cleavage stage embryos frozen	Number of zygote or cleavage stage embryos (i.e. up to 4 days since fertilisation) frozen.
Number of blastocysts frozen	Number of blastocyst embryos (i.e. >4 days since fertilisation) frozen.
Number of embryos donated to someone else or exported from the unit of treatment	To minimise the number of required fields in the data collection, this field serves two purposes: 1. Records the number of embryos to be donated to someone else (donor cycle); or 2. Records the number of embryos to be exported from the current unit to another unit.
Number of potentially usable frozen embryos discarded	Potentially usable embryos disposed of in accordance with patient or government request.
Clinical pregnancy	A pregnancy that fulfils one of the following criteria: 1. Known to be ongoing at 20 weeks; 2. Evidence by ultrasound of an intrauterine sac (with or without a fetal heart); 3. Examination of products of conception reveal chorionic villi; or 4. A definite ectopic pregnancy that has been diagnosed laparoscopically or by ultrasound.
Date pregnancy ended	Date on which delivery, miscarriage or termination takes place.
Number of fetal hearts	Number of fetal hearts seen on first ultrasound (intrauterine only).
Ectopic pregnancy	Yes—pregnancy is an ectopic pregnancy, or a combined ectopic and uterine (heterotopic) pregnancy.
	No—pregnancy not ectopic or heterotopic.
Elective termination of pregnancy	Yes—pregnancy is terminated.
	No—pregnancy not terminated.
Selective reduction performed	Yes—selective reduction was performed owing to fetal abnormality.
	No—selective reduction not performed.
Fetal abnormality in a	Details of elective terminations of pregnancy and fetal

Variable	Data domain
pregnancy ending <20 weeks or in a fetus removed by selective reduction	reductions due to fetal abnormality.
Maternal complications of pregnancy	Describes morbidity related to pregnancy.
Number of babies delivered	Include all liveborn and stillborn babies.
Caesarean delivery	Yes—delivery by planned or emergency caesarean section.
	No—other.
Baby 1 outcome	Liveborn, stillborn or neonatal death.
Baby 1 sex	Male or female.
Baby 1 birthweight	Weight in grams.
Baby 1 abnormality	Describes any known congenital malformation.
Baby 1 date of neonatal death	Date of neonatal death.
Baby 2 outcome	Liveborn, stillborn or neonatal death.
Baby 2 sex	Male or female.
Baby 2 weight	Weight in grams.
Baby 2 abnormality	Describes any known congenital malformation.
Baby 2 date of neonatal death	Date of neonatal death.
Baby 3 outcome	Liveborn, stillborn or neonatal death.
Baby 3 sex	Male or female.
Baby 3 weight	Weight in grams.
Baby 3 abnormality	Describes any known congenital malformation.
Baby 3 date of neonatal death	Date of neonatal death.
Baby 4 outcome	Liveborn, stillborn or neonatal death.
Baby 4 sex	Male or female.
Baby 4 weight	Weight in grams.
Baby 4 abnormality	Describes any known congenital malformation.
Baby 4 date of	Date of neonatal death.

Variable	Data domain
neonatal death	
Admitted with ART morbidity	Yes—woman is admitted to hospital with any condition (excluding any pregnancy-related issues, such as ectopic pregnancy) that could be in any way related to fertility treatment.
OHSS	Yes—admission to hospital is due to symptoms of OHSS.
Morbidity detail	Describes symptoms of treatment-related morbidity.