Systematic review of the health risks to the mother, child and family associated with the use of intracytoplasmic sperm injection (ICSI)

Report to the Ministry of Health
from the New Zealand Guidelines Group
undertaken by the Cochrane Menstrual Disorders and Subfertility Group

Principal authors:
Jane Marjoribanks
Cindy Farquhar
Catherine Marshall

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Executive Summary

Introduction

The Ministry of Health identified a need for an in-depth assessment of the possible risks associated with intracytoplasmic sperm injection (ICSI) and contracted the New Zealand Guidelines Group (NZGG) to undertake this. Following initial scoping meetings and teleconferencing, NZGG subcontracted the Auckland-based Cochrane Menstrual Disorders and Subfertility Group to prepare this systematic review. This review aims to assess the safety of ICSI for mother, child and family and, where possible, quantify the health risks of ICSI versus in vitro fertilisation (IVF) and/or versus spontaneous conception.

Background

ICSI is a fertilisation technique whereby a single sperm is injected directly into a single oocyte. This technique is used for male infertility and unexplained infertility, as well as when fertilisation has failed with conventional IVF. It is also an additional step in the usual sequence of procedures for IVF. If no ejaculated sperm is available, surgically retrieved immature sperm from the epididymis or testis may be used. ICSI is a highly invasive procedure and there has been ongoing debate about its safety.

Objectives

1. To assess the health risks to the mother and child associated with the use of ICSI, including intergenerational health risks, but excluding the risks associated with undergoing fertility treatment as a whole.
2. To assess the health risks to the mother and child associated with the use of ICSI compared to spontaneous conception

Search strategy for identification of studies

A literature search was conducted for studies assessing the health risks to the mother, child and family associated with the use of ICSI. This included the output of a New Zealand Health Technology Assessment search on assisted reproductive technology (ART) outcomes commissioned by the Ministry of Health in October 2003 and updated in July and November 2004. It was supplemented by a search of the Cochrane Library and online fertility journals in December 2004 and by checking the reference lists of articles retrieved.

Outcomes of interest

- Obstetric outcomes (ie, multiple pregnancy, miscarriage, ectopic pregnancy, caesarean delivery, stillbirth, premature birth, low birth weight)
- Neonatal/infant complications
- Chromosomal abnormalities
- Congenital malformations (ie, birth defects)
- Child development (physical, psychomotor and cognitive)
- Psychological outcomes for child and family
- Epigenetic disorders (eg, imprinting)

Methods

Studies that addressed the outcomes of interest were selected from those returned by the search. They were assessed and graded for quality using pre-determined criteria and data were extracted. Where studies were considered for inclusion, but subsequently excluded, the study reference and reason for exclusion were documented. Where cohort studies compared ICSI with IVF and were rated as of higher quality, dichotomous data for each study were expressed as an odds ratio with 95% confidence intervals. Data were pooled in meta-analysis where more than one suitable study reported the same outcome. The findings of lower quality studies that compared ICSI with IVF were reported solely in the text, as were the findings of studies comparing ICSI with spontaneous conception. This was due to the likelihood of such studies being seriously biased by confounding.

Description of studies

Ninety-two publications were retrieved by the search, of which 3 systematic reviews and 42 publications relating to 35 different primary studies were selected for inclusion. The primary studies comprised 17 cohort studies (of which 8 used registry data), 10 case-control studies, 6 case series and 2 case studies. Among the 27 controlled studies, 21 included a control group having IVF and 6 had only spontaneously-conceived controls. All the included studies had methodological limitations. The higher quality studies were generally the larger primary cohort or registry cohort studies. The registry studies had the advantage of large sample sizes, but were inevitably limited by the impossibility of guaranteeing that data were complete.

Nine studies were rated as of reasonable or fair quality (referred to in the text as *higher quality*) while 25 were rated as of questionable or poor quality (referred to in the text as *lower quality*).

Results

Please see the text and tables for detailed results of individual studies reporting each outcome. A highly simplified summary of the results is presented in Appendix 3.

Conclusions

- *Obstetric outcomes*

IVF and ICSI children both have an increased risk of adverse obstetric outcomes compared to spontaneously-conceived children. This is largely, but not entirely, due to the high rate of multiple births in ART pregnancies. ICSI children do not appear to be at any greater risk of adverse obstetric outcomes than IVF children.

- *Neonatal outcomes*

ICSI and IVF were comparable with respect to overall neonatal complications, though when multiple births were considered separately, ICSI had a significantly *lower* complication rate than IVF. Moreover, there was a significantly lower incidence of neonatal/infant death in the ICSI group, for no obvious reason.

- *Chromosomal abnormalities*

Children conceived by ICSI may have a higher rate of chromosomal abnormalities than those

conceived by IVF, but there have been no large well-controlled studies reporting this outcome. Compared to spontaneously conceived children, ICSI children appear to have a three- to four-fold increase in risk of both inherited and de novo (newly occurring) chromosomal abnormalities. The absolute risk of the diagnosis of a prenatal de novo chromosomal abnormality is around 1.6% for ICSI conceptions compared with 0.5% for spontaneous conceptions.

De novo abnormalities in ICSI foetuses consist mainly of an increased number of sex chromosomal abnormalities, though structural abnormalities are also increased above population rates. Such abnormalities are more common where the father has a low sperm concentration or motility. Affected children are usually phenotypically normal at birth but have an increased risk of developmental problems and infertility that is difficult to quantify. Couples having ICSI require careful genetic counselling and may choose to undergo prenatal testing, particularly where the male partner has low sperm concentration. In some cases preimplantation genetic diagnosis may be appropriate.

The ICSI sons of men with a Y chromosome microdeletion will inherit the same deletion and are likely to be infertile. They will require ICSI themselves if they wish to father a child. It is currently unclear whether other abnormalities may also be associated with Y chromosome microdeletion as most children born as a result of ICSI are not yet sexually mature.

- *Congenital malformations*

There is reasonably good evidence that there is no significant difference between ICSI and IVF in the rate of major malformations diagnosed during the first year of life, but a recent case-control study has raised the possibility that ICSI children, especially boys, may be more likely to have urogenital or other malformations diagnosed later in childhood. Moreover, there is evidence of a 30–40% increased risk of major and minor birth defects associated with both IVF and with ICSI, compared to spontaneous conception. Assuming an underlying prevalence of major abnormalities of 4%, the absolute risk increases to 5.2–5.6% for babies conceived by ART.

- *Child development*

There is very little reliable evidence measuring the physical, motor and cognitive development of ICSI children, and none for children older than five years. What evidence is available suggests that young children conceived by ICSI do not differ significantly from children conceived by other means except that children conceived by ICSI or IVF are more likely to have a major childhood illness or need to use health care resources.

- *Psychological outcomes*

The very limited evidence available found that parents reported similar temperaments and levels of behaviour problems in their children regardless of their mode of conception. The incidence of marital difficulties, mental health problems and family stress were also similar.

- *Epigenetic disorders*

Currently, very little is known about human epigenetic regulation. It is suspected that ART children are prone to rare imprinting disorders and a large case-control study has shown a significant link between Beckwith-Weidemann Syndrome (BWS) and ART. Although BWS is in most cases associated with a good long-term outcome and the absolute risk of BWS for ART children remains low (around 1/4000 births), imprinting disorders can cause severe

disability and other large controlled studies will be required to confirm the extent of risk to ART children and to indicate whether ICSI increases the risk of such disorders more than IVF alone. Moreover, it has been suggested that epigenetic errors may also account for a wider spectrum of ART-related complications than is currently recognised, such as low birth weight.

Implications for research

These conclusions are based on the very limited evidence that is currently available. As the first ICSI births were as recent as 1992, long-term follow up will be required to monitor the fertility of ICSI children and their ongoing sexual, physical and intellectual development. The overall safety of ICSI cannot be reliably assessed without well-controlled and adequately powered cohort studies with sufficient follow up to measure these outcomes.

Contents

Introduction

The New Zealand Guidelines Group has subcontracted the Auckland-based Cochrane Menstrual Disorders and Subfertility Group to undertake:

- a background overview of intracytoplasmic sperm injection (ICSI), including an outline on the different ICSI techniques used in New Zealand
- a literature search for completed studies reporting health risks of assisted reproductive technology (ART) for mother and child and including ICSI as an intervention, using the NZHTA search to November 2004.

They will:

- identify whether or not outcomes of interest are affected by ICSI in comparison to the underlying risk from in vitro fertilisation (IVF).
- where possible, quantify health risks of ICSI versus IVF (and/or versus normal conception) for outcomes of interest.

This process has been addressed by means of the following systematic review.

Background

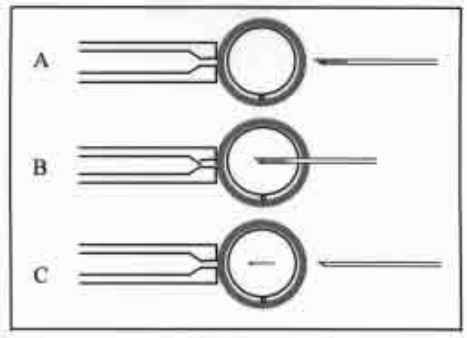
The use of ART

Since the birth in 1978 of the first baby conceived by the revolutionary technique of IVF, assisted reproductive technology (ART) has expanded rapidly and over a million ART children have now been born world wide (Wennerholm 2004).

IVF aims to achieve fertilisation outside the body by retrieving oocytes (eggs) from the ovaries and mixing them with ejaculated sperm in a laboratory glass Petri dish (*in vitro* meaning *in glass*). After fertilisation, one or more of the resulting embryos are transferred into the woman's uterus. IVF has revolutionised the treatment of infertility caused by female factor infertility (ie, conditions such as abnormal fallopian tubes). However, it is relatively ineffective where semen parameters in the male partner are impaired (male factor infertility) as fertilisation commonly fails and there are no embryos for replacement (Van Steirteghem 2002). IVF requires at least 10,000–100,000 good quality motile sperm per oocyte for fertilisation to succeed (Tesarik 1998).

The advent of ICSI

A second revolution in ART was marked with the birth in 1992 of the first child conceived by intracytoplasmic sperm injection (ICSI). ICSI is a fertilisation technique whereby a single sperm is injected directly into a single oocyte, and is an additional step in the usual sequence of procedures entailed in IVF. ICSI has enabled a much wider range of conditions to be treated by ART: although ICSI was developed initially for male factor infertility it is increasingly used for unexplained infertility and where fertilisation has failed with conventional IVF (Kurinczuk 2004, Barnes 2004). ICSI has superseded earlier assisted fertilisation techniques such as partial zona dissection (PZD) and subzonal insemination (SUZI).



The ICSI procedure (Sparks 2002)

A. The oocyte is held by applying gentle suction through a glass holding pipette. A single, immobilised sperm is held in the injection pipette.
B. The injection pipette pierces the oocyte and the sperm is injected into the cytoplasm.
C. The injection pipette is withdrawn from the oocyte.

Different types of ICSI

Initially, a single sperm from ejaculated semen was used for ICSI, but it is also possible to use surgically retrieved immature sperm from the epididymis or testis. The indications for these different types of ICSI are explained below.

ICSI with ejaculated sperm is indicated where viable sperm are present in the ejaculate but their number is low or their motility and/or morphology are poor (oligoasthenoteratospermia). This may apply in the following situations:

- where there is a high concentration of sperm antibodies
- in cases where semen was banked prior to cancer treatment or vasectomy
- in men with spinal cord injury, ejaculatory disturbances or retrograde ejaculation
- in cases of repeated fertilisation failure after conventional IVF.

ICSI with microsurgical epididymal sperm aspiration (MESA) is indicated where there are no sperm in the ejaculate due to an obstruction in the excretory ducts (obstructive azoospermia or OA). This may be caused by:

- congenital bilateral absence of the vas deferens (CBAVD)
- scarring in the epididymis from trauma, surgery or infection
- Young's syndrome (which comprises bronchiectasis, rhinosinusitis and reduced fertility).

ICSI with testicular sperm extraction (TESE) is indicated where there is epididymal scarring or impairment of the production or maturation of germ cells (non-obstructive azoospermia or NOA). Common causes of NOA include the following:

- Klinefelter's syndrome
- radiotherapy
- torsion (twisting injury)
- mumps orchitis (aute inflammation of the testis secondary to post-pubertal mumps)
- crypto-orchidism (undescended testes).

(Devroey 2004, Nicopoullos 2004)

The attrition rate of ICSI cycles

Occasionally, CSI cycles are unable to proceed: in about 1% of planned cycles no suitable oocyte is retrieved and in about 1% of cycles there is no sperm available (Bonduelle 1999). The scenario of no available sperm occurs most commonly where the indication for ICSI is non-obstructive azoospermia, as direct sperm retrieval by biopsy or aspiration techniques is successful in only about 41% of such cases (Friedler 2002).

In addition, there is a high attrition rate throughout the ICSI/IVF process. Up to 10% of oocytes are damaged by the injection procedure, about 33% of injected oocytes fail to fertilise and about 25% of those fertilised fail to develop into embryos of sufficient quality for embryo transfer. With further losses during implantation and pregnancy, the eventual live delivery rate per ICSI cycle is about 25% (Van Steirteghem 2002).

As with IVF, embryos arising from ICSI can be frozen and used in subsequent ART treatment where they are thawed and transferred to the uterus (Bryant 2004).

The risks of conventional IVF

Children conceived by ART have a higher incidence of obstetric and perinatal problems than those conceived spontaneously, although the higher proportion of multiple pregnancies in ART conceptions explains most of the increased risk. Obstetric and neonatal outcomes are consistently worse for multiple conceptions: for example, twins have a shorter gestation and lower birth weight than singletons – these being two of the leading predictors of foetal and neonatal wellbeing. Twins also have a higher risk of cerebral palsy and of developmental delay than singletons (Kurinczuk 2003).

In New Zealand and Australia, 18.9% of ART deliveries in 2002 involved twins or triplets, in comparison with a rate of 1.7% in the general population. Although the triplet rate is declining, the proportion of twin deliveries (21.1%) changed little over the 10 years to 2002 (Bryant 2004). Bonduelle (2003) notes that ‘as long as ART produces more twins than in the general population, the developmental outcome of the children will be worse in ART… this is simply a consequence of the policy in most ART programmes of transferring two or more embryos’.

However, even when multiple pregnancies are excluded from consideration, studies comparing singleton pregnancies conceived by ART with those conceived spontaneously have shown higher rates of antenatal complications, prematurity, low birth weight, small-for-gestational-age babies and perinatal mortality compared to the general population (Doyle 1992, Olivennes 1993, Schieve 2002, Tan 1992, Verlaenen 1995). It is currently unclear whether these risks stem from the IVF process itself or from parental factors, since there are many confounding variables and much is unknown about the basic mechanisms of early human development. In the meantime, the overall short term outcomes of IVF have been considered satisfactory and the technique has become very popular (Bonduelle 2002, Medical Research Council 2004).

Potential risks of ICSI (in addition to risks of IVF alone)

ICSI is a more invasive procedure than conventional IVF and moreover it permits fertilisation by spermatozoa that could not otherwise have been used. Thus, there has been ongoing debate about its safety (De Rycke 2002). Like IVF, it was adopted very rapidly into widespread clinical practice without a prolonged experimental phase. There have been many thousands of births worldwide as a result of ICSI but as the oldest children are only 13 some long-term outcomes, such as fertility, have not yet been measured.

Concerns about ICSI has focussed on potential obstetric problems, chromosomal abnormalities, congenital malformations, developmental problems and psychological issues. The rationale for each of these concerns is discussed below.

Obstetric problems

It has been suggested that despite apparently normal growth in vitro, ICSI embryos might be at increased risk of abnormal development after implantation, resulting in increased pregnancy losses. This concern has been addressed by comparison of miscarriage rates for ICSI and IVF (Retzloff 2003).

Chromosomal abnormalities

ICSI bypasses the natural selection procedure that is thought to occur both during spontaneous conception and in conventional IVF, resulting in a greater risk of fertilisation involving an abnormal sperm or oocyte. As a result, children conceived by ICSI may be at increased risk of chromosomal abnormalities either inherited from their parents or newly occurring (de novo) (Gekas 2001).

Inherited chromosomal abnormalities

The prevalence of chromosomal abnormalities is known to be increased in infertile men, with aberrations increasing as sperm counts decrease (Van Assche 1996, Clementini 2005). The most common chromosomal abnormalities associated with male infertility are outlined below.

Numerical sex chromosomal abnormalities

It is currently unclear whether ICSI children are at risk of inheriting sex chromosomal aberrations such as Klinefelter's syndrome (Denschlag 2004, Aittomaki 2004).

Translocations

If the father is carrier of a balanced translocation, it will not necessarily be inherited by the offspring or alternatively may be inherited in either a balanced or an unbalanced form. If a balanced translocation is inherited, the offspring may be infertile. Inheritance of an unbalanced form may result in spontaneous abortion or in a child with severe congenital malformations and mental retardation. The risk of inheritance of an unbalanced translocation varies, but can be up to 20% (Aittomaki 2004).

Y chromosome microdeletions

Sub-microscopic loss of genetic material (deletion) from the Y chromosome is a de novo aberration prevalent in an estimated 10–15% of subfertile men with low sperm counts. It is unidentifiable in normal karyotyping and requires a special test. Although, this condition cannot be inherited in spontaneous conception it will be inherited by all male offspring conceived by ICSI, who are thus likely to be infertile themselves. It has been suggested that Y chromosome microdeletions may possibly become more severe if passed on to successive generations (Kurinczuk 2003)

CFTR gene mutations

Congenital bilateral absence of the vas deferens (CBAVD) accounts for about 2% of all cases of infertility. In some cases, CBAVD is caused by a genetic mutation in the cystic fibrosis transmembrane conductance regulator gene (CFTR gene) and is the only manifestation of cystic fibrosis. Offspring of a male with CBAVD due to CFTR mutations will always inherit a mutated gene. If the female partner is also a carrier, both daughters and sons are at 25% risk of cystic fibrosis (CF) and (for males) CBAVD. There is a significant risk of the female partner also being a carrier, since the carrier frequency of CF is about one in 25 in many Caucasian populations (Aittomaki 2004, Kurinczuk 2003).

Other conditions

An increasing number of mutations are being recognised as the cause of sperm disorders and ICSI may create a substantial risk of transmitting other genetic defects that are associated with male infertility. However, the causes of disordered spermatogenesis are poorly defined at present (Devroey 2004).

De novo chromosomal abnormalities

Chromosome analysis (karyotyping) is generally conducted on white blood cells. Although ICSI parents may have a normal white blood cell karyotype, their offspring may develop de novo chromosomal aberrations resulting from an abnormal egg or sperm. Infertile men, especially those with low sperm concentration, have a high frequency of aneuploid sperm (having the wrong number of chromosomes), which particularly affects the sex chromosomes (Clementini 2005, Rubio 2001). The female partners of infertile men may also have chromosomal aberrations that cannot be identified by standard clinical evaluation, and in some cases fertilisation failure may be the only sign of chromosomal abnormality in men or women (Gekas 2001).

In addition to these parental factors, there is also concern that cell damage caused by the ICSI procedure itself could increase the risk of de novo chromosomal aberrations in ICSI offspring.

Epigenetic disorders

Epigenetics refers to a process whereby developmental patterns in the genome are reprogrammed at critical phases of maturation. These patterns are passed on at cell division without affecting the genetic (DNA) code. Recent studies have suggested that children conceived by either IVF alone or IVF/ICSI may have a higher incidence of disorders related to epigenetic reprogramming. However, few of the factors involved in this process have yet been identified (De Rycke 2002, Lucifero 2004).

One critical reprogramming phase is known as imprinting. A crucial phase of imprinting occurs during the development of sperm and oocytes, before fertilisation. This process ensures that only one of the parental copies of a gene is expressed and the other is silenced. A further period of imprinting takes place after fertilisation, during the pre-implantation period: changes at this stage can result in transgenerational effects: ie, the offspring of the offspring can be affected. Imprinted genes play key roles in embryonic growth and behavioural development and are also involved in carcinogenesis (De Rycke 2002).

It has been suggested based on animal studies that ART children may be at increased risk of epigenetic disorders, due to the use of synthetic embryo culture medium during a time window when epigenetic reprogramming occurs. Moreover, ICSI children may be at greater risk than children having IVF alone due to the use (in some cases) of surgically retrieved immature sperm which may not have completed early imprinting at the time of fertilisation, or due to mechanical damage to the oocyte or contamination from injection of a small amount of culture medium or other foreign material into the oocyte (Bonduelle 2002, Van Steirteghem 2002).

Epigenetic disorders may increase the risk of congenital abnormalities, which could cause loss of pregnancy or birth of an affected child. Anomalies might be clinically evident at birth or might cause more subtle changes in gene expression which would not become evident until a later stage. As noted above, there may also be trans-generational effects, thus it has been suggested that epigenetic disturbances may be responsible for low birth weight in ART children, which might in due course be transmitted to their offspring (De Rycke 2002).

Congenital malformations and developmental problems

As noted above, there are concerns that the invasive ICSI procedure and/or factors related to parental infertility put ICSI children at potentially greater risk than IVF children of obstetric, chromosomal and epigenetic aberrations, which may manifest as congenital malformations (malformations present at birth) or as developmental problems.

Psychological issues

It has been suggested that there may be unique stresses in families where children have been conceived with ICSI compared to families where children have been conceived by conventional IVF alone, due to anxiety about any increased risk to offspring and possibly other issues such as lack of disclosure about male factor infertility and/or a longer period of childlessness prior to conception (Barnes 2004).

ICSI in Australia and New Zealand

ICSI is used in New Zealand ART clinics for the treatment of male factor infertility and failed IVF. Sperm surgically retrieved from the epididymis or testes is used for fertilisation if ejaculated sperm is not suitable.

The use of ICSI in Australia and New Zealand increased more than 8-fold between 1993 and 2002 and is now more commonly used than IVF alone. In 2002, 9627 fresh non-donor cycles were initiated using ICSI alone, comprising 48.4% of all such cycles initiated. Live delivery per cycle was similar for ICSI and IVF (20.6% vs 21.2%), as was live delivery per embryo transfer (24% vs 22.8%). The mean age of women and men having fresh non-donor ICSI was 35 and 38 respectively (Bryant 2004).

Objectives of this review

Our primary objective in undertaking this review was to assess the health risks to the mother and child associated with the use of ICSI, including intergenerational health risks but excluding the risks associated with undergoing fertility treatment as a whole (ie, the risk of IVF).

As a secondary objective we assessed the health risks to the mother and child associated with the use of ICSI compared to spontaneous conception (SC).

Search strategy for identification of studies

A literature search was conducted for completed primary and secondary studies reporting the health risks of ICSI for mother and child. This included the output of an NZHTA search on ART outcomes commissioned by the Ministry of Health in October 2003 and updated in July and November 2004 (see Appendix 1 for search strings). It was supplemented by a search on 22nd December 2004 of the Cochrane Central Register of Controlled Trials (CENTRAL) on the *Cochrane Library* Issue 4 2004, and the following journals: *American Journal of Obstetrics, American Journal of Human Genetics, Fertility and Sterility, Human Reproduction, Human Reproduction Update, Molecular and Cellular Endocrinology, Placenta, Reproduction, Journal of Medical Genetics, Molecular Human Reproduction, Journal of Assisted Reproduction and Genetics, Fetal and Maternal Medicine, BMJ, The Lancet, NEJM*. The reference lists of articles retrieved were also checked.

Criteria for considering studies for the review

Types of study

Studies assessing the health risks to the mother, child and family associated with the use of intracytoplasmic sperm injection (ICSI).

Study design

Included

The following types of study comparing ICSI with IVF were eligible for inclusion. The list is ranked according to the quality of evidence provided by each study design (abridged from Phillips 2001).

- Systematic reviews of randomised controlled trials
- Systematic reviews of cohort studies
- Individual randomised controlled trials
- Individual cohort studies
- Systematic reviews of case-control studies
- Individual case-control studies
- Case series (for inclusion only if no controlled studies were found)

For comparisons of ICSI versus spontaneous conception, our secondary objective, we included only studies using the highest quality design available for each outcome of interest.

Excluded

- Studies that did not describe their design
- Studies of assisted reproductive technology (ART) which did not consider ICSI separately from IVF or other ART techniques
- Studies not published in the English language

Study participants

- Infertile couples and/or their offspring

Study intervention

- ICSI

Study controls

- IVF alone
- Spontaneous conception
- No comparison (if no controlled studies of acceptable quality were found)

Outcomes of interest

Included outcomes

- Obstetric outcomes (ie, multiple pregnancy, miscarriage, ectopic pregnancy, caesarean delivery, stillbirth, premature birth, low birth weight)
- Neonatal/infant complications
- Chromosomal abnormalities
- Congenital malformations (ie, birth defects)
- Child development (physical, psychomotor and cognitive)
- Psychological outcomes for child and family
- Epigenetic disorders (eg, imprinting)

Excluded outcomes

- Fertility outcomes (implantation rates, pregnancy rates, birth rates)
- Economic outcomes
- Ethical issues
- Outcomes related to use of pre-implantation genetic diagnosis (PGD)
- Outcomes reported by studies but not pre-specified as primary or secondary outcomes

Quality criteria

The following quality criteria were used:
Criteria 1–5 below were adapted from the Newcastle-Ottawa Scale for assessing the quality of non-randomised studies (Wells 2005)

1. Selection of ICSI and non-ICSI Groups

a. Was the recruitment method prospective, retrospective or unclear?
b. Design: was a complete conception/birth cohort recruited?
c. Participation rate of eligibles – and were those eligible but declining to participate similar to participants with respect to prognostic variables?

2. Comparability of groups on basis of design or analysis

a. Did the study report the balance between the groups of prognostic variables such as singleton/twin status, maternal age, parity, maternal education etc., where it was likely to affect the outcome measured?
b. Were the groups balanced, matched or statistically adjusted for prognostic variables (also known as confounders)?

3. Outcome assessment

a. Were the outcomes assessors blinded to the conception method?
b. Were the same level of scrutiny, the same ascertainment method and the same definition of outcomes used in both groups?
c. Did the study use an appropriate numerator and denominator for each outcome measured?

4. Timing of follow up

a. Was outcome assessment appropriately timed and was there sufficient length of follow up for outcomes to occur?

5. Completeness of follow up

a. Was a high proportion of each group followed up?
b. Was the group lost to follow up similar to the group whose outcomes were known, with respect to prognostic variables?

6. Sample size

a. Was the study large enough to be likely to have the statistical power to show a clinically significant difference between the groups for the outcomes measured?

7. Quality of reporting

a. Were the trial design, methods and findings clearly reported or was important information missing or unclear?

Notes on quality assessment

Study design and selection of participants

Randomised controlled trials (RCTs) facilitate objective comparison between groups because prognostic factors (both known and unknown) are likely to be evenly split between the groups by the randomisation process. However, for ethical and logistic reasons RCTs are rarely feasible for measuring long-term safety and in their absence controlled observational studies are the best alternative. As anticipated, no RCTs were found that measured our outcomes of interest.

A prospective study was defined as one in which participants were recruited to the study before the outcome of interest had occurred and were monitored over time. Prospective recruitment reduces the risk of selection bias.

Data collection in a prospectively recruited study may be prospective or retrospective. Prospective data collection is preferable, as it is likely to be more reliable than, for example, a questionnaire that relies on memory of past events.

A cohort design was defined as the comparison of a complete ICSI cohort (eg, all couples conceiving by ICSI within a defined time period) with a complete control cohort (eg, all couples conceiving by IVF within the same time period). This study design reduces the potential for selection bias and confounding.

A case-control design was defined as the comparison of an ICSI cohort with a control group selected to 'match' the ICSI cases. A case-control study could be prospective, eg, if the groups were matched for prognostic factors before the outcomes of interest had occurred. The matching process is unavoidably subjective, which increases the likelihood of a biased result compared to the cohort design.

Comparability of cohorts on basis of design or analysis

The challenge of evaluating the safety of ICSI from observational studies is that the role of the ICSI procedure may be confounded by underlying factors related to infertility itself. The ICSI population is inherently more at risk of adverse outcomes than the general population due to a higher mean maternal age, higher incidence of multiple births and whatever pathology underlies the infertility. Comparison with an IVF-alone group provides some control for these factors, but the ICSI group still differs somewhat because it comprises couples for whom IVF alone is ineffective due to severe male factor infertility or has been tried and found ineffective for unknown reasons.

In observational studies there is no ideal method of ensuring the comparability of cohorts, mainly because many potential confounding factors are unknown. Statistical adjustment for known confounders creates the risk of adjusting for variables that are actually part of a causal chain of events. Moreover, case-control designs that involve matching have to exclude ART children for whom matches cannot be found, causing information to be lost and increasing the potential for bias (Kurinczuk 2004).

Outcome assessment

The feasibility of comparing ICSI with IVF varies according to the outcome measured. It is relatively straightforward to compare obstetric outcomes such as rates of miscarriage and stillbirth as they are routinely monitored, clinically obvious and do not require extended follow up. However, other outcome measures are more prone to participation bias, losses to follow up and differential assessment, as discussed below.

Measurement of chromosomal abnormality rates at prenatal diagnosis are highly subject to selection bias since only a minority of couples agree to undergo prenatal diagnosis, generally those perceiving themselves most at risk.

ART children generally undergo much more intensive screening and scrutiny than spontaneously-conceived children, either because of the history of their conception or because

of a clinical condition associated with prematurity or multiple birth, with the result that malformations are more likely to be diagnosed and registered in ART children (ascertainment bias) (Bonduelle 2005).

Outcomes assessed using registry data may be less reliable than data collected within a standardised study protocol unless the registry data is mandatory and collected prospectively. Otherwise, it may be impossible to ascertain what proportion of data is missing.

Different studies use different classification systems for congenital malformations, which means that event rates cannot be compared between different studies, but only *within* studies. Moreover both groups within the same study must be assessed using the same classification system and the use of differing systems within the same study has been shown to bias results (Kurinczuk 1997). Similarly, event rates obtained from medical records cannot be compared to rates in population registers as a substantial number of malformations in medical notes are not contained in registers: this method would tend to overestimate the malformation rate from ICSI (Wennerholm 2000). Malformation rates reported by cohort studies are likely to be maximum estimates compared to studies using registry data, due to the level of scrutiny employed (Bonduelle 2002).

Timing and length of follow up

At least 30% of congenital malformations are missed at birth (Bonduelle 2005). Most surveys of ART children stop at two years, which means problems that manifest later in development, such as some imprinting errors, may not be identified (De Rycke 2002). A related issue is that measures of congenital malformations should include live births, stillbirths and therapeutic terminations of pregnancy (TOPs), since an estimation based on live births alone excludes the group most at risk of having a serious or lethal defect (survivor bias) (Kurinczuk 2004).

With respect to developmental outcomes, measures of intelligence in the first two years of life tend to measure perceptual motor skills rather than true intellectual ability. Developmental delay in the first two years is not always strongly predictive of later cognitive impairment. Tools developed for older children are able to provide a more robust assessment of long-term intelligence – thus greater weight should be accorded to studies of five year olds than of younger children as they are more likely to reflect true differences in mental ability. Moreover, measures comparing the proportion of children with developmental delay may be clinically more meaningful than comparisons of overall IQ (Leslie 2004).

Completeness of follow up

It is likely that children followed up differ in outcome from those lost to follow up. A high loss to follow up may bias findings in either direction, as the loss may be due to survivor bias, which favours those with favourable outcomes, or alternatively to increased vigilance in those with abnormalities (Van Steirtegheim 2002a).

Power

Individual studies require a large sample size in order to achieve the statistical power to show significant results for outcomes that are relatively unusual such as congenital malformation. When comparing the safety of ICSI and IVF a non-statistically-significant difference (usually reported as a P value >0.05) does not indicate that the interventions are equally safe *if the study is underpowered* (Kurinczuk 2004).

Methods

The reviewer read the abstracts of studies returned by the search, read the full text of those appearing to meet the inclusion criteria and made a final selection. Relevant data on the outcomes of interest were extracted and tabulated. Studies were assessed for quality using the criteria described above and were graded according to the overall quality of the evidence provided. See Table 1 for a brief outline of the included studies and related publications and see Table 2 for quality assessment. For more details on characteristics of the included studies see Appendix 2.

Where studies were considered for inclusion and subsequently excluded, the study reference and reason for exclusion were documented: see Table 3 for a list of excluded studies.

Statistical analysis

Where cohort studies compared ICSI with IVF and were rated as of higher quality (See Table 2), dichotomous data for each study were expressed as an odds ratio with 95% confidence intervals. If there was more than one suitable study reporting the same outcome, results were combined for meta-analysis with RevMan software using the Peto Mantel-Haenszel method and a fixed effect model. Continuous data were expressed as a weighted mean difference (WMD) with 95% confidence intervals and were combined for meta-analysis using a fixed effect model.

Odds ratios were not calculated for comparisons of ICSI with IVF in lower quality studies, nor for any primary studies comparing ICSI with spontaneous conception, as such studies were more likely to be subject to confounding. The findings of these studies are reported solely in the text.

Description of studies

Ninety-two publications were retrieved by the search, of which 3 systematic reviews and 41 publications relating to primary studies were selected for inclusion.

Systematic reviews

Three systematic reviews were found which included assessments of the safety of ICSI (Tanbo 2002, Wennerholm 2004, Hansen 2005).

Tanbo 2002, an online publication, was a systematic review of ICSI prepared for the Norwegian Department of Health. It was in Norwegian with an English summary. The clinical outcomes of interest were as follows: congenital malformations, growth disturbances, neurological problems, developmental problems, chromosomal abnormalities and transmission of subfertility to male offspring. Thirty controlled studies were included, out of 443 retrieved from a search of electronic databases. Of these 30 studies, 13 had adequate or well-defined control groups and were rated as of acceptable quality. The reviewers found few data of acceptable quality on any of the endpoints apart from congenital malformations.

Wennerholm 2004 is a systematic review of IVF based on 113 of 635 studies retrieved by a PubMed search from 1978 to May 2003. One objective (of several) was to report on the differences between IVF and ICSI. Cohort and case-control studies were included that met (unspecified) quality criteria. No meta-analysis was conducted. ICSI and IVF were compared for obstetric outcomes, congenital malformations, chromosomal aberrations, and cognitive development. The authors commented on the limitations of existing research on ART outcomes, with most studies being underpowered and poorly controlled.

Hansen 2005 was a systematic review of ART and the risk of congenital malformations. All controlled studies comparing ICSI and IVF with spontaneous conception were pooled in a meta-analysis, regardless of quality. A second meta-analysis included only studies selected by a team of expert independent blinded reviewers as being of suitable quality for meta-analysis. The reviewers' quality criteria concerned sample size, whether assessment was blinded and objective, whether both groups were subject to the same intensity of surveillance, and whether statistical adjustment was appropriate. Comparisons of ICSI versus IVF were not addressed in this review, but a sub-group analysis included studies of ICSI versus spontaneous conception. Five studies of ICSI versus spontaneous conception were included in the initial meta-analysis, but only one (Hansen 2002) passed the quality assessment.

Primary studies

Eighty-nine publications relating to primary studies were retrieved, of which 42 publications relating to 35 different primary studies were selected for inclusion. The 42 included publications represent the most recent findings from each study.

Six studies accounted for 13 publications. Four studies published more than one paper about the same cohort of children (Barnes 2004, Bonduelle 2005 and Ponjaert-Kristoffersen 2005; Bonduelle 2004 and Ponjaert-Kristoffersen 2005; Bowen 1998 and Leslie 2003; Pinborg 2004 and 2004a). A further two studies had multiple publications which reported outcomes from overlapping cohorts (Bonduelle 2002 and 2003; Sutcliffe 2001 and 2003). A single publication was included for each of the other 29 studies. Two uncontrolled case series investigating chromosomal and epigenetic defects have been considered as separate studies, although they were conducted among the same participants as one or more of the above-mentioned studies (Bonduelle 2002a, Manning 2000).

In addition, 21 publications were found which reported preliminary findings in the 35 included studies (see Table 1) and 26 publications were excluded from analysis (see Table 3).

Prospective cohort studies

A research group at the Dutch-speaking University in Brussels have followed up children born since the first successful use of ICSI there in 1992. They have published many papers on this cohort, including findings for a range of outcomes in ICSI children at prenatal testing, birth, two months and two years (Bonduelle 2002 and 2003).

Five Australian cohort studies were included, three of which used registry data. The largest study comparing ICSI with IVF for congenital malformations was an unpublished study from Sydney, Australia. This used Australian and New Zealand registry data and reported congenital malformations at birth in 8325 ICSI offspring conceived from 1990 to 1999 (Lancaster 2004). Two other Australian studies used data from the same registry: Bryant et al. (2004) used 2002 data and reported miscarriage rates and obstetric outcomes and Hurst et al.

(2001) used 1998 to 1999 data and reported congenital malformation rates. Two other Australian studies also measured congenital malformation rates, one a registry study from Western Australia (Hansen 2002) and the other a smaller prospective cohort study that also reported developmental outcomes, measuring these at one year and five years (Bowen 1998, Leslie 2003)

There were several other prospective cohort studies comparing ICSI with IVF which measured the incidence of congenital malformations as their primary outcome, including an unpublished primary study from Germany (Paulus 2004), registry studies from Sweden (Ericson 2001) and Denmark (Pinborg 2004 and 2004a) and a tiny Japanese study (Kuwata 2004). Pinborg et al. (2004 and 2004a) also reported the utilisation of hospital care.

The above cohort studies all compared ICSI with IVF. Some also included a case-control comparison with spontaneously-conceived children (see below). One Egyptian cohort study compared 430 ICSI children with spontaneously-conceived children *without* an IVF control group: this study reported on chromosomal abnormalities at birth (Aboulghar 2001).

Retrospective cohort studies

A small US cohort study reported chromosomal abnormality rates in the products of conception in women undergoing dilatation and curettage for early miscarriage. The sample comprised 21 women after ICSI and 38 after IVF (Lathi 2004). Other small retrospective cohort studies comparing obstetric outcomes and congenital malformations at birth in ICSI and IVF babies were conducted in Israel (Bider 1999, Orvieto 2000) and in Spain (Van Golde 1999.

Case-control studies

ICSI vs IVF

A European five-nation cohort study measured a range of outcomes including malformation rates, child development and family outcomes at five years (Barnes 2004, Bonduelle 2005, Ponjaert-Kristoffersen 2005).

A large US study described outcomes after ICSI for 2059 children and compared them with IVF controls for congenital malformation rates. Other measures in this study were not controlled (Palermo 2000). A large unpublished German registry study reported the prevalence of major congenital abnormalities among 85 ICSI and 2002 IVF births on a population register (Weisel 2003).

Four smaller case-control studies also compared ICSI and IVF children. One, with 145 ICSI children, was conducted in Brussels at the French-speaking University (Govaerts 1998) and measured miscarriage rates, obstetric outcomes and congenital malformations at birth. A second smaller study conducted by the same group measured child development at in children aged from nine months to five years (Place 2003). Another small US study measured child development at 4-48 months (Squires 2003) and a tiny Greek study measured both child development and maternal psychological wellbeing at one year (Papaligoura 2004).

ICSI vs SC:

Several of the above-mentioned studies of ICSI versus IVF also had a control group of spontaneously-conceived children (Bonduelle 2002 and 2003; Barnes 2004, Bonduelle 2005 and Ponjaert-Kristoffersen 2005; Bowen 1998 and Leslie 2003; Hansen 2002; Kurinczuk

2003; Papaligoura 2004; Place 2003). The studies varied as to the means used of 'matching' the spontaneously-conceived group to the IVF and ICSI groups: see Appendix 2 for characteristics of included studies.

Four other case-control studies compared outcomes in ICSI and spontaneously-conceived children, with no IVF control group. The largest of these was an international study with 300 singletons born after ICSI in Belgium, Sweden and the US and 266 spontaneously-conceived matched controls. They were assessed for growth, general health and psychological outcomes at five years (Bonduelle 2004, Ponjeart-Kristoffersen 2004). Another case-control study with 208 children in the ICSI group measured congenital malformations and child development in children aged one to two years in the UK and subsequently in a smaller group in Australia (Sutcliffe 2001 and 2003). Two other small case-control studies reported on child development (Katagiri 2004) and parent-child interaction (La Sala 2004).

Case series and case studies

The group from the Dutch-speaking University of Brussels reported a case series on chromosomal testing of 2586 ICSI foetuses (Bonduelle 2002a) and a Turkish group reported the same outcome on 632 ICSI foetuses (Joswiak 2004).

The large Belgian group also reported a case series on the prevalence of imprinting defects in 92 children born after ICSI (Manning 2000). Three other case series, from the USA/Germany, France and the UK reported the prevalence of ART children on registers of Beckwith-Weidemann Syndrome (BWS), a rare disease that can be associated with an imprinting error (De Baun 2003, Gicquel 2003, Maher 2003). Another case series reported the incidence of ART children among the patients at a Dutch clinic specialising in retinoblastoma (Moll 2003)

Two case studies described two cases and a single case (respectively) of Angelman Syndrome (AS) in children conceived by ICSI (Cox 2002, Orstavik 2003). AS is another rare developmental disease that can be associated with an imprinting error.

Quality of included studies

Systematic reviews

The three included systematic reviews all searched the literature extensively, systematically evaluated the quality of the included trials and reported their findings clearly and objectively. The main focus of Wennerholm et al. (2004) and of Hansen et al. (2005) was the safety of ART in general (versus spontaneous conception) rather than specifically the safety of ICSI.

Tanbo et al. (2002) was concerned solely with ICSI outcomes and provided a meta-analysis of ICSI versus IVF for the outcome of congenital malformations. Several of the studies included in the meta-analysis have since been updated and the largest included study (Hurst 1999) included all micro-insemination techniques in the ICSI group and has therefore been excluded from the current review. Only the summary of this review is available in English.

Primary studies

All the included studies had methodological limitations. Many had insufficient power to detect a statistically significant or clinically significant difference between the groups yet reported non-significant P values as evidence that the groups were similar. The higher quality studies were generally the larger primary cohort or registry cohort studies. The registry

studies had the advantage of large sample sizes, but were inevitably limited by the impossibility of guaranteeing that data was complete.

The evidence of four studies was rated as of reasonable quality: these were all prospective studies of whole conception or birth cohorts which measured chromosomal abnormality rates (Aboulghar 2001), obstetric outcomes (Bryant 2004), congenital malformations (Hansen 2002) and all of above plus child development (Bonduelle 2002 and 2003). These studies had sample sizes large enough to show a significant difference between the groups for their primary outcomes.

The evidence of five studies was rated as of fair quality. One European case-control study of congenital malformations and child development was limited by a low or variable response rates in some of the trial centres (Barnes 2004, Bonduelle 2005 and Ponjaert-Kristoffersen 2005).

A smaller study of child development was largely prospective, but recruited extra ART children retrospectively at the five year follow up in order to increase study power for the primary outcome (Bowen 1998 and Leslie 2003). Three large registry studies (Hurst 2001, Lancaster 2004, Pinborg 2004 and 2004a) of congenital malformation rates were limited by the difficulty in ascertaining the completeness of the registry data and only one (Pinborg 2004 and 2004a) followed up children beyond birth.

The evidence of eleven studies was rated as of questionable quality. One was a large Swedish registry study known to have incomplete ascertainment and in which the comparison of ICSI with IVF was not clearly reported as this was not the primary outcome of the review (Ericson 2001). Another large cohort study from Germany reported obstetric and neonatal outcomes in ICSI versus spontaneously conceived children, but the groups were very unbalanced with respect to prognostic features such as maternal age and health. These measures were a secondary outcome of the study (Katalinic 2004). A third much smaller cohort study reported chromosomal abnormality rates in the products of conception of US women undergoing dilatation and curettage after early miscarriage. A whole cohort was followed up but the total sample size was only 59. It was not stated what proportion of all women who miscarried subsequently underwent dilation and curettage (Lathi 2004). The other eight were case-control studies with poor, unknown or unreported participation rates in one or more groups (Bonduelle 2004 and Ponjaert-Kristoffersen 2004; Govaerts 1998; Sutcliffe 2001 and 2003) and/or it was unclear whether recruitment was consecutive (Govaerts 1998), small retrospective cohort studies (Bider 1999, Orvieto 2000, Van Golde 1999), and two unpublished studies which gave little information as only the abstract was currently available (Paulus 2004, Weisel 2003).

The evidence of another seven controlled studies was rated as poor. These studies were clearly too small to show a clinical difference between the groups (Katagiri 2004, La Sala 2004, Place 2003, Papaligoura 2004), failed to describe their design (Squires 2003), reported events which they failed to include in analysis (Palermo 2000) and showed evidence of referral bias (Kuwata 2004).

Eight uncontrolled studies were included: the quality of their evidence was rated as poor. One of these was a case series reporting the prevalence of chromosomal abnormalities in ICSI prenatal tests (Bonduelle 2002a) and the other seven were case series and case reports on epigenetic disorders (Cox 2002, De Baun 2003, Gicquel 2003, Maher 2003, Manning 2000, Moll 2003, Orstavik 2003).

Results

Results are presented in the text below for each outcome of interest, with reference to tables where relevant. Results are presented for comparisons of ICSI with IVF, followed by comparisons of ICSI versus spontaneous conception. Where evidence was available from systematic reviews this precedes the results for primary studies for each outcome. However, for most outcomes of interest, other than congenital malformation rates, the systematic reviews found insufficient high quality evidence to reach any conclusion. Results of higher quality studies are reported first.

A highly simplified summary of the results is presented in Appendix 3.

Where a primary study had multiple current publications reporting findings for different outcomes, the single relevant publication has been referenced in the text below.

ICSI versus IVF

1. Obstetric outcomes ICSI vs IVF

Findings of systematic reviews:
The only systematic review (Wennerholm 2004) that compared ICSI and IVF for obstetric outcomes reported that they were comparable. These findings derived from three controlled studies (Bonduelle 2002, Govaerts 1998, Ludwig 2003).

Findings of primary studies:

- **Multiple pregnancy**

As stated in the literature, risk of conception of a multiple pregnancy is directly related to the number of embryos replaced and the only strategy to reduce the incidence of multiple pregnancies after ART is single embryo transfer (Kurinczuk 2003, De Sutter 2003). Only one primary study was found measuring multiple pregnancy that reported the mean number of transferred embryos in each group. This was a case-control study of questionable quality which analysed 145 ICSI and 145 IVF pregnancies. In both groups, the mean number of embryos transferred per participant was 2.7. The number of multiple gestations was similar in both groups (ICSI 35%, IVF 31%) (Govaerts 1998).

- **Miscarriage**

Two higher quality (ie, graded as reasonable or fair) cohort studies reported the incidence of miscarriage, defined as loss of clinical pregnancy under 20 weeks' gestation. There was no statistically significant difference between ICSI and IVF for this outcome (OR 1.00, 95% CI 0.90 to 1.11). See Table 4 1.1.

Three lower quality cohort studies and one case control study measured this outcome. Two of the cohort studies (Bider 1999, Van Golde) and the case-control study (Govaerts 1998) found no statistically significant difference between the groups. However, the third cohort study, of questionable quality, found a statistically significant difference which favoured the ICSI group (Orvieto 2000).

- **Ectopic pregnancy**

Two higher quality cohort studies reported the incidence of ectopic pregnancy per clinical pregnancy. There was no statistically significant difference between ICSI and IVF for this outcome (OR 0.78, 95% CI 0.57 to 1.06). See Table 4 1.2.

Two lower quality studies measured this outcome, one cohort (Orvieto 2000) and one case-control (Govaerts 1998): again no statistically significant difference was found between the groups.

- **Caesarean delivery**

No higher quality cohort studies measured caesarean rates as a pre-specified outcome.

Two lower quality cohort studies (Orvieto 2000, Van Golde 1999) and one case-control study (Govaerts 1998) measured this outcome and found no statistically significant difference between the groups.

- **Stillbirth**

Two higher quality cohort studies reported the incidence of stillbirth per child born. There was no statistically significant difference between ICSI and IVF for this outcome (OR 1.22, 95% CI 0.85 to 1.74). See Table 4 1.3.

Similarly, a lower quality cohort study (Van Golde 1999) and a case-control study (Govaerts 1998) that measured this outcome found no statistically significant difference between the two groups.

- **Premature birth (<37/40) per live born child or per delivery**

One large reasonable quality cohort study reported the incidence of premature birth per live child born (Bonduelle 2002). For singleton and multiple births combined (n=5795) there was no statistically significant difference between ICSI and IVF for this outcome (OR 1.12, 95% CI 1.00 to 1.25). However, the study also analysed over 4000 multiple births separately and found significantly more premature births among multiple births in the ICSI group compared with the IVF group (OR 1.27, 95% CI 1.09 to 1.48). See Table 4 1.4.

A small case-control study of questionable quality measured prematurity in 123 ICSI and 124 IVF conceptions and reported significantly more premature births among IVF twins than ICSI twins (p=<0.05): however, the total sample of twin births was tiny (n=61). No such difference was found between the groups for singleton births (Govaerts 1998). A small cohort study, also of questionable quality, reported prematurity rates per delivery in 87 ICSI deliveries and 96 IVF deliveries balanced for singleton/multiple status. No statistically significant difference was found between the groups (Van Golde 1999).

- **Low birth weight**

One large reasonable quality cohort study reported the incidence of low birth weight per live child born (Bonduelle 2002). Overall, there was no statistically significant difference between ICSI and IVF for this outcome (OR 1.01, 95% CI 0.90 to 1.14). When singleton births and multiple births were analysed separately this did not affect the significance of the results. See Table 4 1.5.

The small case-control study described above (Govaerts 1998) found a statistically significant difference in rates of low birth weight in twins, with ICSI twins being significantly heavier (p=<0.05), which was in accordance with their higher gestational age at delivery. No such difference was found between the groups for singleton births.

2. Neonatal/infant complications ICSI vs IVF

- **Neonatal complications requiring intervention**

One large reasonable quality cohort study (Bonduelle 2002) reported the incidence of neonatal complications or surgical interventions requiring hospitalisation in a neonatal unit. Overall, there was no statistically significant difference between ICSI and IVF for this outcome (OR 0.92, 95% CI 0.80 to 1.04). However, when singleton and multiple births were analysed separately, the complication rate was significantly lower for ICSI multiple children (n=1341) than for IVF multiple children (n=1399) (OR 0.82, 95% CI 0.70 to 0.97) (Bonduelle 2002) See Table 4 2.1.

- **Neonatal death**

Two large reasonable-quality cohort studies reported this outcome (Bonduelle 2002, Bryant 2004). There was a statistically significant difference between ICSI and IVF in the incidence of death before the age of 2 months, with a higher incidence in the IVF group (OR 0.33, 95% CI 0.20 to 0.54). Analyses by singleton/multiple status (Bonduelle 2002) did not change the significance of the results. See Table 4 2.2.

Two lower quality cohort studies (Bider 1999, Van Golde 1999) and one case-control study (Govaerts 1998) also measured this outcome. They found no statistically significant difference between the groups.

3. Chromosomal abnormalities ICSI vs IVF

Systematic review
Wennerholm et al (2004) found that ICSI-conceived children possibly have a slight increase in sex chromosomal abnormalities and that they may inherit the same Y chromosome microdeletion as their fathers. The authors did not attempt to quantify the risk. They cited one study comparing ICSI with IVF for this outcome (Bonduelle 2002a).

Primary studies
There were three studies comparing the incidence of chromosomal abnormalities in ICSI versus IVF foetuses undergoing prenatal diagnosis. Odds ratios were not calculated and results were not pooled as only a small proportion of each group were karyotyped and/or the total sample size was very small.

In the large Belgian cohort study (Bonduelle 2002), only 49.7% of ICSI foetuses and 16.6% of IVF foetuses were karyotyped. Of foetuses tested, 42/1437 (2.9%) of the ICSI group and 15/439 (3%) of the IVF group were abnormal. In the IVF group the abnormalities were mostly related to a higher maternal age, but in the ICSI group over 60% of the mothers were aged under 35 (Bonduelle 2002a). The authors noted that only 16.5% of the IVF group were tested, compared with 50% of the ICSI group and that the difference in uptake rate would have been expected to result in a significantly lower percentage of phenotypically abnormal

children in the ICSI group at birth (given that TOP was performed for foetuses with a major anomalies). However did not eventuate and there was an equal number of phenotypically abnormal children (n=3) born in each group. Although this study was graded as of reasonable quality overall, the findings for this outcome are of questionable value due to the low participation rate.

The evidence of another cohort study was also of questionable quality, due to the small sample size. The products of conception were karyotyped in 59 women who miscarried in the first trimester after conceiving by ICSI or IVF. There was a statistically significant difference between the groups (p=<0.01), with chromosomal abnormalities being detected in the products of conception of 76% of the ICSI group (16/21) and 41% of the IVF group (16/48). Most of the abnormalities were autosomal aneuploidies (having the wrong number of chromosomes) (Lathi 2004).

Another small lower quality study also reported this outcome. Van Golde et al. (1999) karyotyped 30% of pregnancies conceived by ICSI or IVF and detected abnormalities in 2/28 ICSI pregnancies and none of 29 IVF pregnancies. The mean maternal age was similar in both groups (33 and 34 years respectively).

4. Congenital malformations ICSI vs IVF

Systematic reviews:

Tanbo et al. (2002) found eleven controlled studies, including two Australian registry studies, which were of acceptable quality and compared ICSI versus IVF for the incidence of congenital malformations. Nine of these were pooled in a meta-analysis which included both cohort and case-control studies. Both cohort and case-control studies were included in the meta-analysis. Overall, the relative risk of major malformations for ICSI versus IVF was 1.13 (95% CI 1.00 to 1.29), which is not statistically significant, but supports the possibility of a small increased risk after ICSI. Separate meta-analyses on specific categories of malformations did not indicate any increased risk after ICSI for any particular malformation.

Wennerholm et al. (2004) found that ICSI children do not seem to be at any significant additional risk of congenital malformation, compared to IVF children. Their findings were based on controlled studies from Sweden (Ericson 2001) and Belgium (Bonduelle 2002), three Australian registry studies (Hurst 1999 and 2001, Hansen 2002) and the above-mentioned Norwegian Health Technology Assessment (Tanbo 2002).

Primary studies:

- **Major congenital malformations ICSI vs IVF**

Birth to two years' follow up

Five higher quality cohort studies (including two registry studies) compared the incidence of major congenital malformations in children/foetuses conceived as a result of ICSI or IVF. Three reported abnormalities diagnosed at birth (Bonduelle 2002, Hurst 2001, Lancaster 2004), while two included follow-up to one year of age (Bowen 1998, Hansen 2002).

In three cases analysis included not only births, but also pregnancy data – ie, affected foetal deaths and therapeutic terminations of pregnancy for foetal abnormality (TOPs) (Bonduelle 2002, Hurst 2001, Lancaster 2004). One study included TOPs though not other affected foetal deaths (Hansen 2002).

One of these studies, which is unpublished, did not report the size of the IVF sample (Lancaster 2004). The other four studies reported data suitable for meta-analysis and were pooled. There was no statistically significant difference in the incidence of congenital malformations between the two groups at birth, at one year or overall (overall result: OR 0.91, 95% CI 0.74 to 1.10). See Table 4 3.1.

As mentioned above, one reasonable quality cohort study (Lancaster 2004) was unsuitable for meta-analysis. This large unpublished Australian registry study analysed 4260 births and TOPs after ICSI and compared them with births and TOPs after IVF. The investigators found that the rate of major malformations at birth was similar between the two groups, at around 2.5%.

Bonduelle et al. (2002) analysed multiple births separately for this outcome – again, this did not affect the results for ICSI versus IVF (OR 0.82, 95% CI 0.56 to 1.20). The investigators reported that there were significantly more malformations in multiple births than in singletons in both groups (Bonduelle 2002). However, Hansen et al. (2002) found that restricting analysis to singletons did not materially affect the likelihood of malformations in either group.

Among the lower quality studies measuring the outcome were two cohort studies (Van Golde 1999, Paulus 2004) that measured congenital malformation rates at birth and at one year respectively and two case-control studies (Govaerts 1998, Palermo 2000) that measured rates at birth. None of these studies found any statistically significant difference between the groups.

Five years' follow up

A multi-centre European case-control study of fair quality (Bonduelle 2005) compared major malformation rates in ICSI with IVF, as well as, spontaneously-conceived children in the UK, Belgium, Sweden, Denmark and Greece. The ICSI and IVF groups included children from the UK and Belgium who had already been assessed at two years (Bonduelle 2002, Sutcliffe 2001). The proportion of children with major malformations was 33/540 (6%) in the ICSI groups and 18/437 (4%) in the IVF group. This difference was not statistically significant.

However, the investigators noted that although all three groups had comparable rates of major malformation at birth (according to their medical records), there were relatively more congenital malformations in the ICSI group by the age of five years. This increase was related to increased urogenital malformations and was higher in ICSI boys (8.2%) than ICSI girls (3.6%). Participation rates in this study varied between study centres from 25% to 96%, but the investigators noted that the Swedish study centre, with virtually complete data, had results consistent with other countries (Bonduelle 2005).

- **Major and minor congenital malformations ICSI vs IVF**

Two higher quality cohort studies, one a registry study of twins only (Pinborg 2004), compared the incidence of major and minor congenital malformations in children born as a result of ICSI or IVF. These data include live births only. They reported abnormalities diagnosed at birth (Bonduelle 2002, Pinborg 2004).

Meta-analysis of these studies found a statistically significant difference overall between the groups, favouring the ICSI group (OR 0.67, 95% CI 0.56 to 0.79). However, there was

significant statistical heterogeneity in these results (I^2 69.7% for singletons and multiples combined, I^2 87.5% for multiples only). This finding was attributed to a high rate of minor malformations detected on routine screening ultrasounds among multiple births in the IVF group in the largest of the two studies (Bonduelle 2002). Bonduelle et al. (2002) reported that when they excluded the children who had routine examinations at birth, the rate of malformations diagnosed dropped from 6.7 to 4.4% in the ICSI group and from 9 to 5.3% in the IVF group, the difference between the groups becoming non-significant. Moreover, when singletons were considered separately there was no significant difference between the groups.

Four lower quality studies reported this outcome (Ericson 2001, Weisel 2003, Bider 1999, Kuwata 2004). Ericson et al. (2001), a Swedish registry study, found an excess of malformations in the ICSI group with 118/1652 (7.1%) in the ICSI group and 398/7523 (5.3%) in the IVF group in infants aged up to two years. The primary comparison of interest in this study was ART versus spontaneous conception and the investigators did not comment specifically on the excess of malformations diagnosed in the ICSI group. They reported that ascertainment of malformations was incomplete, but that missing data was hopefully random. Weisel et al. (2003) was a large German case-control study that was considered of uncertain quality as it was unpublished and gave few details on methodology in the abstract. In this study, rates of major congenital malformations at birth were measured in 20,161 live-births, stillbirths, miscarriages and TOPs registered on the Mainz Birth Registry from 1994 to 2001 and events were analysed according to mode of conception. The odds for being diagnosed with a malformation were significantly higher in ICSI than in IVF children (OR 2.4, 95% CI 1.1 to 5.2) (Wiesel 2003).

Bider et al (1999) and Kuwata (2004) were two small cohort studies which measured malformation rates in twins. Neither found any significant difference between the groups.

Five years' follow-up: ICSI vs IVF

The above-mentioned multi-centre European case-control study also reported rates of major and/or minor malformation in 540 ICSI children and 437 IVF children. The proportion of children with any malformation was the same (33%) in both groups (Bonduelle 2005).

- **Specific congenital malformations**

Several cohort studies listed the incidence of specific malformations diagnosed in ICSI and IVF children. In a single case, a registry study of uncertain quality, a statistically significant difference was found between ICSI and IVF children (Ericson 2001), namely an increased risk of hypospadias in the ICSI children: 10/1652 (0.6%) vs 18/7523 (0.23%). However, a meta-analysis of the two higher quality cohort studies that reported the incidence of hypospadias showed no significant difference in incidence between the ICSI group and the IVF group (OR 1.35, 95% CI 0.80 to 2.30). This meta-analysis included 3664 in the ICSI groups and 5485 in the IVF groups. See graph 4.3.

One lower quality cohort study (Kuwata 2004) also measured the incidence of hypospadias and found no statistically significant difference between the groups.

5. Child development ICSI vs IVF

- **Physical and psychomotor development**

This outcome was reported by three higher quality studies, comprising one cohort study, one registry cohort study and one case control study (Bowen 1998, Pinborg 2004a, Bonduelle 2005 and Ponjaert-Kristoffersen 2005)

Bowen et al. (1998) compared 92 ICSI and 86 IVF Australian one-year-old children and found no statistically significant difference in their physical development nor in their scores in formal psychomotor testing. This study also measured hospital admission rates, as did a large Danish registry study of 835 ICSI and 2558 IVF twins aged from two to seven years (Pinborg 2004a). Neither study found a statistically significant difference between the groups. See Table 5 5.1.

The multicentre European case-control study assessed the physical health of 540 ICSI and 437 IVF children aged five and found no statistically significant difference between them for use of health care resources or for other physical measures such as height, weight and childhood illnesses (Bonduelle 2005). Similarly, measures of motor development in this study found no statistically significant difference between the ICSI and IVF groups (Ponjaert-Kristoffersen 2005).

Among the lower quality studies, an unpublished German cohort study (Paulus 2004) reported no statistically significant difference in the growth and development of 434 ICSI and 356 IVF one year old children and a tiny Belgian case-control study assessed full-term singleton preschoolers for hospital admission rates and found no statistically significant difference between the groups (Place 2003). A Spanish cohort study of 120 ICSI and 132 IVF children aged 6 to 18 months found no difference in the proportion of children with developmental delay in the two groups (Van Golde 1999). A small US case-control study administered a developmental questionnaire to the parents of 141 ICSI children and 144 IVF children aged from 4 to 48 months: 24/141 (17%) of ICSI children and 14/144 (9.7%) of IVF children were designated ‘at risk of developmental delay’. However, the participation rate of eligible children was unknown and there was no independent evaluation of development (Squires 2003).

- **Cognitive development**

Three higher quality studies used cognitive development measures to compare ICSI and IVF children. These comprised two cohort studies (Bowen1998 and Leslie 2003; Bonduelle 2003) and one case-control study (Ponjaert-Kristoffersen 2005)

The Australian cohort study (Bowen 1998) found a statistically significant difference between ICSI and IVF one-year-olds tested with the Bayley Mental Development Index, with a greater proportion of ICSI children assessed as mildly or significantly delayed in mental development. However, at the age of five, 84% of the original cohort were retested using the Wechsler scale, along with additional groups of children in the ICSI and IVF groups to increase study power, and no statistically significant difference was found between the groups in the proportion of five-year-olds with delayed cognitive development (Leslie 2003). See Table 5 5.2 .

The large Belgian cohort study tested 439 ICSI and 207 IVF two-year-olds, using the Bayley Mental Development Index. Only 27% of the original birth cohort were tested: statistical analysis of prognostic factors indicated that the group tested were representative of singletons in the original cohorts, but only a small sample of ICSI twins participated. No statistically significant difference was found between the groups in overall scores, nor in the proportion of children with delayed cognitive development (Bonduelle 2003). See Table 4 4.2.

In an unpublished paper, the multi-centre European case-control study assessed cognitive development in 483 ICSI and 399 IVF five-year-olds. They found no statistically significant difference in Wechsler scale scores for intelligence. Moreover, each group had a similar proportion of children with delayed cognitive development: 21/483 (4.3%) in the ICSI group and 17/399 (4.2%) in the IVF group (Ponjaert-Kristoffersen 2005).

Two lower quality studies, both case-controls, also reported this outcome. The tiny Belgian case-control study assessed full-term singleton preschoolers for hospital admission rates and for child development, using the Brunet-Lezine scale for 9 and 18 month olds and the Wechsler scale for three- and five-year-olds. Most children were tested twice. No statistically significant difference was found between the ICSI and IVF groups at any time-point (Place 2003). Similarly, a tiny Greek case-control study reported no statistically significant difference in Bayley scores for mental development between 34 ICSI and 24 IVF one-year olds who were assessed blindly by a psychologist (Papaligoura 2004).

6. Psychological outcomes **ICSI vs IVF**

The above-mentioned multi-centre European case-control study, which was of fair quality, assessed family functioning and children's socio-emotional development at five years in 540 ICSI and 437 IVF five-year-olds. Children were tested with the Bene-Anthony scale to measure their perception of their parents and the parents completed a battery of questionnaires relating to general health, parenting stress, marital relationships, and current commitment to parenting, as well as a questionnaire about their child's temperament and behaviour. No statistically significant difference was found between the groups for any of these measures (Barnes 2004).

7. Epigenetic disorders **ICSI vs IVF**

No studies were found which compared ICSI with IVF for this outcome.

ICSI versus spontaneous conception or with no control group

For comparisons of ICSI with spontaneous conception, which is our secondary outcome, we have reported below only the highest level of evidence available for each outcome of interest.

1. Obstetric and neonatal outcomes **ICSI vs spontaneous conception**

No higher quality controlled studies were found for this outcome.

A large cohort study from Germany measured obstetric and neonatal outcomes in ICSI versus spontaneously-conceived children as a secondary outcome (the primary outcome of the study being congenital malformation rates). The prognostic factors of the two groups differed markedly and so the evidence of this study was graded as questionable. There were significantly more obstetric and neonatal complications in the ICSI group. Even when singletons were considered separately, the ICSI group had significantly higher rates of vaginal bleeding, placental problems, oligohydramnios, anaemia, pre-eclampsia, premature labour and birth, and low birth weight and they had a lower mean gestational age (Katalinic 2004)

A case-control study, also of questionable quality, conducted by a UK team compared obstetric outcomes in with 208 ICSI and 221 spontaneously-conceived singleton pregnancies (Sutcliffe 2001) and in 58 ICSI and 38 spontaneously-conceived singleton pregnancies (Sutcliffe 1999). Outcomes were similar apart from a higher rate of caesarean deliveries in the ICSI group. However, a small case-control study, also of questionable quality (Place 2003), compared caesarean section rates in 66 full-term ICSI singletons with 59 spontaneously-conceived IVF singletons and reported that the rates were similar.

2. Chromosomal abnormalities **ICSI vs spontaneous conception (or no controls)**

Systematic reviews:
As noted above, Wennerholm et al. (2004) found that children conceived by ICSI may be at increased risk of sex chromosomal aberrations and may inherit the same Y chromosome microdeletion as their fathers. They cited one study comparing ICSI with population rates (Aboulghar 2001).

Tanbo et al. (2002) reported that although infertility will be inherited by the sons of males with Y chromosome microdeletions (and possibly also by the sons of other men with severely compromised sperm quality), theoretical models suggest that ICSI will not result in any substantial increase in male infertility. The reviewers cited two uncontrolled genetic studies (Cram 2000, Kent-First 1996).

Primary studies:
One reasonable quality cohort study karyotyped 430 ICSI and 430 spontaneously-conceived and consecutively delivered babies at birth and compared the incidence of chromosomal abnormalities. The groups were balanced for the proportion of mothers over 35. There were no chromosomal abnormalities in the spontaneously-conceived group and 15/430 (3.5%) in the ICSI group. This was statistically significant (p=<0.001). Six babies had sex chromosome

abnormalities (including XO, XXY, XYY, and XXX), eight had autosomal abnormalities and one had both. Parents in this study were not systematically karyotyped so it is unclear whether the abnormalities in ICSI children were de novo (Aboulghar 2001).

A case-series published by the Belgian group compared chromosomal abnormality rates in 1586 ICSI foetuses with general population rates reported in the literature. The group tested comprised 60% of foetuses of over 12 weeks' gestation and were considered to be a fairly representative sample of the total ICSI cohort. Among ICSI foetuses, 22/1586 (1.4%) had inherited abnormalities, compared with a rate of 0.3–0.4% among prenatally tested foetuses in the general population: this difference was statistically significant (p=<0.001). In addition, 25/1586 (1.6%) had de novo abnormalities versus 0.5% among women of the same age undergoing amniocentesis in the general population: this difference was also statistically significant (p=<0.007). Among the 25 de novo abnormalities, 10 were sex chromosomal abnormalities of which 6 were numerical (XXX, XXY or XYY) and 4 were mosaic. Fifteen of the de novo abnormalities were autosomal (eight numerical and seven structural). De novo abnormalities were significantly more common among men with a sperm concentration of $<20X10^6$ ml and/or <50% motile sperm. The incidence of sex chromosomal abnormalities was compared with reports from the literature citing rates in unselected newborn populations, the authors suggesting that these rates were comparable because sex chromosomal aberrations are not life threatening. The rate of 1.6% in the ICSI foetuses was about three times the rate reported in very large population studies (Bonduelle 2002a).

3. Congenital malformations **ICSI vs spontaneous conception**

Systematic reviews
Wennerholm et al. (2004) reviewed controlled studies of this outcome published up to 2003, and concluded that both IVF and ICSI children were at a slightly increased risk of congenital malformation compared to naturally conceived children. The reviewers concluded that the absolute risk "seems to be small and might be acceptable to couples who would otherwise not achieve pregnancy" (p 42).

A recent rigorously conducted Australian systematic review of ART and the risk of congenital malformations (Hansen 2005) included a subgroup analysis comparing event rates in ICSI versus spontaneous conception. Five relevant cohort and case-control studies were found: four of these are described elsewhere in this review as they included comparisons with IVF (Hansen 2002, Bowen 1998, Sutcliffe 2001, Sutcliffe 2003) and one was a large German study that compared ICSI with spontaneous conception (Ludwig 2002). However, only one study was judged to be of acceptable quality for meta-analysis: this study (Hansen 2002) found a significantly increased risk of major congenital abnormality in the ICSI group (OR 2.0, 95% CI 1.3 to 3.2). When lower quality studies (n=4) were also included, the increased risk from ICSI remained, though the odds ratio was lower (OR 1.28, 95% CI 1.14 to 1.43).

Hansen et al. (2005) included studies published up to 2003. Since then Bonduelle et al. (2005) have published their multi-centre case-control study of European five-year-olds, which included 540 ICSI and 538 spontaneously-conceived children, matched for maternal age and education, socio-economic status, gender and birth order. They found a significantly increased risk of a major congenital malformation in the ICSI group (OR 2.77, 95% CI 1.41 to 5.46) compared to the spontaneously-conceived group.

4. Child development ICSI vs spontaneous conception

- **Physical development**

One higher quality study measured this outcome: a multi-centre European case-control study of 540 ICSI and 538 matched spontaneously-conceived children measured physical health in five-year-olds. The ICSI group were significantly more likely to have had a significant childhood illness or need health care resources than the spontaneously-conceived group. However, measures of motor development in this study found no statistically significant difference between the ICSI and IVF groups (Bonduelle 2005, Ponjaert-Kristoffersen 2005).

Among lower quality studies, a European/US case-control study compared 300 ICSI and 266 spontaneously-conceived five-year-old singletons, matched for gender, child age and maternal age). Participation rates were low in some centres and the Belgian cohort in this study (n=100) may overlap with the study mentioned above. The main outcome measure was growth: ICSI children were found to have a significantly lower birth weight and a higher rate of health care interventions than the spontaneously-conceived group, but at five years their height and weight were not significantly different, indicating catch-up growth (Bonduelle 2004).

- **Cognitive/psychomotor development**

Two higher quality studies measured this outcome. An Australian cohort study found that significantly more ICSI children than spontaneously-conceived children experienced developmental delay at one year. However, retesting at five years showed no significant difference between the groups (Bowen 1998 and Leslie 2003). The multi-centre European case-control study of 540 ICSI and 538 matched spontaneously-conceived children found no significant differences in scores for cognitive development between ICSI and spontaneously conceived five-year-olds, nor was there any difference in the proportion of children with developmental delay (Ponjaert-Kristoffersen 2005, unpublished).

Six lower quality case studies also reported this outcome (Ponjaert-Kristofferssen 2004, Sutcliffe 2001 and 2003, Katagiri 2004, La Sala 2004, Place 2003, Papaligoura 2004). The European/US case-control study of 300 ICSI and 266 matched spontaneously-conceived five-year-old singletons found no statistically significant difference in their overall cognitive development. In psychomotor measures, some differences were found between the groups that were attributed partly to subtest differences between the study sites. There was a persistent statistically significant trend for ICSI children to have lower fine motor development than spontaneously-conceived children: however, the groups were unbalanced for maternal age, with ICSI parents being significantly older. Outcomes assessment was blinded at only one of the three study centres (Ponjeart-Kristoffersen 2004).

Sutcliffe et al. (2001 and 2003) measured neuro-development in case-control comparisons of 208 ICSI and 221 spontaneously-conceived singleton British children aged from one to two years and 58 ICSI and 28 spontaneously-conceived Australian singletons aged 13 months. In neither case was any statistically significant difference found between the groups.

Four other small case-control studies also found no statistically significant difference between the groups in their overall psychomotor and/or cognitive development. These included a US study of 1010 ICSI and 57 spontaneously conceived five-year-olds (Katagiri 2004), an Italian study of 50 ICSI and 51 spontaneously-conceived children one- to two-year-olds (La Sala

2004), a tiny Greek study of 34 ICSI and 29 spontaneously-conceived one-year-olds (Papaligoura 2004) and a small Belgian study of 66 ICSI and 59 spontaneously-conceived preschoolers (Place 2003). The Belgian study noted lower scores for three and five-year-olds for intellectual development, but the differences were no longer significant when statistical adjustment for differences in parental education was made.

5. Psychological outcomes **ICSI vs spontaneous conception**

The multi-centre European case-control study comparing 540 ICSI and 538 matched spontaneously-conceived children found few differences between them. The only significant differences were that mothers in the ICSI group were less likely to express hostility or aggression about their child and reported higher levels of commitment to parenting than mothers of spontaneously conceived children (Barnes 2004).

The above-mentioned European/US case-control study of 300 ICSI and 266 matched spontaneously-conceived five-year-old singletons also measured emotional/behavioural development in children and parenting stress levels. Results showed significant subtest differences by study site (Brussels, New York and Goteborg): the investigators suggested that these might be attributable to differences in culture and in study recruitment procedures, related to the different medical systems operating in Belgium, the USA and Sweden. After adjustment for study site, there were no significant differences between the groups for child emotional-behavioural development. Scores on the Parental Stress Index were significantly higher (ie, worse) for parents of spontaneously-conceived children than parents of ICSI children. However the investigators noted that these differences were probably not clinically significant. The quality of this study was questionable, with a low or unknown participation rate of eligible children in Belgium and the USA (Ponjaert-Kristoffersen 2004).

A poor quality Italian study (La Sala 2003) measured the quality of parent-child interaction in 50 ICSI and 50 spontaneously-conceived children, using blinded assessment of video-taped parent-child interaction. The investigators reported less sensitive and more controlling parenting patterns among ICSI mothers and fathers (respectively) of one-year-olds than among spontaneously-conceived parents, but no difference between families in the two groups whose children were aged two.

6. Epigenetic disorders **ICSI/IVF versus general population controls**

No controlled studies were found reporting epigenetic disorders in ICSI versus spontaneous conception.

The only imprinting disorders about which any relevant evidence was found were Angelman Syndrome, Prader-Willi Syndrome, Beckwith-Weidemann Syndrome and Retinoblastoma. Six case series or case reports were found. One measured the prevalence of specific imprinting disorders in children born after ICSI (Manning 2000) and five described ICSI or IVF children affected by imprinting disorders and compared them to population norms (Cox 2002, Orstavik 2003, De Baun 2003, Maher 2003, Gicquel 2003). All reported ICSI outcomes separately.

Manning et al. (2000) investigated the incidence of specific imprinting disorders in 92 children born after ICSI. The investigators tested the chromosome associated with Angelman and Prader-Willi Syndromes, which are neurodegenerative diseases that can be caused by an imprinting defect. None of the children was found to have any abnormality. However, the authors noted that in a normal population only 1 in 15,000 newborns is affected and thus far more data were needed.

Two studies outlined below described children conceived by ICSI who were found to have Angelman Syndrome (AS), which is characterised by severe mental retardation and other developmental problems (Cox 2002, Orstavik 2003):

- Cox et al. (2002) described two ICSI children diagnosed with AS after being referred for assessment of developmental delay. Both were found to have imprinting errors. Their parents were chromosomally normal though both fathers had oligospermia (low sperm count). The authors suggested that the assumption of a causal relationship between ICSI and AS was not unreasonable, for the following reasons:
  a. the incidence of imprinting defects causing AS in the general population of newborns is 1/300,000
  b. there was no evidence that the defects were inherited
  c. it is scientifically plausible that ICSI may interfere with imprinting
  d. animal studies have reported imprinting disorders related to in-vitro tissue culture

- Orstavik (2003) described a third similar case, though in this instance the father had normal sperm.

Three studies outlined below suggested a link between ART and Beckwith-Weidemann Syndrome (BWS), which is a congenital disorder associated with overgrowth and a predisposition to embryonic cancer (De Baun 2003, Maher 2003, Gicquel 2003) and is caused by an imprinting disorder in about 60% of cases (Maher 2003).

- De Baun et al. (2003) reported finding seven children conceived by ART among children in a US BWS registry set up to monitor cancer incidence. After noting that four patients on the register were born after ART, the researchers specifically requested data about ART for patients joining the register from 2001. A further 3 children with BWS were identified out of 65 joining the register from 2001, giving a prevalence of 5% among children conceived by ART compared to 0.8% among the background population, thus suggesting a 6-fold increased prevalence in ART children. In five of the six cases for which samples were available, BWS was associated with an imprinting mutation. Five of the seven cases were conceived by ICSI, which was used in 42% of IVF cycles in the general population: the study was too small to determine whether ICSI increased the risk of BWS over and above IVF alone.

- Maher et al. (2003) reviewed the notes of those patients referred to a UK BWS group for whom detailed clinical information was available. They reported that 6/49 were conceived by ART, three after ICSI and three after IVF alone. Estimating that 1% of children resulted from ART conceptions, the authors estimated a three-fold increase in the expected prevalence of BWS among such children. Two cases were assessed for imprinting errors and both were positive.

- Gicquel et al. (2003) reported strikingly similar results, with 6/149 BWS patients diagnosed at their reference centre in France having been born following ART, 2/6 after ICSI. In accordance with Maher et al. (2003), the authors suggested a three-fold increase in risk associated with ART, based on a prevalence of 4% in ART patients in their series compared with 1.3% in the general French population. All six patients in this study had BWS associated with imprinting defects.

Moll et al. (2003) reported the incidence of retinoblastoma in children born in the Netherlands after ART. Retinoblastoma is a tumour of the retina which in some cases is linked to epigenetic mechanisms (Niemitz 2004). The authors inferred a relative risk of 4.9 (95% CI 1.6 to 11.3), assuming that 1.5% of births were after IVF. One out of the five cases of retinoblastoma diagnosed in ART patients involved ICSI as well as IVF. However, the authors did not report whether the diagnoses were associated with imprinting errors.

Discussion

Quality
All the included studies were limited by methodological shortcomings. Most primary studies had small sample numbers with insufficient power and were poorly controlled, especially for longer term outcomes such as child development. The larger registry studies had more statistical power, but were subject to an unknown degree of ascertainment bias. Some findings were potentially misleading due to insufficient follow up: thus several studies assessed congenital malformation rates only at birth, whereas a substantial proportion of malformations become apparent during the first year of life or later. There were no controlled studies of children older than five years. There is a clear need to monitor the long-term safety of ICSI by collecting data from large cohorts with proper control groups.

Obstetric outcomes
ICSI and IVF resulted in comparable rates of miscarriage, ectopic pregnancy, stillbirth and low birth weight. Prematurity rates were also comparable among singletons, though there was evidence in one large study of an increased prematurity rate among multiple births in the ICSI group. The reason for this finding was uncertain, as although the ICSI mothers were older and more likely to be primigravidae, other outcomes were similar (Bonduelle 2002). The included studies provided little evidence about caesarean section rates or multiple pregnancy rates: however, as noted above, rates of multiple pregnancy are determined largely by the number of embryos transferred.

Neonatal outcomes
ICSI and IVF were also comparable with respect to overall neonatal complications measured by intervention rates. However, when multiple births were considered separately, the only study of reasonable quality found that ICSI had a significantly *lower* complication rate than IVF: this was based on an analysis of 2754 multiple births (Bonduelle 2002). Moreover, a meta-analysis of Bonduelle et al. (2002) with another large cohort study (Bryant 2004) found a significantly lower incidence of neonatal/infant death in the ICSI group. This was partly attributable to high death rates among multiple pregnancies in the IVF group in one of the studies, but the risk remained significantly lower in the ICSI group when singletons were considered separately in this study (Bonduelle 2002).

Chromosomal and genetic abnormalities
Lancaster et al. (2004, unpublished data) note the lack of systematic prenatal or postnatal cytogenic studies on chromosomal abnormality rates after ICSI.

Bonduelle et al. (2002) compared karyotypes among 1437 ICSI and 493 IVF foetuses and found the rates similar (2.9% vs 3%). However, this finding is of questionable value given the low uptake of prenatal testing, especially in the IVF group. The case-series published by the same group (Bonduelle 2002a) found a three- to four-fold increase in inherited and de novo (newly occurring) chromosomal abnormalities among ICSI foetuses compared to general population rates. This study reported that de novo abnormalities particularly affected the sex chromosomes and were related to sperm concentration and motility.

A higher rate of congenital abnormalities was found in ICSI foetuses after early miscarriage, compared to IVF (Lathi 2004) and Aboulghar et al. (2001) found a significantly increased

chromosomal abnormality rate in ICSI children compared to spontaneously-conceived children.

Chromosomal abnormalities in ICSI offspring may be inherited or de novo. An inherited anomaly is commonly a structural defect exactly the same that carried by one of the parents. The children may be phenotypically normal at birth but may be subject to a slight increase in mental retardation and/or malformation due to minor chromosomal imbalances secondary to the structural anomaly (Bonduelle 2002a).

De novo abnormalities in ICSI offspring are often sex chromosomal aneuploidies which are probably associated with sperm defects, even though the father may be karyotypically normal. Children born with sex chromosomal abnormalities usually have a normal physical appearance and an IQ within the normal range but they are often infertile and there is also a moderate risk of developmental problems in the areas of speech, motor skills and learning abilities. De novo structural abnormalities may be less benign and carry more risk of mental retardation (Bonduelle 1998, 2002a).

Careful genetic screening for chromosomal abnormalities and Y choromosome microdeletions is recommended for couples contemplating ICSI, along with the offer of prenatal diagnosis where pregnancy is achieved. Other interventions such as preimplantation genetic diagnosis (PGD) or even use of donor gametes (eggs or sperm) may be the best option in some cases (Bonduelle 2002a, Kurinczuk 2003).

It appears that all sons of men with Y chromosomal microdeletions will inherit the anomaly and are thus likely to be infertile themselves. It had been assumed until recently that infertility was the only problem likely to ensue, but there are some concerns that more serious chromosomal disorders, undetectable by standard karyotyping, may be found in association with Y chromosome microdeletions. Similarly, congenital absence of the vas deferens may be the only clinical symptom of mutations in the cystic fibrosis gene which could be inherited by ICSI offspring in a more severe form if the mother is also a carrier (Kurinczuk 2003).

Congenital malformations
The evidence on congenital malformation rates is difficult to interpret. Rates of major malformations diagnosed during the first year of life were similar after ICSI and IVF. However, there were no cohort studies measuring this outcome beyond two years and the only higher quality study with longer follow up, a case-control study, found that although there was no statistically significant difference between the groups at five years, relatively more congenital malformations had become evident in the ICSI children. The additional malformations were mainly urogenital abnormalities in boys (Bonduelle 2005).

One study (Ericson 2001) reported a statistically significant increase in hypospadias in ICSI two-year-olds. However this study was limited by ascertainment bias and no other study found a statistically significant increased risk of hypospadias or any other specific abnormality.

For the outcome of *any* malformation (major or minor), meta-analysis of two large studies showed evidence of a significantly higher rate in the IVF group at birth. However, this finding apparently related to heart problems in multiples in the IVF group in the largest study (Bonduelle 2002), these being short-term problems which resolved spontaneously. Two large

registry studies also found higher rates of major and/or minor malformations in children after ICSI compared to IVF, but one was poorly reported with only the abstract currently available (Weisel 2003) and the other reported problems with ascertainment (Ericson 2001).

Several studies have investigated whether outcomes for ICSI children differ according to the pathology underlying infertility or the quality of sperm used. Two studies found that obstetric outcomes were generally similar regardless of the sperm origin, sperm quality or and whether it is fresh or frozen (Aytoz 1998 and 1999, Wennerholm 2000a), though one of these studies reported a higher rate of intrauterine death in the ICSI group when ejaculated sperm of very poor quality was used (Aytoz 1998). For the outcome of congenital malformations, no differences were found in relation to the indication for ICSI (Vernaeve 2003, Ludwig 2003) or the origin of the sperm (ejaculated, epididymal or testicular) (Bonduelle 2002, Joswiak 2004, Ludwig 2003). When sperm parameters were investigated, some studies found no differences in malformation rates in relation to sperm concentration (Ludwig 2003, Wennerholm 2002) or sperm morphology (Bonduelle 2002). However, one study found that major congenital malformations were more frequent where sperm motility was below 50% (Bonduelle 2002) and another found a higher frequency of chromosomal abnormalities where sperm motility and/or concentration were low (<50%; $<20X10^6$ respectively) (Bonduelle 2002a).

Although, as noted above, no reliable studies have shown a significantly increased risk of congenital malformations after ICSI compared to IVF, there is increasing evidence of a significantly increased risk of major malformations after ICSI *compared to spontaneous conception*. Two recent higher quality publications, one a systematic review, calculated odds ratios of 2.0 (95% CI 1.3 to 3.2) and 2.77 (95% CI 1.41 to 5.46) respectively for major congenital malformations (Hansen 2005, Bonduelle 2005). Hansen et al. (2005) suggest that clinicians counselling their patients should calculate their absolute risk of a congenital malformation in terms of the number needed to harm, and should base this on a 30–40% increase of risk over and above the baseline prevalence for their population. The baseline prevalence varies according to the population and the definition of malformation used. The Victorian Perinatal Data Collection Unit (2004) reported an underlying rate of notifiable birth defects of 3.9% in 2002. Most minor defects were excluded from this calculation. Assuming an underlying prevalence of major malformations 4%, 62 children would need to be conceived by ART for one additional child to be born with a major congenital malformation (Hansen 2005).

Child development

There was very little reliable evidence available for this outcome, and none for children older than five years. There were only two higher quality studies measuring child development in ICSI children beyond the age of two (Barnes 2004, Bonduelle 2005 and Ponjaert-Kristoffersen 2005; Leslie 2003).

From the evidence available, ICSI children appeared to be similar to IVF children in their physical development and use of health care resources, though compared to spontaneously conceived children they were significantly more likely to have had a major childhood illness or need health care resources (Bowen 1998 and Leslie 2003; Pinborg 2004 and 2004a; Barnes 2004, Bonduelle 2005 and Ponjaert-Kristoffersen 2005).

Although, one study reported that one-year-old ICSI children were more likely to experience developmental delay, a follow up study at five years was reassuring (Bowen 1998 and Leslie 2003). No other studies found evidence of any major difference between the ICSI and IVF groups with respect to motor or cognitive development. Regression analysis in one study showed that the factors influencing cognitive development in two-year-old singletons were sex, pregnancy duration, parity and child's age: method of conception and sperm parameters were not significant factors (Bonduelle 2003).

Psychological outcomes

In the single higher quality study reporting this outcome, parents reported similar temperaments and levels of behaviour problems in their children regardless of their mode of conception (IVF, ICSI or spontaneous). Nor did mode of conception affect the incidence of marital difficulties, mental health problems or family stress (Barnes 2004).

Epigenetic disorders

It is unclear from the handful of case reports and small studies published to date whether ART increases the frequency of epigenetic abnormalities and if so whether ICSI is specifically implicated. As all such disorders are rare, a sample large enough to detect minor increases is likely to require linkage of multiple large population-based disease registers to population-based registers of ICSI offspring (Kurinczuk 2003). It is feasible that imprinting errors may account for a wider spectrum of ART-related complications than is currently recognised, as faulty imprinting is suspected to play a role in neuro-behavioural disorders such as autism, bipolar affective disorder and schizophrenia. It is also associated with certain cancers (De Rycke 2002). As such disorders may only manifest themselves in older children or adults, long term follow up will be required. Further animal studies may also help to explain the pathogenesis of epigenetic disorders (Lucifero 2004).

A recent large controlled study (Halliday 2004) compared the incidence of BWS in ART versus spontaneously-conceived children, using Australian registry data and matching mothers for age. BWS was nine times more common in the ART population than in the general population, which was statistically significant ($p=0.06$). This study did not report what proportion of the ART children had been conceived using ICSI. The authors note that the overall risk of BWS in children conceived using ART remains low, and that BWS is in most cases associated with good outcome (Halliday 2004).

With regard to all the above outcomes, the questions raised by the evidence to date reinforce the need for large well-designed cohort studies, ongoing follow up and appropriately-timed outcome assessment.

Conclusion

IVF and ICSI children both have an increased risk of adverse obstetric and perinatal outcomes compared to spontaneously-conceived children. This is mainly due to the high rate of multiple births in ART pregnancies, associated with multiple embryo transfer. However, the risk is also increased for ART singletons, who have a higher rate of prematurity and low birth weight than spontaneously-conceived singletons. This applies even after adjustment for maternal age and other background variables (Wennerholm 2004). ICSI children do not appear to be at any greater risk than IVF children of adverse obstetric and perinatal outcomes.

Children conceived by ICSI may have a higher rate of chromosomal abnormalities than those conceived by IVF, but there have been no large well-controlled studies reporting this outcome. Based on comparison with population data, children conceived by ICSI were found to have a three- to four-fold increase in risk of inherited or de novo chromosomal abnormalities compared to spontaneously-conceived children. The individual's risk of inherited abnormality can generally be assessed if the parental karyotypes are known but the risk of a de novo abnormality is less predictable: it appears to be higher where sperm concentration and motility are low. The *absolute* risk of a de novo chromosomal abnormality diagnosed prenatally is around 1.6% for ICSI conceptions versus 0.5% for spontaneous conceptions (Bonduelle 2002a).

De novo abnormalities in ICSI foetuses consist mainly of an increased number of sex chromosomal abnormalities, though structural abnormalities are also increased. Such abnormalities are frequently relatively mild and affected children are usually phenotypically normal at birth, but they have an increased risk of developmental problems and infertility which is difficult to quantify. Couples having ICSI require careful genetic counselling and may choose to undergo prenatal testing, particularly where the male partner has low sperm concentration. In some cases, preimplantation genetic diagnosis may be appropriate (Bonduelle 2002a).

The ICSI sons of men with a Y chromosome microdeletion will inherit the same deletion and are likely to be infertile. They will probably require ICSI themselves if they wish to father a child, although there are no data available yet as the oldest ICSI children are only 12- to 13-years old. It is currently unclear whether other abnormalities may also be associated with Y chromosome microdeletions.

There is reasonably good evidence that there is no significant difference between ICSI and IVF in the rate of major malformations diagnosed during the first year of life, but a recent case-control study has suggested that ICSI children, especially boys, may be more likely to have malformations diagnosed later in childhood (Bonduelle 2005). However, there is reasonably good evidence of a 30–40% increased risk of major and minor birth defects associated with ART compared to spontaneous conception (Hansen 2005). This could be due to differences between the ART population and the general population but there could also be an independent procedure-related risk. Assuming an underlying prevalence of major abnormalities of 4%, this would increase the absolute risk to 5.2–5.6% for babies conceived by ART.

With respect to epigenetic abnormalities, currently very little is known about human epigenetic regulation. It is suspected that ART children are prone to rare imprinting disorders

and a large case-control study has shown a significant link between Beckwith-Weidemann Syndrome and ART. Although BWS is in most cases associated with a good long-term outcome and the absolute risk of BWS for ART children remains low (around 1/4000 births), imprinting disorders can cause severe disability and other large controlled studies will be required to confirm the extent of risk to ART children and indicate whether ICSI increases the risk of such disorders above IVF alone. Moreover, it has been suggested that epigenetic errors may also account for a wider spectrum of ART-related complications such as low birth weight (Halliday 2004, De Rycke 2002).

These conclusions are based on the very limited evidence that is currently available, much of which derives from a single research group (Bonduelle et al) who had the foresight to initiate prospective clinical follow-up of all couples in their ICSI and IVF programmes. Although the long-term safety of ICSI cannot be reliably assessed without properly controlled and adequately powered studies with ongoing follow up, a survey reported that the majority of clinics offering ICSI had instigated no systematic follow-up for ICSI offspring (Kremer 2001). However, what evidence there is to date suggests that, to date, most ICSI children are healthy and their growth and cognitive development are comparable with both IVF and spontaneously-conceived children at the same ages. There is no data on the adult health of children born of ICSI.

Table 1: Included studies

Included study	Study design	Control	Country	Earlier/related publications
Aboulghar 2001	Cohort	SC	Egypt	
Barnes 2004/Bonduelle 2005/Ponjaert-Kristoffersen 2005	Case-control	IVF and SC	Belgium, UK, Sweden, Denmark, Greece	
Bider 1999	Cohort	IVF	Israel	
Bryant 2004#	Registry cohort	IVF	Oz	
Bonduelle 2002/2003	Cohort	IVF	Belgium	Aytoz 1998, Bonduelle 1995, 1996, 1996a, 1998,
Bonduelle 2002a	Case series	N/A	Belgium	1998a, 1998b, Kurinczuk 1997, Vernaeve 2003
Bonduelle 2004/Ponjaert-Kristoffersen 2004	Case control	SC	Belgium/Sweden/US	
Bowen 1998/Leslie 2003	Cohort	IVF and SC	Australia	Leslie 2002
Cox 2002	Case study	N/A	Germany	
De Baun 2003	Case series	N/A	USA	
Ericson 2001	Registry cohort	IVF	Sweden	Wennerholm 2000, 2000a, Bergh 1999
Gicquel 2003	Case series	N/A	France	
Govaerts 1998	Case control	IVF	Brussels	
Hansen 2002	Registry cohort	IVF and SC	Australia	Kurinczuk 2003a
Hurst 2001#	Registry cohort	IVF	Australia	
Katagiri 2004	Case control	SC	USA	Neri 2004
Katalinic	Registry cohort	SC	Germany	
Kuwata 2004	Cohort	IVF	Japan	
La Sala 2004	Case control	**SC**	Italy	
Lancaster 2004#	Registry cohort	IVF	Australia	
Lathi 2004	Cohort	IVF	USA	
Maher 2003	Case series	N/A	UK	
Manning 2000	Case series	N/A	Belgium	As for Bonduelle 2002 (above)
Moll 2003	Case series	N/A	The Netherlands	
Orvieto 2000	Cohort	IVF	Israel	
Orstavik 2003	Case study	N/A	Norway	
Palermo 2000	Case control	IVF	NY, USA	Palermo 1996, 2000
Papaligoura 2004	Case control	IVF and SC	Greece	
Paulus 2004	Cohort	IVF	Germany	
Pinborg 2004/2004a	Registry cohort	IVF	Denmark	Pinborg 2003, Loft 1999
Place 2003	Case control	IVF and SC	Belgium	
Squires 2003	Case control	IVF	USA	
Sutcliffe 2001/2003	Case control	**SC**	UK & Australia (2005 only)	Sutcliffe 2001a
Van Golde 1999	Cohort	IVF	Spain	
Weisel 2003	Registry cohort	IVF and SC	Germany	

#Bryant 2004, Hurst 2001 & Lancaster 2004 all use AIHW register: Bryant 2004 uses 2002 data, Hurst 2001 uses 1998, Lancaster 2004 uses 1990-99

Table 2: Quality of included studies

Studies were graded for overall quality of evidence using the following criteria:

*Reasonable**	Large prospective cohort studies with adequate sample size for outcomes measured, well reported, limitations acknowledged and/or statistically explored/adjusted for
*Fair**	Relatively large cohort or case-control studies with adequate sample size for outcomes measured, limitations acknowledged and/or statistically explored
*Questionable**	Smaller studies, likelihood of selection bias (applies to case-control comparisons of ICSI vs. SC), deficiencies in reporting and/or limitations not discussed/explored or adjusted for
*Poor**	Very small or uncontrolled studies, poor/deficient reporting, inappropriate exclusions and/or clear selection bias

- Studies graded as *reasonable* or *fair* are referred to in the text as *higher quality studies*. Studies graded as *questionable* or *poor* are referred to as *lower quality studies.*

Table 2: Quality of included studies

Study	Aboulghar 2001	Barnes 2004/ Bonduelle 2005/ Ponjaert-Kristoffersen 2005	Bider 1999	Bonduelle 2002/2003	Bonduelle 2002a
Comparison of interest	ICSI vs. SC	ICSI vs IVF ICSI vs SC	ICSI vs IVF	ICSI vs IVF	N/A
Selection	Prospective	Cross-sectional	Retrospective	Prospective	N/A
Design	Cohort	Case-control	Cohort	Cohort	Case series
Participation rate of eligibles	100%	Variable between centres: 25%-96%	100%	Whole cohorts enrolled	Only 47% ICSI fetuses karyotyped
Comparability	Unbalanced for plurality	SC group younger & better educated	No s/s differences	Well balanced	N/A
Outcomes	Chromosomal anomalies	Child development Congenital abnormalities Family functioning	Obstetric outcomes, Congenital malformations	Child development, Congenital malformations	Chromosomal abnormalities
Blinded outcome assessment	Not stated	Only in one centre	Not stated	Blinded paediatrician for child development	N/A
Same ascertainment method all groups	Yes	Yes	Yes	Yes	N/A
Same level of scrutiny all groups	Yes	Yes	Yes	Yes	N/A
Appropriate denominator for all outcomes	No TOP/miscarriage data	No TOP/miscarriage data	No TOP/miscarriage data	Yes	N/A
Timing of follow-up	OK	OK	Malformations only to one week	OK	N/A
Completeness of follow up	100%	N/A	100%	98% at birth >77% at 2 months 27% at 2 yrs	N/A
ICSI sample size	430 children	540 children	265 pregnancies 60 sets twins	2889 births 439 at 2 yrs	1586 fetuses
Controls sample size	430 SC children	437 IVF, 538 SC children	387 pregnancies 80 sets twins	2995 births 207 at 2 yrs	N/A
Quality of reporting	Good	Good	Fair	Good	Good
Reviewers' grade	Reasonable	Fair	Questionable	Reasonable	Poor

Table 2: Quality of included studies

Study	Bonduelle 2004/ Ponjaert-Kristoffersen 2004	Bowen 1998/ Leslie 2003	Bryant 2004	Cox 2002	De Baun 2003
Comparison of interest	ICSI vs SC	ICSI vs IVF ICSI vs SC	ICSI vs IVF	N/A	N/A
Selection	Cross-sectional	Mostly prospective	Prospective	N/A	N/A
Design	Case-control	Cohort (ICSI vs. IVF) Case-control (ICSI vs. SC)	Registry cohort	Case study	Case series
Participation rate of eligibles	Up to 45% in ICSI children Unknown in SC children	100% initially, only 12% among additional SC children at 5 yrs	Whole cohort	N/A	N/A
Comparability	ICSI parents older	ICSI vs. IVF: ICSI maternal education level lower ICSI vs.: More ICSI non-English speakers	Unclear – no data on some variables	N/A	N/A
Outcomes	Child development Psychological outcomes	Congenital malformations Child development	Obstetric outcomes	Angelman syndrome	Beckwith Weidemann Syndrome
Blinded outcome assessment	Only in one centre	Blinded at 5 yrs	No	N/A	N/A
Same ascertainment method all groups	Yes	Yes	Yes	N/A	N/A
Same level of scrutiny all groups	Yes	Possibility of more scrutiny of ART children	Yes	N/A	N/A
Appropriate denominator for all outcomes	Yes	No TOP/miscarriage data	Yes	N/A	N/A
Timing of follow-up	OK	OK	OK	N/A	N/A
Completeness of follow up	N/A	97% at 1 yr 84% at 5 yrs (plus additions)	Completeness of register uncertain	N/A	N/A
ICSI sample size	300 children	92/97 children at 1 yr/5yrs	2575 pregnancies	Single case	7 cases
Controls sample size	266 SC children	84/80 IVF at 1yr/5 yrs 86/110 SC at 1 yr/5 yrs	2028 pregnancies	N/A	N/A
Quality of reporting	Good	Good	Good	Good	Good
Reviewers' grade	Questionable	Fair	Reasonable	Poor	Poor

Table 2: Quality of included studies

Study	Ericson 2001	Gicquel 2003	Govaerts 1998	Hansen 2002	Hurst 2001	Katagiri 2004
Comparison of interest	ICSI vs IVF	N/A	ICSI vs IVF	ICSI vs IVF	ICSI vs IVF	ICSI vs SC
Selection	Prospective	N/A	Retrospective	Prospective	Prospective	Cross-sectional
Design	Registry cohort	Case-control	Case-control	Registry cohort	Registry cohort	Case control
Participation rate of eligibles	Whole cohort	N/A	Unclear	Whole cohorts	Whole cohorts	Not stated
Comparability	No information	N/A	ICSI mothers younger, more ICSI primips	ICSI mothers younger than IVF, older than SC	No information	Not stated
Outcomes	Congenital malformations	Beckwith Weidemann Syndrome	Obstetric outcomes, congenital malformations	Congenital malformations	Congenital malformations	Motor and cognitive development
Blinded outcome assessment	No	N/A	No	No	No	Unclear
Same ascertainment method all groups	Yes	N/A	Yes	Yes	Yes	Yes
Same level of scrutiny all groups	Possibility of more scrutiny of ART children	N/A	Yes	Yes	Yes	Yes
Appropriate denominator for all outcomes	No TOP data	N/A	Yes	Miscarriages not included	Yes	Yes
Timing of follow-up	Malformations only to birth	N/A	Malformations only to birth	OK	Malformations only to birth	OK
Completeness of follow up	Known omissions in register	N/A	100%	Completeness of register uncertain	Completeness of ascertainment uncertain	N/A
ICSI sample size	1652 babies	6 cases	133 clinical pregnancies	301 children	1816 ICSI >20/40 fetuses	101 children
Controls sample size	7523 IVF	N/A	124 clinical pregnancies	837 children	1995 >20/40 fetuses and TOPs	57 children
Quality of reporting	Fair	Good	Reasonable	Good	Good	Poor
Reviewers' grade	Questionable	Poor	Questionable	Reasonable	Fair	Poor

Table 2: Quality of included studies

Study	Katalinic 2004	Kuwata 2004	La Sala 2004	Lancaster 2004	Lathi 2004	Maher 2003	Manning 2000
Comparison of interest	ICSI vs SC	ICSI vs IVF	ICSI vs SC	ICSI vs IVF	ICSI vs IVF	N/A	N/A
Selection	Prospective	Prospective	Cross sectional	Prospective	Retrospective	N/A	N/A
Design	Registry cohort	Cohort	Case-control	Registry cohort	Cohort	Case study	Case series
Participation rate of eligibles	Whole cohorts	Excluded referrals with suspected abnormality	41-63% but criteria unclear	Whole cohorts	Whole cohort	N/A	N/A
Comparability	Poor	Appears balanced; Fair information	ICSI parents older, more twins in ICSI group	No information	Balanced for maternal age	N/A	N/A
Outcomes	Obstetric and neonatal outcomes	Congenital malformation in dichorionic twins	Child development, Family functioning	Congenital abnormalities	Chromosomal abnormalities	Beckwith-Weidemann Syndrome	Imprinting errors
Blinded outcome assessment	No	No	Yes	No	Unclear	N/A	N/A
Same ascertainment method all groups	Yes	Yes	Yes	Yes	Yes	N/A	N/A
Same level of scrutiny all groups	Yes	Yes	Possibility of more scrutiny of ART children	Yes	Yes	N/A	N/A
Appropriate denominator for all outcomes	Yes	Excludes pregnancy data	ICSI children with congenital abnormalities excluded	Yes	Yes	N/A	N/A
Timing of follow-up	OK for outcomes of interest	Malformations only at birth	OK	Malformations only to birth	Abnormalities 1st trimester only	N/A	N/A
Completeness of follow up	100%	100%	N/A	Completeness of ascertainment uncertain	N/A	N/A	N/A
ICSI sample size	3372	42 twin pregnancies	50 children	8325 >20/40 fetuses and TOPs	21 ICSI miscarried fetuses	6 cases	92 children
Controls sample size	8016	74 twin IVF pregnancies	51 children	Not stated (whole cohort)	38 IVF miscarried fetuses	N/A	N/A
Quality of reporting	Good	Fair	Poor	Fair (unpublished)	Good	Good	Fair
Reviewers' grade	Questionable	Poor	Poor	Fair	Questionable	Poor	Poor

Table 2: Quality of included studies

Study	Moll 2003	Orvieto 2000	Orstavik 2003	Palermo 2000	Paulus 2004	Papaligoura 2004	Pinborg 2004/ 2004a
Comparison of interest	N/A	ICSI vs IVF	N /A	ICSI vs IVF	ICSI vs IVF	ICSI vs IVF	ICSI vs IVF
Selection	N/A	Retrospective	N/A	Retrospective	Prospective	Retrospective	Retrospective
Design	Case series	Cohort	Case study	Case control	Cohort	Case-control	Registry cohort
Participation rate of eligibles	N/A	Whole cohorts	N/A	No information about IVF group	Unclear	97-100%, though selection criteria unclear	Whole cohorts
Comparability	N/A	ICSI mothers younger. Little information	N/A	No information	Little information	Lower IVF birth weight & less IVF twins	Balanced for plurality, no other information
Outcomes	Retinoblastoma	Obstetric outcomes	Angelman Syndrome	Congenital malformations	Congenital malformations	Cognitive development	Hospital admissions Congenital malformations in twins
Blinded outcome assessment	N/A	No	N/A	No	Not stated	Yes	No
Same ascertainment method all groups	N/A	Yes	N/A	Unclear	Yes	Yes	Yes
Same level of scrutiny all groups	N/A	Yes	N/A	Unclear	Yes	Yes	Yes
Appropriate denominator for all outcomes	N/A	Yes	N/A	Large numbers of events not analysed	No TOP/miscarriage data	Yes	No TOP/miscarriage /stillbirth data
Timing of follow-up	N/A	Yes	N/A	Malformations only to birth	Yes	Yes	Yes
Completeness of follow up	N/A	Complete	N/A	Complete	Unclear	Complete	Uncertain whether ascertainment complete
ICSI sample size	1 case	100 conceptions	Single case	2059 children	434 children	34 ICSI children	1282 singletons 835 twins
Controls sample size	N/A	100 conceptions	N/A	1796 children	356 children	26 IVF children	3848 singletons 2558 twins
Quality of reporting	Good	Fair	Good	Poor	Fair (abstract only)	Poor	Fair
Reviewers' grade	Poor	Questionable	Poor	Poor	Questionable	Poor	Fair

Table 2: Quality of included studies

Study	Place 2003	Squires 2003	Sutcliffe 2001/2003	Van Golde 1999	Weisel 2003
Comparison of interest	ICSI vs IVF ICSI vs SC	ICSI vs IVF	ICSI vs SC	ICSI vs IVF	ICSI vs. IVF and SC
Selection	Cross sectional	Unclear	Retrospective	Retrospective	Retrospective
Design	Case-control	Case-control	Case-control	Cohort	Case-control
Participation rate of eligibles	40-70% of those invited. Criteria unclear	Unclear	Unclear for SC children	Only 30% karyotyped Complete cohort for other variables	Unclear
Comparability	SC parents better educated	Little information	ICSI parents older, ICSI parity lower, ICSI children younger	ICSI mothers younger	No information
Outcomes	Congenital malformations Hospital admissions Child development	Child development	Congenital malformations, Child development	Karyotypes Obstetric outcomes Congenital malformations Child development	Major congenital malformations at birth
Blinded outcome assessment	Not stated	No	No	Not stated	No
Same ascertainment method all groups	Yes	Yes	Yes	Yes	Unclear
Same level of scrutiny all groups	Yes	Yes	Possibility of more scrutiny of ART children	Yes	Unclear
Appropriate denominator for all outcomes	No TOP/miscarriage data	Yes	No TOP/miscarriage /stillbirth data	No miscarriage/TOPs data	Includes TOP/miscarriage /stillbirth data
Timing of follow-up	Wide age range	Wide age range	Yes	Wide age range	Measures malformations to birth only
Completeness of follow up	Unclear	Unclear	90-99%	92% to 6-18 months	Completeness of ascertainment uncertain
ICSI sample size	66 children	141 children	208 (UK) 58 (Australia)	120 pregnancies >20/40	85 ICSI births/miscarriages/TOPs
Controls sample size	52 IVF children 59 SC children	144 children	221 (UK) 38 (Australia)	132 pregnancies >20/40	202 IVF and 19,211 SC births/miscarriages/TOPs
Quality of reporting	Poor	Poor	Fair	Good	Fair
Reviewers' grade	Poor	Poor	Questionable	Questionable	Questionable

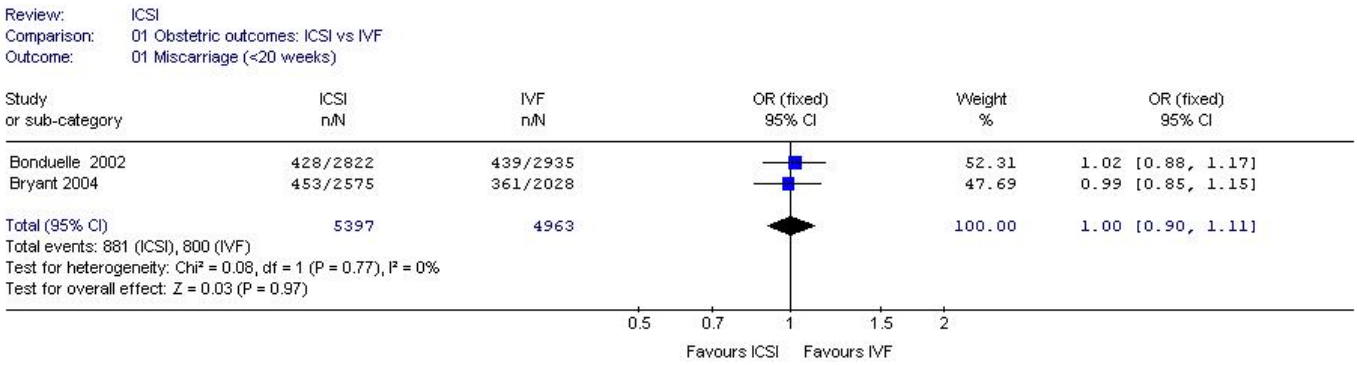
Table 3: Excluded studies

Study	Reason for exclusion
Aytoz 1999	Compares fresh versus frozen ICSI, no IVF controls
Bhattacharya 2001, UK	Measures fertility outcomes : not of interest to this review
Bonduelle 1994, Belgium	No control group
Bonduelle 1999, Belgium	No control group
Causio 1999, Italy	No control group
Cederblad 1996, Sweden	No separate ICSI group
Dumoulin 2005, The Netherlands	No clinical outcomes
Dumoulin 2001, The Netherlands	No clinical outcomes
ESHRE 1998, Belgium	Not controlled
Friedler 2001, Israel	No outcomes of interest
Hurst 1999, Australia	Includes any type of micro-insemination, not just ICSI
In't Veld 1995, Belgium	No controls
Lin 2004, China	In Chinese
Ludwig 1999, Germany	Not controlled
Ludwig 1999a , Germany	Not controlled
Ludwig 2003, Germany	Measures congenital malformations in ICSI versus spontaneously conceived group and evidence of systematic review is available for this outcome. No IVF controls
Manning 2001, Belgium	No clinical outcomes
Nyboe Andersen 2004, Belgium	No outcomes of interest for which ICSI reported separately from IVF
Oldereid 2003	In Norwegian
Olivennes 2004	No separate consideration of ICSI children
Pinborg 2004, Denmark	ICSI not reported separately from IVF
Pruksanonda 2004, Thailand	No control group
Tartalzis 1998, Greece	Survey data – no defined control group
Van Steirteghem 1998 Belgium	No control group
Wennerholm 1996, Sweden	Not controlled
Wisanto 1995, Belgium	No control group

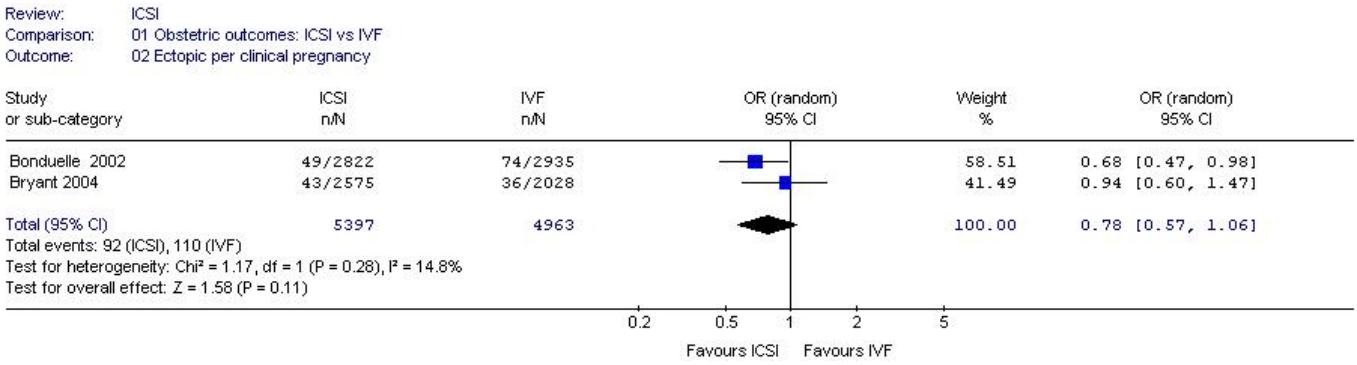
Table 4: Forest plots

1. Obstetric outcomes: ICSI versus IVF

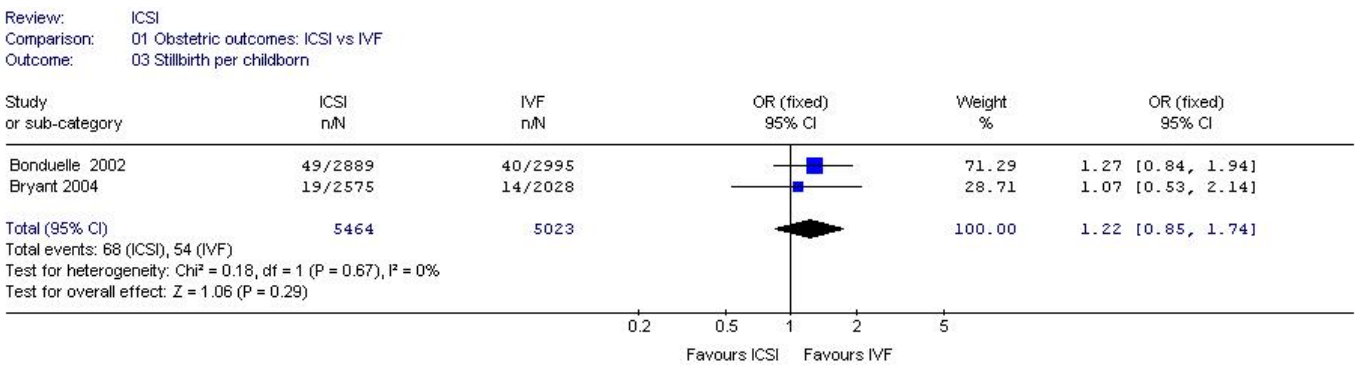
1.1 Miscarriage



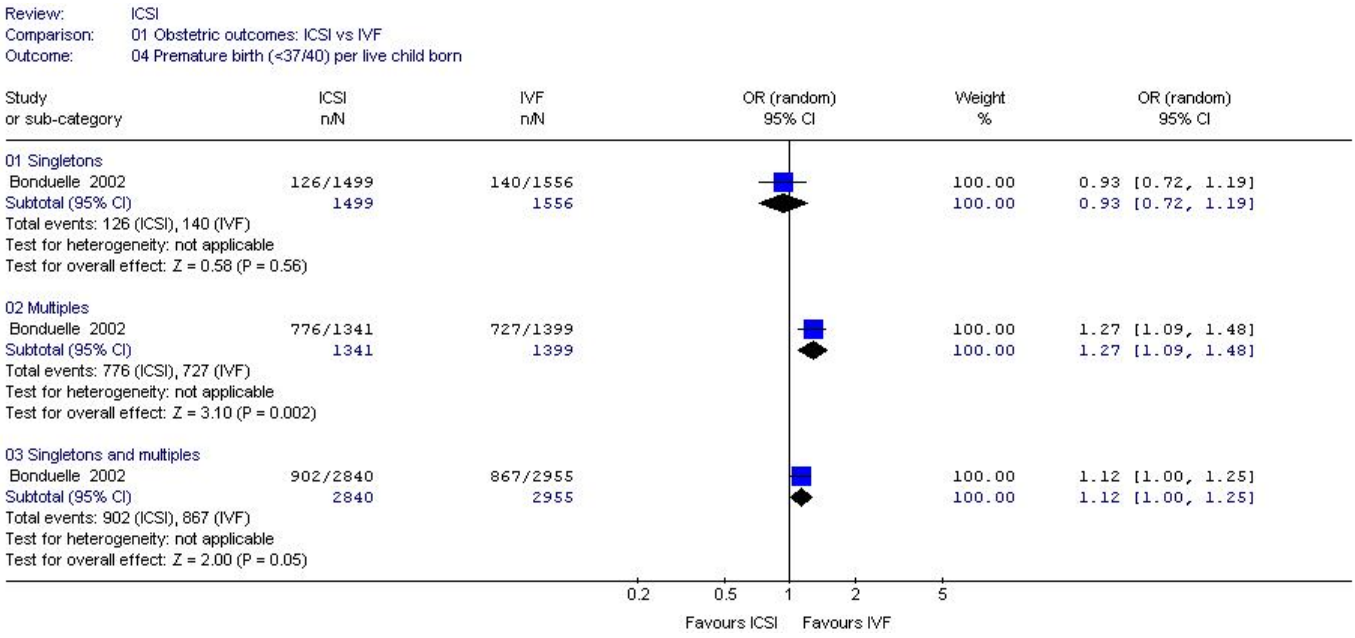
1.2. Ectopic pregnancy



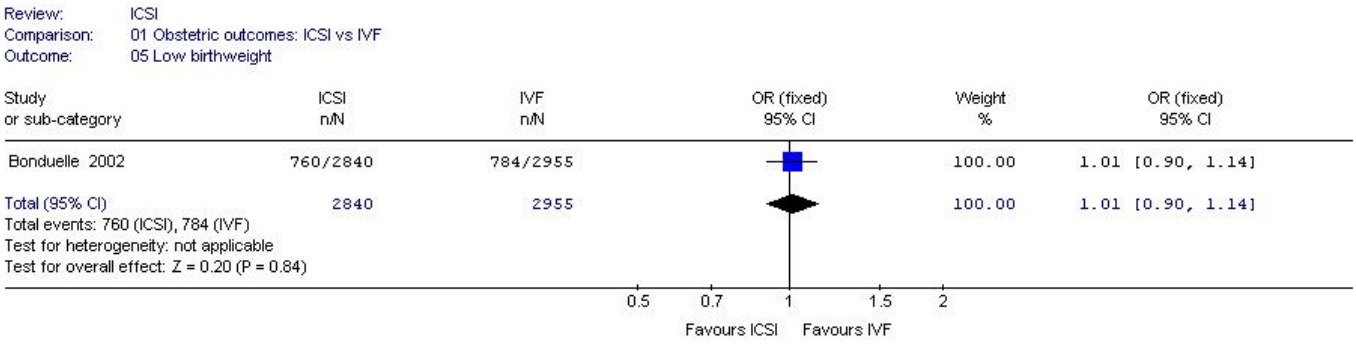
1.3 Stillbirth



1.4 Premature birth (<37/40)

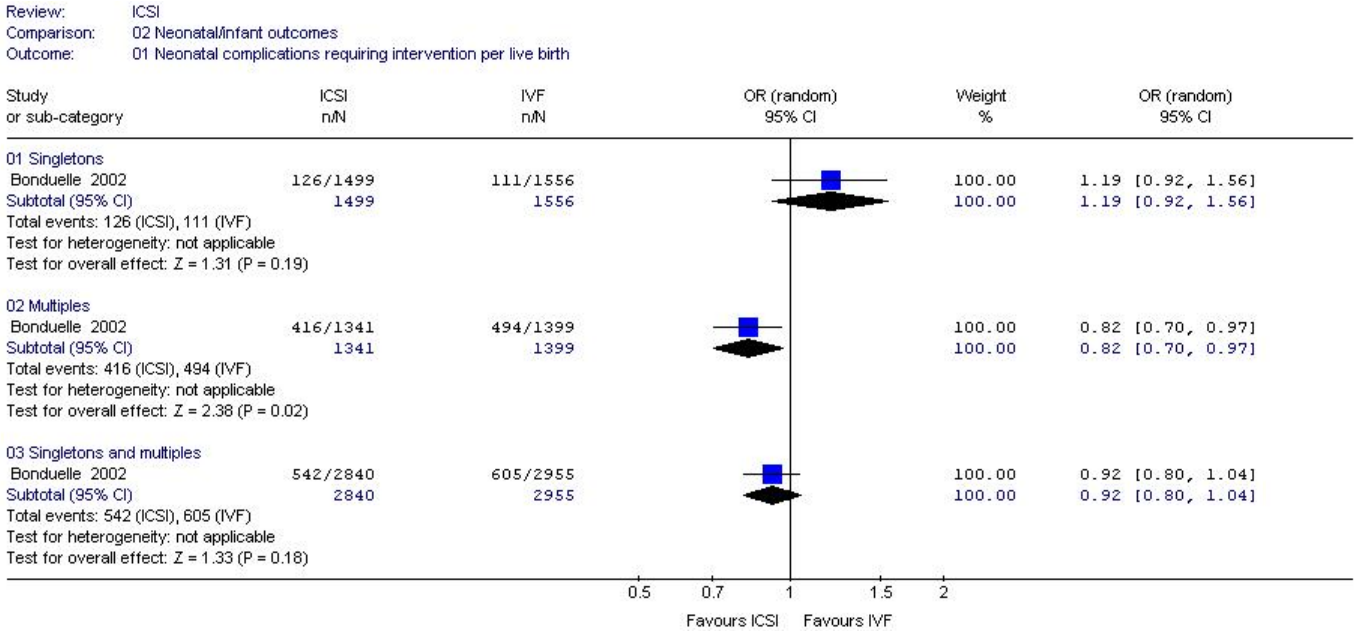


1.5 Low birth weight (<2500 gms)

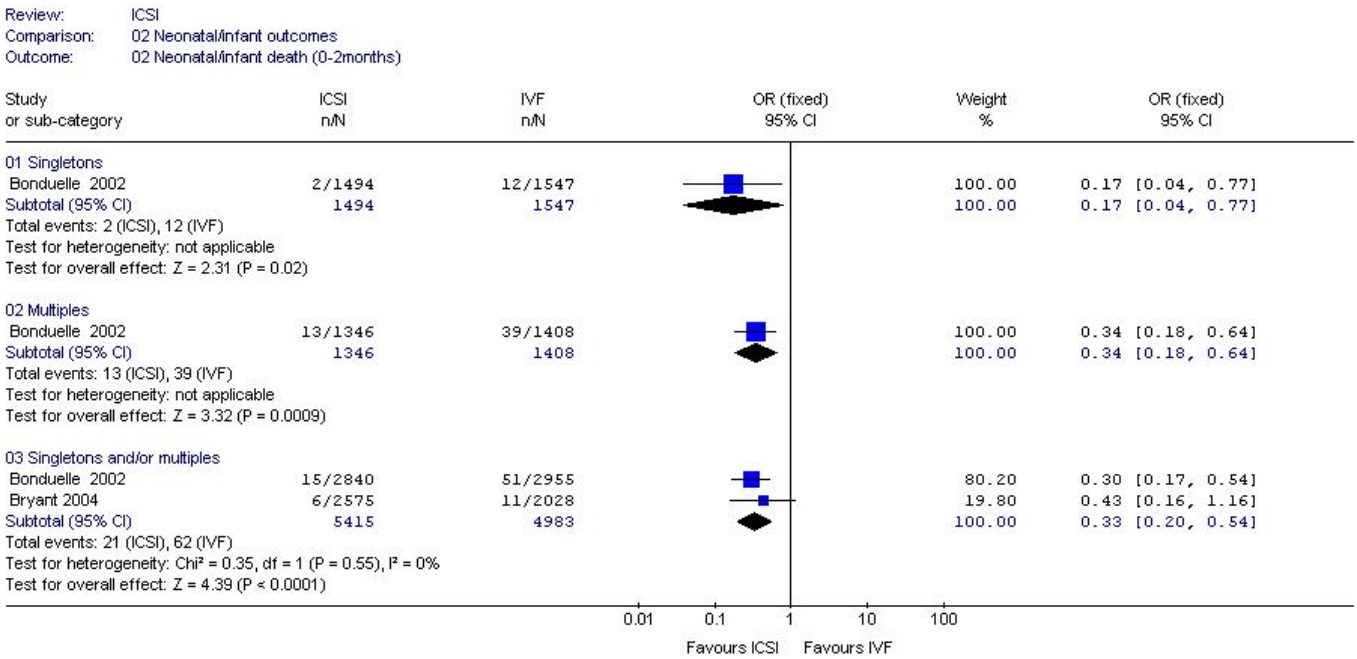


2. Neonatal/Infant complications: ICSI versus IVF

2.1 Neonatal complications requiring intervention

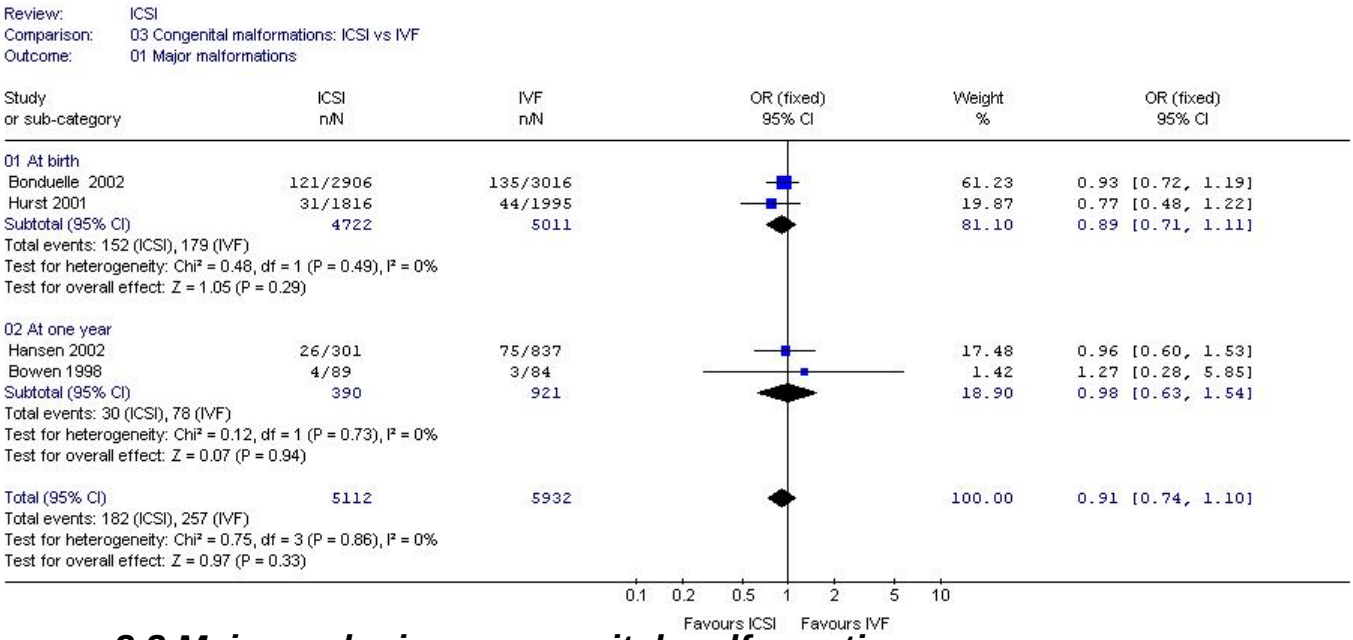


2.2 Neonatal/infant death (0-2 months)

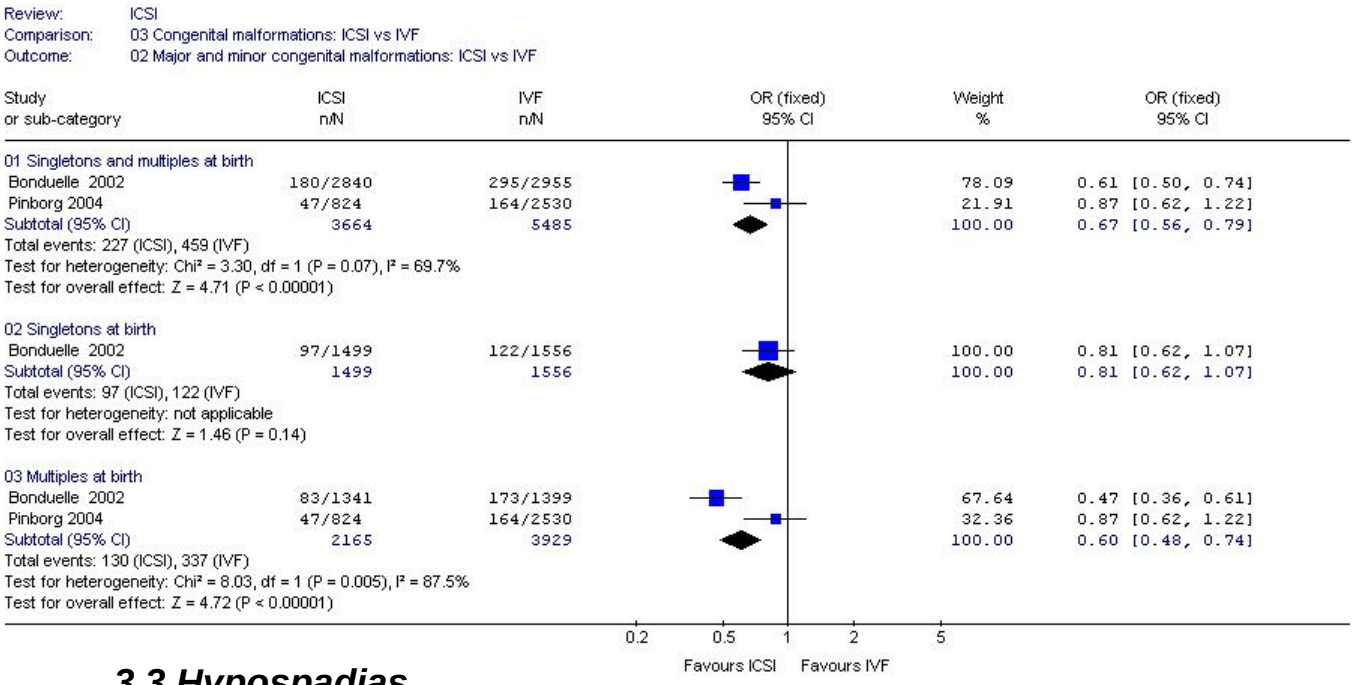


3. Congenital malformations: ICSI versus IVF

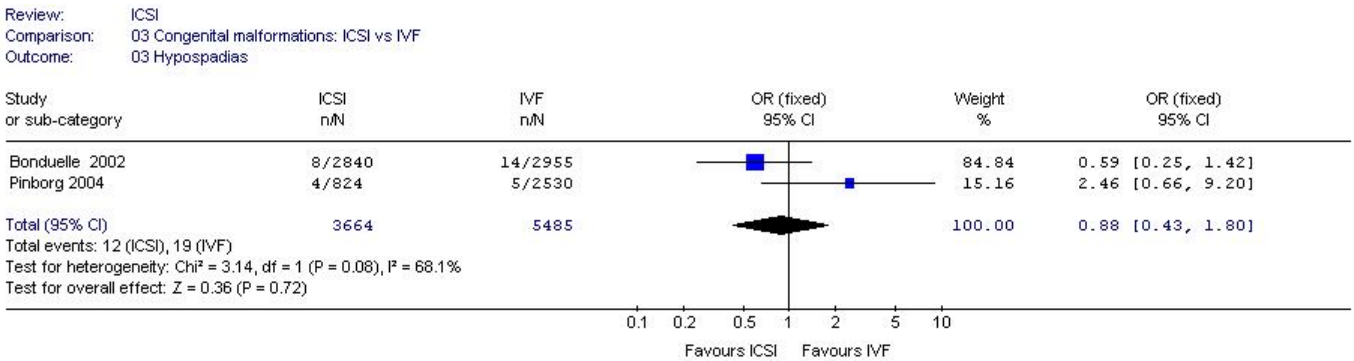
3.1 Major congenital malformations



3.2 Major and minor congenital malformations

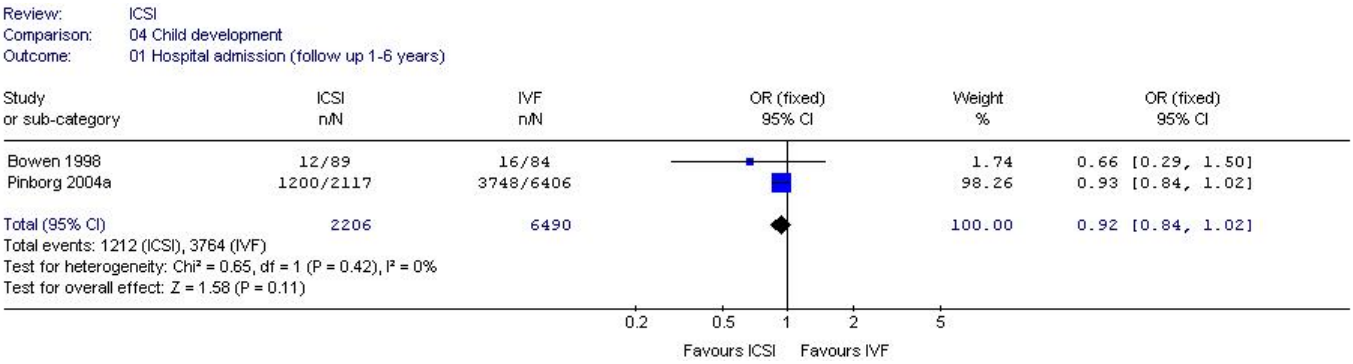


3.3 Hypospadias

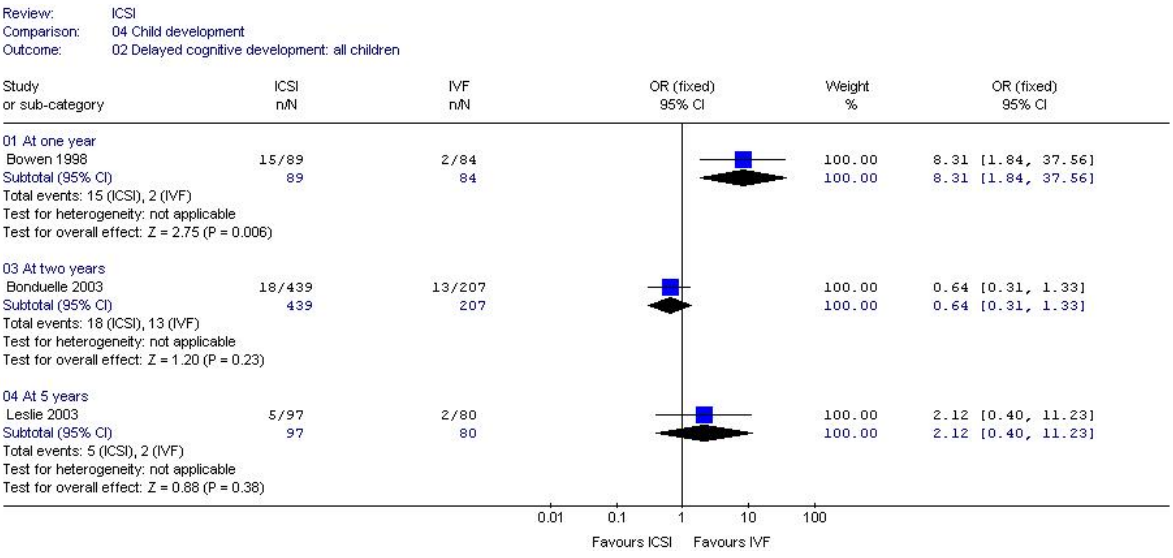


4. Child development: ICSI versus IVF

4.1 Hospital admission



4.2 Delayed cognitive development



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Appendix 1: Search strings

Devised and conducted by NZHTA in October 2003 and updated in July and November 2004

Medline/Cochrane Controlled Trials Register

1 exp Reproductive Techniques, Assisted/ (31315)
2 Sperm Injections, Intracytoplasmic/ (1150)
3 exp fertilization in vitro/ (17169)
4 Preimplantation Diagnosis/ (746)
5 Cryopreservation/ or Embryo Transfer/ (15840)
6 or/1-5 (37853)
7 Abnormalities/ (17410)
8 Fetal Diseases/ (21192)
9 7 or 8 (37253)
10 6 and 9 (411)
11 limit 10 to yr=1990-2003 (285)
12 limit 11 to english (251)
13 ae.fs. (794168)
14 6 and 13 (2262)
15 from 12 keep (selected references)(134)
16 remove duplicates from 15 (119)
17 from 16 keep 1-119 (119)
18 exp Reproductive Techniques, Assisted/ae or Sperm Injections, Intracytoplasmic/ae or exp fertilization in vitro/ae or Preimplantation Diagnosis/ae or (Cryopreservation/ae or Embryo Transfer/ae) (1159)
19 Developmental Disabilities/ (6987)
20 exp "congenital, hereditary, and neonatal diseases and abnormalities"/ or abnormalities/ (605196)
21 19 or 20 (610400)
22 18 and 21 (147)
23 limit 22 to (english language and yr=1990-2003) (110)
24 23 not 16 (73)
25 from 24 keep (selected references)

Embase

1 Fertilization in Vitro/ (12353)
2 infertility therapy/ or embryo transfer/ or intracytoplasmic sperm injection/ (8438)
3 Cryopreservation/ (6745)
4 Preimplantation Embryo/ (2222)
5 genetic disorder/di (2184)
6 preimplantation genetic diagnosis.mp. (469)
7 4 and 5 (98)
8 1 or 2 or 3 or 6 or 7 (21540)
9 exp congenital disorder/ or exp congenital malformation/ (181991)
10 8 and 9 (996)
11 limit 10 to (english and yr=1990-2003) (864)
12 animal/ or mouse/ (323430)
13 CATTLE/ (40285)
14 11 not (12 or 13) (829)
15 Case Report/ (544642)
16 Letter/ (261656)
17 14 not (15 or 16) (616)
18 from 17 keep (selected references)

Cinahl

1 exp Reproduction Techniques/ (871)
2 fertilization in vitro/ (344)
3 intracytoplasmic sperm injection.mp. (18)
4 Cryopreservation/ or embryo freezing.mp. (20)
5 preimplantation genetic diagnosis.mp. (12)
6 or/1-5 (892)
7 exp abnormalities/ (6329)
8 developmental disabilities/ (901)
9 7 or 8 (7185)
10 6 and 9 (21)
11 ae.fs. (41023)
12 6 and 11 (84)
13 10 or 12 (98)
14 limit 13 to yr=1990-2003 (92)
15 from 14 keep (selected references)

Current Contents/Science & Social Science Citation Indexes

TS=(in vitro fertili* OR assisted reproduct*)
TS=preimplantation genetic diagnosis
TS=(embryo freezing OR intracytoplasmic sperm injection)
#1 OR #2 OR #3
TS=(abnormalities OR anomalies OR malformations)
TS=(children OR infants OR neonates OR offspring)
#4 AND #5
#4 AND #6
#7 OR #8

Other databases

Combinations of the keywords in the strategies above were used to search other databases and sources for which strategies are not given.

Appendix 2: Characteristics of included studies

Study	**Aboulghar 2001, Egypt**
Design	Prospective cohort study
ICSI cases	430 ICSI children (220 singletons, 198 twins, 12 triplets)
Controls	430 spontaneously conceived children (406 singletons, 12 twins)
Recruitment	Recruited in pregnancy (unclear at what gestation)– all babies born in one centre from consecutive deliveries
Prognostic balance	Balanced for % of women aged >35 and for consanguinity
Outcomes of interest	Chromosomal abnormalities at birth
Data collection	All babies karyotyped at birth
Follow-up	Complete
Strengths	Prospective recruitment of two complete cohorts. Powered to detect 2.5% difference between the groups
Weaknesses	Parents not karyotyped – uncertain which abnormalities in children were de novo. Unbalanced for twin/singleton status No information on gestational age at recruitment – appears to exclude terminations or miscarriages during pregnancy which could be associated with chromosomal abnormality)

Study	**Barnes 2004, Bonduelle 2005, Ponjaert-Kristoffersen 2005 UK/Belgium, Sweden/Denmark, Greece**
Design	Case control
ICSI cases	540 ICSI children from the UK, Belgium, Sweden, Denmark and Greece, singleton, Caucasian, born at >32 /40, mostly first or second born, speaking native language of research centre, plus their parents (Greek parents not assessed)
Controls	437 IVF first or second born children, matched for age, sex and maternal age to ICSI group (see Bonduelle 2005) 538 spontaneously conceived (SC) first of second born children, matched to ART groups for maternal age and education, parental socio-economic status, gender and birth order
Recruitment	ICSI children recruited partially from established cohorts, with additional children from major ART clinics (in UK and Belgium), and from ART clinic records (in Sweden Denmark & Greece) All ICSI children were consecutive births IVF children invited by letter if child fulfilled matching criteria (thus not necessarily consecutive births) SC children from local schools and nurseries (UK, Belgium, Greece), from medical birth registry (Sweden) and from participating hospital's birth registry (Denmark)
Prognostic balance	All groups balanced for mean child age, parental occupation and family social class. ICSI vs IVF: Balanced for parental age and education. ICSI vs SC: Maternal age lower and parental education level higher in SC group
Outcomes of interest	Socio-emotional development in 5 year old child, family functioning (Barnes 2004), physical development and congenital abnormalities by age 5 (Bonduelle 2005), motor and cognitive development at age 5 (Ponjaert-Kristoffersen 2005)
Data collection	Paediatric examination of children and administration of child development scales and Bene-Anthony Family Relations Test to children. Questionnaires

	completed by parents to measure parental well-being, parent-child relationship and child's socio-emotional development. Paediatrician blinded in Sweden only
Follow-up	Cross-sectional
Strengths	Large scale, multisite. ICSI and IVF groups well balanced. For each questionnaire, data analysed only if total response rate for country was at least 50% in Belgium/UK (where sample sizes were largest) or at least 60% in other centres. Detailed participation and response rates of parents in each country reported.
Weaknesses	Variable participation rate between countries (25-96%). No data included from Greece. No information on non-participants - families experiencing difficulties may have been less likely to participate. Variable response rate to questionnaires – low response from fathers. Socio-emotional questionnaires completed by parents rather than an objective source.
Note	Same groups assessed for physical health (Bonduelle 2005) and for cognitive and motor development (Ponjaert-Kristoffersen 2005) Described by investigators as cohort study but matched IVF group selected by fertility centres for invitation
Study	**Bider 1999, Israel**
Design	Retrospective cohort study
ICSI cases	60 multiple pregnancies from 265 pregnancies after ICSI Dec. 1994 - Oct. 96
Controls	80 multiple pregnancies from 387 pregnancies after IVF Dec. 1994 - Oct. 96
Recruitment	Recruitment method unclear but apparently identified retrospectively from clinic records
Prognostic balance	Similar no of embryos replaced: ICSI: 3.4 (SD 1.1), IVF 3.3 (SD 2) ICSI mothers of twins younger (31.8 vs 35.1), more likely to be primigravidae (69% vs 54%), but differences not statistically significant
Outcomes of interest	Obstetric outcomes and major congenital malformations in multiple births
Data collection	Chart analysis
Follow-up	Complete follow up
Strengths	Complete cohort followed up
Weaknesses	Small sample size, some prognostic imbalance, primary outcome of limited relevance since routine transfer of 3 embryos not practised in NZ, malformation rate only to one week of life, and malformation rate in miscarriages and neonatal deaths not reported.
Note	Pregnancy defined as rising HCG levels. *Clinical* pregnancy not defined, but miscarriage defined as loss of clinical pregnancy <20 weeks. Up to 3 embryos transferred. Large number of reductions reported – 6 ICSI and 7 IVF quintuplets reduced to twins, 1 ICSI and 1 IVF triplets reduced to twins.

Study	**Bonduelle 2002, Belgium** (Dutch speaking Brussels Free University): **See also Bonduelle 2003, below**
Design	Prospective cohort
ICSI cases	2822 clinical pregnancies (2889 children) after ICSI (99%) or SUZI (1%) 1990-1999, using fresh embryos and ejaculated (87%) or surgically obtained (13%) sperm
Controls	2935 clinical pregnancies (2995 children) after IVF 1983-1999 using fresh embryos
Recruitment	All couples invited before starting ICSI or IVF to join clinical follow-up study of children
Prognostic balance	Balanced for pregnancy medications, pregnancy duration, maternal educational level. *Both groups:* Mean mat age slightly lower in multiple than in singleton pregnancies *ICSI group*: More first pregnancies and first babies, more smokers, mean age 6 months older (32.7 vs 32.2)
Outcomes	*ICSI vs IVF:* Miscarriage, ectopic pregnancy, stillbirth, abnormal karyotype, congenital malformations *Ejaculated vs non-ejaculated sperm:* congenital malformations
Data collection	Data obtained largely from medical specialists, partly from parents. Children examined at birth & 2 months
Follow-up	Birth data >98% complete 2-month data >81% complete for ICSI, >77% for IVF.
Strengths	Prospective enrolment and same methodology for both groups, homogenous population, prognostic features similar, (any differences did not favour ICSI), same rate of multiple pregnancies in both groups. Includes pregnancy data for malformations
Weaknesses	Only 49.7% ICSI and 16.6% IVF foetuses karyotyped. 20% children lost to follow up by 2 months (a time during which congenital malformations commonly become apparent) IVF data starts 7 years earlier than ICSI data (unclear what effect this might have).
Note	See also Bonduelle 1996, 1996a, 2002a

Study	**Bonduelle 2002a, Belgium** (Dutch speaking Brussels Free University)
Design	Case series
ICSI cases	1586 ICSI foetuses tested for fetal karyotype 1990-2001, from fresh embryo transfer
Controls	Uncontrolled.
Recruitment	Prenatal diagnosis offered to all couples attending fertility centre
Outcomes of interest	Abnormal fetal karyotype, according to sperm parameters and sperm origin
Data collection	Findings of chorionic villus sampling (698 foetuses) or amniocentesis (888 foetuses)
Follow-up	47% of pregnancies ongoing beyond 12/40 weeks were tested
Strengths	Large sample prospectively enrolled. Subgroup analysis of mothers <35 in order to eliminate bias of age
Weaknesses	Selection bias: low acceptance rate. Those mothers tested were probably those at higher risk of abnormality (37% were aged >/= 35) Comparison of outcomes according to sperm origins include relatively small samples of non-ejaculated sperm
Note	Authors cite general population stats (as reported in the literature) for comparison with ICSI findings. Same 1990-99 data compared with IVF group in Bonduelle 2002

Study	**Bonduelle 2003, Belgium** See also Bonduelle 2002 above
Design	Prospective cohort
ICSI cases	439 children born after ICSI reaching the age of 24-28 months between 1995-2002 (378 singletons, 61 twins) assessed
Controls	207 children born after IVF (138 singletons, 69 twins) assessed
Recruitment	All couples invited before starting ICSI or IVF to join clinical follow-up study of children
Prognostic balance	*Between ICSI and IVF groups tested:* Balanced for maternal age, maternal education, parity, obstetric and neonatal characteristics and malformation rates *Between 2 year olds Bayley Scale tested and those not tested, among 2 year olds attending for follow up:* Balance for maternal education and birth weight, Group Bayley-Scale-tested had lower proportion of twins than those not tested and only a small sample of ICSI twins tested *Between children from birth cohort followed up and those lost to follow up by 2 years* Group followed up at 2 years had higher gestational age, lower proportion of twins, higher birth weight, higher malformation rate and lower maternal age than those lost to follow up. Maternal education, parity and % neonatal complications were similar.
Outcomes of interest	Child development (perception, cognition and language): Analyses outcomes of ICSI vs IVF and (within ICSI singleton group) according to sperm parameters of father.
Data collection	By paediatrician trained in clinical psychology
Follow-up	Of birth cohort, 46.6% ICSI children and 42.1% IVF children attended 2 year follow-up. Of these, 27% had assessment with Bayley Scale.
Strengths	Prospective follow up, relatively large sample size, appropriate control group, blind assessment using objective measure.(Bayley Scale), Singletons and twins reported separately. Analysis of prognostic factors suggests that singletons are a representative sample of ICSI and IVF children
Weaknesses	Low proportion of birth cohort Bayley tested at two years. Only a small proportion of ICSI twins followed up
Note	Results are more robust for singletons than for twins. Results reported are for *mental* scale of Bayley Scale only (motor scale not reported in publication). Most common reason for failure of children followed up at 2 years to be Bayley-Scale-tested was difficulty fitting in with limited time schedule of paediatrician performing tests. Results for developmental delay outcome are corrected for gestational age <36 weeks (this affects scores for twins only)

Study	**Bonduelle 2004, Ponjeart-Kristofferson 2004**, **Belgium, Sweden, USA**
Design	Matched international case-control study. 300 singleton ICSI children and 266 singleton naturally-conceived children matched for gender, child age and maternal age. (In Sweden, also for maternal education)
ICSI cases	300 singleton children born after ICSI in Brussels (100), Goteberg (98) or New York (102) assessed
Controls	266 spontaneously conceived children assessed
Recruitment	ICSI children recruited via fertility centres, invited in consecutive birth order. Control children recruited from schools (Brussels), from birth registry (Sweden) and by advertising (USA)
Prognostic balance	ICSI mothers and fathers significantly older than parents of controls. Educational level of parents similar
Outcomes	Primary outcome: growth (Bonduelle 2004). Psychological outcomes (Ponjaert-Kristoffersen 2004) Secondary: general health (Bonduelle 2004)
Data collection	Questionnaire on medical history up to age 5 completed by parents, medical records (Sweden only), battery of psychological tests and paediatric examination at age 5
Follow-up	Cross sectional
Strengths	Consecutive recruitment of ICSI children, multiple sites aim to increase generalisability
Weaknesses	Participation bias: large proportion of ICSI children not reached (23%) or refused to participate (10%) in Belgium (23%) and in USA (6%, 45% respectively). Control group participation rates not possible to calculate for USA (69% in Belgium, 78% in Sweden). Selection bias: difference in recruitment methods for ICSI children and controls. Investigators blinded in Sweden only.
Note	No ICSI vs IVF comparison. No adjusted results. Findings discussed in text The two publications report different outcomes of the same cohort

Bonduelle 2005: see Barnes 2004

Study	**Bowen 1998, Leslie 2003, Australia**
Design	Prospective cohort
ICSI cases	92 ICSI children (72 singletons, 10 twins) enrolled before birth. An additional 24 ICSI children enrolled for 5 year assessment
Controls	86 IVF children (60 singletons, 24 twins) enrolled 82 spontaneously conceived (SC) children (62 singletons, 20 twins) enrolled before birth. An additional 50 children enrolled for 5 years assessment.
Recruitment	ICSI: all live born children conceived by ICSI May 1993-June 1995. IVF: live born children conceived by IVF whose mothers attended research centre for obstetric care from 28/40. SC: offspring of primips aged >27 IVF whose mothers attended research centre for obstetric care from 28/40. Additional ICSI children enrolled were the next singleton or twin children conceived in the same programme after those enrolled in the study. Additional SC children were enrolled from preschools that matched the demographics of the ICSI cohort.
Prognostic balance	All groups balanced for gestation, parental occupation, *ICSI vs IVF*: ICSI maternal education level lower, father's occupational level less skilled *ICSI vs SC:* More non-English-speaking families in ICSI group
Outcomes of interest	Congenital malformations (at one year), child development (at one and five years)
Data collection	One year tests conducted by one of two (unblinded) investigators. Five year tests conducted by investigators blinded to one year results and to study group.
Follow-up	100% of children assessed at birth for obstetric and neonatal history. >97% reassessed at one year for malformations and Bayley Scale testing. 84% of original cohort reassessed at 5 years, plus additional children enrolled, as above,.
Strengths	Initial acceptance 100% in ICSI group, 80% in IVF group, about 70% in SC group. Demographic characteristics of children lost to follow up not different to those followed up. High acceptance rate of additional ICSI children at 5 years (86%). Objective measures used (Bayley Scale at 1 year, Wechsler Scale at 5 years), with blinded assessment at 5 years. Power of 100% to detect a clinically meaningful difference in IQ at 5 years, in terms of educational needs.
Weaknesses	Low acceptance rate of additional SC children at 5 years (approx 12%). Congenital malformation rates exclude pregnancy losses.
Note	Authors note that 5 year results on Wechsler Scale likely to provide more robust assessment of long term intelligence than Bayley Scale, which is designed to assess perceptual motor skills in 1-2 year olds.

Study	**Bryant 2004, Australia**
Design	ART registry data
ICSI cases	2575 fresh non-donor ICSI clinical pregnancies in 2002
Controls	2028 fresh non-donor IVF clinical pregnancies in 2002
Recruitment	Identified from registers
Prognostic balance	Similar multiple birth rates (ICSI 16.5%, IVF 17.3%), similar % of mothers aged >34 (ICSI 54.3%, IVF 56.5%)
Outcomes of interest	Obstetric outcomes
Data collection	Australian and New Zealand Assisted Reproduction Database (ANZARD). Data collected at all Australian (n=25) and New Zealand (n=4) fertility centres at time of ART treatment. Staff follow up patients and/or clinicians for pregnancy and birth outcomes
Follow-up	"Small proportion" of follow-up data incomplete
Strengths	Large sample, complete cohorts registered prospectively. Same recruitment and ascertainment methods for both groups
Weaknesses	No information on prognostic variables such as maternal education, parity etc. Unclear how much follow-up data missing. Some follow-up data self-reported (though validated with medical records where possible).

Study	**Cox 2002, Germany/USA**
Design	Case study
Cases	Two cases of Angelman Syndrome post ICSI
Data collection	Cases investigated for developmental delay at tertiary clinic
Note	Both cases positive for imprinting error

Study	**De Baun 2003, USA**
Design	Case series
Cases	7 children born after ICSI/IVF on BWS registry
Data collection	Review of BWS registry data
Note	Specific data on conception collected only for 2/8 years of registry. Estimate of prevalence based on 3/65 BWS cases registered since2003, when mode of conception data collection started. Imprinting status available for 6/7 ICSI/BWS cases.

Study	**Ericson 2001, Sweden**
Design	Population registry data
ICSI cases	1652* ICSI babies born in Sweden up to 1997 (see Note below)
Controls	7523* IVF babies born 1982-97 in Sweden (see Note below) 1,690,577 population controls
Recruitment	Complete cohort registered at birth
Prognostic balance	No information
Outcomes of interest	Major congenital abnormalities, specific congenital abnormalities from birth to 2 years
Data collection	All reported ART births tracked (using personal identification number) for entries in Medical Birth registry, Register of Congenital Malformations, and auxiliary registers.
Follow-up	Medical records tracked for >99.5% of infants
Strengths	Large cohort, ascertainment similar for both groups
Weaknesses	Unclear when ICSI births started – IVF data likely to begin about 10 years earlier Ascertainment incomplete – authors found unregistered births and malformations while conducting study No information on terminations of pregnancy No information on balance of prognostic variables (eg, multiple births)
Note	*Number of ICSI and IVF babies calculated from percentages cited in text – Comparison of incidence rates of >75 specific congenital malformations. Only hypospadias reported in this review (as authors found excess incidence in ICSI group **See also Wennerholm 1996, Bergh 1999, Wennerholm 2000 & 2000a**

Study	**Gicquel 2003**
Design	Case study
Cases	6 cases of Beckwith-Weidemann Syndrome post ICSI/IVF
Data collection	Review of notes of 149 patients on BWS register
Note	All 6 cases tested for imprinting status

Study	**Govaerts 1998, Belgium** (French speaking Brussels Free University)
Design	Case control
ICSI cases	145 ICSI pregnancies (22 pre-clinical) from Sep 1993-Jan 1996, using fresh embryos
Controls	145 IVF pregnancies (21 preclinical), using fresh embryos; matched for last menstruation
Recruitment	Recruitment method unclear. Records accessed retrospectively.
Prognostic balance	ICSI mothers significantly younger and more primips. Balanced for plurality and mean infertility duration
Outcomes of interest	Obstetric outcomes, chromosomal abnormalities*, congenital malformations at birth
Data collection	Questionnaire completed by gynaecologists. For malformation rates, routine paediatric reports at birth
Follow-up	Complete
Strengths	Ascertainment similar in both groups. Includes information on fetal abnormalities/therapeutic abortions
Weaknesses	* Only 8/145 IVF pregnancies karyotyped: this outcome not reported in review

Study	**Hansen 2002, Australia**
Design	ART registry study
ICSI cases	301 ICSI infants
Controls	837 IVF infants 40000 spontaneously conceived (SC) babies, randomly chosen from register
Recruitment	Identified from registers
Prognostic balance	ICSI vs IVF: IVF mothers a little older (:34.1 vs 32.6), balanced for parity, marital status, ethnic group, multiplicity ICSI vs SC: ICSI mothers more likely to be older and primiparous, married, white, and urban. ICSI infants more likely to be delivered by caesarean, preterm and of low birth weight.
Outcomes of interest	Major malformations diagnosed by one year
Data collection	All reported >20/40 pregnancies and TOPs for malformation from Reproductive Technology Register linked to W Australian Birth Defects Register. Random selection of SC births from Midwives' Notification System similarly linked.
Follow-up	Birth records available for all infants. Level of ascertainment of Birth Defects Register uncertain, though authors assert that level of ascertainment and accuracy is high.
Strengths	Enrolment of complete cohorts ICSI vs IVF groups fairly well balanced Same method of recruitment and ascertainment for all groups. In an attempt to assess the potential effects of confounding, the following sensitivity analyses were conducted: a) Analyses were adjusted for maternal age and parity, infant gender and correlation of the risk of birth defects between siblings. b) an independent blinded paediatrician suggested which birth defects might be the result of unusually strict surveillance and these were excluded from analysis, c) TOPs of ART babies for fetal abnormality were included in analysis, plus (in the SC group) 14 TOPs for fetal abnormality that were randomly selected from the birth defects registry (based on 3.5/1000 incidence of such terminations in the general population) d) Only singletons were included. None of these measures materially affected the results
Weaknesses	Reliance on registry data provides less potential for quality control of data collection than formal study protocol No information on maternal education
Note	ICSI vs SC comparison reported in text in this review. Same data reported in Kurinczuk 2003 (abstract)

Study	**Katagiri 2004, USA**
Design	Case-control
ICSI cases	101 ICSI children aged 5
Controls	27 spontaneously conceived children
Recruitment	Not described
Prognostic balance	Not described
Outcomes of interest	Physical and cognitive development
Data collection	Weschler IQ test, Peabody Motor Scale
Follow-up	Cross-sectional
Strengths	
Weaknesses	Small, poorly reported
Note	Also measured congenital abnormalities – this outcome not included as systematic review available

Study	Katalinic 2004 Germany
Design	Prospective cohort
ICSI cases	2809 pregnant women (3372 children/foetuses) >16 weeks gestation who conceived by ICSI using fresh embryos
Controls	8010 children/foetuses> 16 weeks who were conceived spontaneously (SC)
Recruitment	ICSI cases recruited consecutively during 16th week of gestation, from August 1998-August 2000 Cohort children's data accessed from population register born Jan 1993-Dec. 2001
Prognostic balance	Compared to SC group, ICSI group had higher maternal and paternal ages, more mothers aged >35, more obesity, lower parity, and less previous malformations or pregnancy losses .
Outcomes of interest	Obstetric and neonatal outcomes (see note below))
Data collection	ICSI couples assessed by phone in week 16, 26, 28 and then 2-4 weekly until delivery and once post-delivery. Controls from population register for which all newborn are examined according to same protocol as study cohort and which also registers miscarriages, stillbirths and terminations from week 16.
Follow-up	2687/2809 (96%) followed up throughout. Sperm analysis available for 2545 (95%) pregnancies (3199 children/foetuses)
Strengths	Large sample - powered to show equivalence in malformation prevalence, which was set at 7%. ICSI group constituted 44% of ICSI children born in Germany during the study period (though authors concede that 100% would have been preferable, as would a nationwide control sample). Data prospectively collected, similar assessment procedure for ICSI and NC groups. Includes pregnancy data. Assessment not blinded.
Weaknesses	Poor prognostic balance. Control data from earlier years (1993-2000) and from a local rather than a nationwide register (whereas ICSI group recruited nationwide)
Note	Primary outcome was major congenital abnormalities at birth/6-8 weeks but these findings not included, because evidence of systematic review is available for this outcome. Chromosomal abnormalities also measured but not reported in this review as uptake of prenatal testing was only 26% in ICSI group and <8% in SC group.

Study	Kuwata 2004, Japan
Design	Prospective cohort
ICSI cases	42 women pregnant with dichorionic twins conceived by fresh ICSI
Controls	74 women pregnant with dichorionic twins conceived by fresh IVF, referred to research hospital for obstetric care <24/40, not referred due to suspicion of abnormality 94 women pregnant with dichorionic twins conceived spontaneously (SC), referred to research hospital for obstetric care <24/40, not referred due to suspicion of abnormality
Recruitment	All women with dichorionic twins referred to research hospital for obstetric care Jan 1990-July 2001 <24/40 (and not referred due to suspicion of abnormality) recruited
Prognostic balance	ICSI vs IVF: balanced for maternal age, parity, gestational age and birth weight ICSI vs SC: maternal age higher in ICSI group and more women primiparous. Balanced for gestational age and birth weight
Outcomes of interest	Congenital abnormality at birth
Data collection	Examined in delivery room by at least 2 deontologists , on postnatal days 1 and 5 and at time of discharge
Follow-up	Complete
Strengths	Prospective follow up
Weaknesses	Possible selection bias – referral criteria to research hospital unclear. Survival bias – excluded referrals with suspected abnormality. TOPs for abnormality not considered. Mentions intrauterine fetal death for abnormality which is not included in analysis (and unclear whether stillbirth or miscarriage)
Note	2 additional comparison groups also included, having GIFT or ovulation induction

Study	**La Sala 2004, Italy**
Design	Case control
ICSI cases	50 ICSI children conceived at single Italian clinic, born 1998-2001, no known congenital abnormality
Controls	51 spontaneously conceived (SC) children aged 12-24 months
Recruitment	ICSI families invited by letter. SC families, randomly selected from cohort (derivation of cohort not stated) and contacted by letter
Prognostic balance	Balanced for parental education, parental occupation, multiple births, infant age. ICSI group had significantly higher rates of caesarean section, neonatal special care admission, and higher maternal and paternal age,
Outcomes of interest	Child development at one and two years, quality of child-parent interraction
Data collection	Parents completed questionnaire, two (blinded) psychologists administered Bayley Scale to children, checked questionnaire and video-taped parent-child play interaction for 3 minutes. Video data coded using standardised instrument (CARE-index) by two (blinded) coders
Follow-up	Cross-sectional
Strengths	Blinded assessment using standardised measures
Weaknesses	Tiny sample size. Participation rate low, comprising 50/120 (41%) invited ICSI children and 51/81 (63%) invited controls. Reasons for non-participation unclear in most cases, except that 4 ICSI children who initially accepted were excluded for known congenital abnormalities. Selection process for SC group unclear. Higher frequency of twins in ICSI group (40% vs 24%) though not statistically significant due to small sample size.

Study	**Lancaster 2004, Australia/NZ**

Design	ART registry study
ICSI cases	8325 ICSI births/TOPs of at least 20 weeks' gestation 1990-99
Controls	IVF pregnancies notified to register – no sample number stated in abstract
Recruitment	ICSI cases identified from register
Prognostic balance	No information given
Outcomes of interest	Major congenital abnormalities at birth, frequency of specific abnormalities
Data collection	Australian and New Zealand Assisted Reproduction Database (ANZARD). Data collected at all Australian and New Zealand fertility centres at time of ART treatment. Staff follow up patients and/or clinicians for pregnancy and birth outcomes
Follow-up	No information given
Strengths	Very large sample, complete cohorts registered prospectively. Same recruitment and ascertainment methods for both groups, includes pregnancy data
Weaknesses	No information on prognostic variables Unclear how much follow-up data missing No follow up beyond birth.
Note	Reports % of events in IVF group but no numerator or denominator (thus unsuitable for meta-analysis) Unpublished (abstract only) Bryant 2004 and Hurst 2001 report data from same register: Hurst data (from 1998) overlaps

Study	**Lathi 2004, USA**
Design	Cohort
ICSI cases	21 women undergoing dilatation and curettage (D&C) for early miscarriage after ICSI
Controls	38 women undergoing dilatation and curettage (D&C) for early miscarriage after IVF
Recruitment	All women undergoing D&C for missed abortion identified from clinic records
Prognostic balance	Balanced for maternal age
Outcomes of interest	Fetal chromosomal abnormalities
Data collection	Review of lab data
Follow-up	Complete
Strengths	Whole cohort, complete outcome assessment
Weaknesses	Small sample, not stated what proportion of women with early miscarriage underwent D&C

Study	**Maher 2003, UK**
Design	Case study
Cases	6 cases of Beckwith-Weidemann Syndrome post ICSI/IVF
Data collection	Review of notes of 149 patients on BWS register
Note	Only 2/6 cases tested for imprinting status

Study	**Manning 2000, Belgium**
Design	Case series
Cases	92 children born after ICSI (52 singletons, 40 twins)
Recruitment	Not described
Outcomes of interest	DNA methylation status in chromosome 15q11q13: site for potential imprinting errors associated with Angelman Syndrome and Prader-Willi Syndrome
Strengths	
Weaknesses	Very small sample: prevalence of methylation defects in normal population is 1:150,000. Unclear whether cases were consecutively recruited
Data collection	Blood samples analysed from children aged 5 months – 4 years.

Study	**Moll 2003, The Netherlands**
Design	Case series
Cases	Five cases of retinoblastoma: 4 post IVF , 1 post ICSI
Data collection	Records of Dutch retinoblastoma clinic which sees 95% of cases of retinoblastoma in the Netherlands

Study	**Orstavik 2003, Norway**
Design	Case study
Cases	One case of Angelman Syndrome post ICSI
Data collection	Imprinting defect found

Study	**Orvieto 2000, Israel**
Design	Retrospective cohort
ICSI cases	100 consecutive fresh ICSI conceptions 1996-97 (128 children: 59 singletons, 42 twins, 27 triplets)
Controls	100 consecutive fresh IVF conceptions 1996-97 (110 children: 45 singletons, 56 twins, 9 triplets)
Recruitment	Identified from clinic database
Prognostic balance	ICSI mother significantly younger. Other prognostic factors not mentioned.
Outcomes of interest	Miscarriage, obstetric outcomes.
Data collection	Clinic database records, plus standardised telephone questionnaire to parents, administered by physician
Follow-up	Complete
Strengths	Whole cohorts followed.
Weaknesses	Groups unbalanced for maternal age, other prognostic factors unclear. Also unclear how much of data of data self-reported
Note	Clinical pregnancy defined as presence of fetal sac on U/S

Study	**Palermo 2000, USA**
Design	ICSI case series, with IVF comparison group for one outcome (congenital malformations)
ICSI cases	2059 children who survived neonatal period, born following ICSI 1993-99 (of 2129 live-born children)
Controls	1796 children born after "standard IVF treatment at our institution"
Recruitment	Complete ICSI cohort; no information about IVF group except
Prognostic balance	No information given
Outcomes of interest	Major malformations at birth (no comparison group for other outcomes)
Data collection	Pregnancy outcome data obtained from obstetricians/gynaecologists and/or paediatricians
Follow-up	All pregnancies followed to term
Strengths	Whole ICSI cohort 1993-9 followed up.
Weaknesses	No description of IVF group. No information on 70 live born ICSI children who did not survive neonatal period. Excludes from analysis of congenital malformations 45 miscarried ICSI foetuses found to have chromosomal abnormalities. No information on ICSI TOPs nor on IVF miscarriages, TOPs or whether all live born IVF children included.
Note	Study not included in meta-analysis as data is missing on large number of ICSI children with highest likelihood of congenital abnormality (ie, 70 neonatal deaths) **See also Palermo 1996. Neri 2004** updates same cohort to 2003 but no control group is described and findings are unintelligible

Study	**Paulus 2004, Germany**
Design	Prospective cohort study
ICSI cases	434 ICSI children (296 singletons, 120 twins, 18 triplets) from one ART centre assessed at one year
Controls	356 IVF children (246 singletons, 104 twins, 6 triplets) at same ART centre assessed at one year
Recruitment	Unclear whether recruited at birth or antenatally
Prognostic balance	Balanced for multiple pregnancies and gestational age
Outcomes of interest	Congenital abnormalities, growth retardation at one year, developmental retardation*
Data collection	Assessment by paediatrician
Follow-up	Not stated how many recruited
Strengths	Prospective follow-up, groups well balanced, same assessment criteria for both groups
Weaknesses	Follow up rate unclear (study reported in abstract only). Does not include TOPs/miscarriages in assessment of congenital abnormalities
Note	*Unclear whether this outcome refers to physical or cognitive development – outcome not included in review (study unpublished)

Study	**Papaligoura 2004, Greece**
Design	Retrospective case-control
ICSI cases	34 ICSI firstborn children (26 singletons, 8 twins).
Controls	26 IVF firstborn children (14 singletons, 12 twins), 29 spontaneously conceived (SC) firstborn children (23 singletons, 6 twins)
Recruitment	ICSI and IVF parents invited by phone call from fertility centre – SC children approached through paediatricians associated with Athens' hospitals
Prognostic balance	All groups balanced for parental education, proportion of twins *ICSI vs IVF*: Balanced for maternal age, length of marriage, *ICSI vs SC:* ICSI mothers significantly older and married for longer, ICSI children had lower birth weight, more caesareans, lower gestational age
Outcomes of interest	Cognitive development of children at one year, psychological health of mothers*
Data collection	Test administered to children at home by (blinded) psychologist. Structured interview of mothers
Follow-up	Cross-sectional
Strengths	High acceptance rate – 97%% in ICSI group, 93% IVF group, 100% SC group. Blind assessment of children using reliable measure (Bayley Scale)
Weaknesses	Tiny sample size. Selection criteria unclear – not stated whether whole ART birth cohorts invited to participate. Proportionally twice as many twins and higher incidence of low birth weight in IVF group (though differences not statistically significant due to small sample size)
Note	* No formal measures of psychological status reported – results not reported in this review.

Study	**Pinborg 2004/Pinborg 2004a, Denmark**
Design	Retrospective registry-based cohort study
ICSI cases	All ICSI singletons (1282) and twins (835) born in Denmark from 1995-2000* >22/40
Controls	IVF singletons (3848) and twins (2558) born in Denmark from 1995-2000 >22/40
Recruitment	Identified from Danish Medical Birth Register, cross-linked with ART register
Prognostic balance	ICSI vs IVF: Balanced for singleton/twin status. No comparison of ICSI vs IVF for other prognostic variables
Outcomes of interest	Major and minor congenital malformations in twins to age at least one year, frequency of specific malformations in twins, hospital admissions in all children
Data collection	Data from birth, ART and Patient registries.
Follow-up	Complete IVF cohort tracked. Danish Patient Registry found acceptable for epidemiological research when assessed in 2003 (Larsen et al) Unverifiable how much data is missing.
Strengths	Whole ART cohort followed. Large sample size.
Weaknesses	Stillbirths (n=45) excluded from analysis as outcomes data not available; these are children with highest likelihood of congenital abnormality. No data on TOPs, miscarriages available Data on abnormalities in ART children leaves 39/3393 unaccounted for (? lost to follow up)
Note	Primary objective of study was to compare ART twins versus spontaneous twin conception (as opposed to ICSI vs IVF) but was stratified for ICSI/IVF for congenital malformations. Main study outcomes controlled with all non-ART twins born in Denmark from 1995-2000 (not of interest to this review) Figures presented in the tables in this review have been calculated from percentages reported by authors See also Loft 1999, Pinborg 2003

Study	**Place 2003, Belgium** (French speaking Brussels Free University)
Design	Cross-sectional case-control (see Note below)
ICSI cases	66 full term singletons conceived by ICSI from Belgian ART clinic
Controls	52 full term IVF singletons, 59 spontaneously conceived (SC)
Recruitment	All children eligible between April 1998 and March 2000 invited by letter. Controls matched as closely as possible for birth date, age and gender of child, age of mother, social class, ethnic background and birth order of child
Prognostic balance	Balanced for all criteria except for levels of education of the parents and length of parental relationship, which favoured the spontaneously conceived group. Unclear whether ICSI and IVF children significantly different from each other for these variables.
Outcomes of interest	Congenital malformations, hospital admissions, child development in preschool children
Data collection	Families had standardized interview and questionnaire administered at home by the same clinical psychologist (not blinded), plus paediatrician completed a questionnaire.
Follow-up	Children assessed for child development at one of more time points, namely 9 months, 18 months, 3 years & 5 years. Authors describe follow up rates of 84-93% - but this excludes children seen only once (12/47 at five years) and those who could not be traced (not quantified). Other assessment apparently cross-sectional.
Strengths	Limitation to term singletons reduces risk of confounding. Objective measures used (Brunet-Lezine scale at 9 & 18 months, Wechsler Scale at 3 & 5 years)
Weaknesses	Unclear what proportion of birth cohort invited. Lowish acceptance rate of those invited (ICSI 70%, IVF 60%, SC 40%). Low power due to small sample numbers, especially at 5 years. Data collection only partially prospective – eg, Unclear how many individual children were assessed at both 3 and 5 years. Educational differences between ART and SC parents. No miscarriages/TOPs/stillbirths included in malformations
Note	Described by authors as prospective cohort study and. large proportion of children assessed at two time points for child development. Other outcomes retrospectively assessed using matched controls not whole cohorts

Ponjaert-Kristoffersen 2004 – see Bonduelle 2004

Ponjaert-Kristoffersen 2005 – see Barnes 2004

Study	**Squires 2003, USA**
Design	Prospective case control study (design not very clearly described)
ICSI cases	141 ICSI children aged 4-48 months
Controls	144 IVF children aged 4-48 months
Recruitment	Recruitment method unclear: 7 US fertility centres invited parents by letter – bit stated how sample selected.
Prognostic balance	Parents balanced for demographic variables. No information on prognostic balance among children
Outcomes of interest	Proportion of children "at risk" of atypical development and needing further evaluation
Data collection	Monitored over 4 years with 4-6 monthly Ages and Stages Questionnaires completed by parents.
Follow-up	Unclear what proportion of children followed up prospectively – mean no of questionnaires per child approx 2. 4 participants withdrew.
Strengths	
Weaknesses	Selection criteria unclear, not stated whether whole birth cohorts invited. No data on families who declined to participate (estimated at 35-50% of those invited). No independent assessment (parents supplied all information). No information on prognostic variables among children (eg, ages, proportion of multiple births, birth weights etc.)..Unclear whether outcome is not clinically relevant Significance testing conducted on number of questionnaires rather than number of children.

Study	**Sutcliffe 2001, UK**
Design	Case-control
ICSI cases	208 singleton children conceived after ICSI at 22 UK clinics (Sutcliffe 2001)
Controls	221 spontaneously conceived singleton children, matched for age, sex, maternal education, social class, geographical region
Recruitment	All ICSI children of eligible age invited to join study. SC children recruited from local day nurseries (85%) or peers of cases (15%)
Prognostic balance	Balanced for maternal education and social class. ICSI mothers significantly older and more likely to be having first child. ICSI fathers significantly older and more likely to be manual workers. ICSI children has lower birth weight and more likely to have had caesarean delivery. Mean age of ICSI children significantly lower at time of assessment.
Outcomes of interest	Congenital abnormalities, child development
Data collection	From ICSI clinic records, child health records held by parents, investigator conducted standardized parent interview, physical examination and administration of developmental test
Follow-up	90% of eligible ICSI children participated. Unclear how many controls invited and what proportion accepted.
Strengths	ICSI children not significantly different from ICSI non-participants with respect to parental age, birth weight or father's sperm analysis.. Powered to show 5% difference in developmental score, a minimum clinically meaningful. Difference. Twins and triplets excluded to reduce confounding. Single outcomes assessor. Standardised measure used (Griffiths Scale of mental development)
Weaknesses	Groups unbalanced for several important prognostic factors, though these would be expected to disadvantage the ICSI group. Unblinded assessment, control group participation rate unclear. No TOP/miscarriage/stillbirth data for outcome of congenital malformations
Note	Same UK ICSI cohort followed up prospectively in Bonduelle 2005 (for 5 year outcomes)

Study	**Sutcliffe 2003, UK**
Design	As Sutcliffe 2001 (see above), with the addition of an Australian cohort
ICSI cases	58 singleton children aged 13-15 months conceived by ICSI
Controls	38 spontaneously conceived (SC) children
Recruitment	ICSI children invited by clinic to join study, SC children recruited from local nurseries, matched as above
Prognostic balance	Australian cases and controls balanced for prognostic factors except that ICSI mothers older and ICSI mean birth weights lower. Australian cases and controls younger at assessment than UK cases and controls (13 months vs 17 months) and a greater proportion of Australian mothers drank alcohol.
Outcomes of interest	Congenital abnormalities, child development – aimed to investigate whether the UK findings applied also in Australia
Data collection	As above, with interview and examinations conducted by a paediatrician.
Follow-up	Unclear what proportion of eligible Australian cases and controls were enrolled, though states that overall participation rate was 85%
Strengths	As above for UK cohort.
Weaknesses	Small sample size in Australia. As above for UK participants

Study	**Van Golde 1999, Spain**
Design	Retrospective cohort study
ICSI cases	120 children conceived by ICSI in 1995: all pregnancies proceeding beyond 20/40
Controls	132 children conceived by IVF in 1995: all pregnancies proceeding beyond 20/40
Recruitment	Couples invited at 20/40 gestation to join study
Prognostic balance	Mean maternal age lower in ICSI group. Groups balanced for multiplicity, mean paternal age, maternal smoking and medication in pregnancy and diseases during pregnancy
Outcomes of interest	Karyotypes at prenatal diagnosis, obstetric and neonatal outcomes, major malformations at birth & child development to 6-18 months,
Data collection	Data collected from clinic records, questionnaire to mothers and by contacting gynaecologist and/or paediatrician if necessary, amniocentesis for karyotyping. Catalan national developmental scale used for developmental assessment.
Follow-up	30% pregnancies karyotyped, 92% followed to 6-18 months
Strengths	Complete cohort. High follow-up rate of infants.
Weaknesses	Small sample size, low proportion karyotyped, comprising those at higher risk. Survivor bias: miscarriages and TOPs <20/40 not eligible.
Note	Validity of developmental scale unknown

Study	**Weisel 2003, Germany**
Design	Case control
ICSI cases	85 live births, stillbirths, miscarriages and TOPs
Controls	202 IVF and 19, 211 SC live births, stillbirths, miscarriages and TOPs
Recruitment	Identified from Mainz Birth Registry
Prognostic balance	No information
Outcomes of interest	Major congenital malformations
Data collection	Registry data.. States standardised examination procedure used, no details.
Follow-up	Completeness of ascertainment uncertain
Strengths	Large dataset. Includes pregnancy data
Weaknesses	Abstract only available – little information on methodology. No follow-up beyond birth

Appendix 3: Summary of evidence

Please note:

- This overview is *highly* simplified! See text for detailed information.
- There is no conclusive evidence on any of these outcomes.

Outcome	ICSI vs IVF	ICSI vs spontaneous conception
Obstetric outcomes	ICSI similar	ICSI worse[1]
Neonatal outcomes	ICSI similar or better[2]	ICSI worse [1]
Chromosomal abnormalities	ICSI probably worse	ICSI worse
Congenital malformations	ICSI similar at birth[3]	ICSI/IVF worse
Child growth	ICSI similar	ICSI similar
Child physical development	ICSI similar	ICSI worse[4]
Child cognitive development	ICSI similar	ICSI similar
Psychological outcomes	ICSI similar	ICSI similar
Epigenetic disorders	No evidence	ICSI/IVF probably worse[5]

1. Mainly due to high multiple rate in ART
2. Evidence of lower neonatal complication and death rate in ICSI group – unclear why
3. More data needed on older children, among whom there is an unproven possibility of increased abnormalities
4. Higher likelihood of childhood illness and higher use of health resources
5. Far more data needed to clarify risk and which specific disorders are involved.