Intracytoplasmic sperm injection versus conventional in-vitro fertilisation in couples with infertility in whom the male partner has normal total sperm count and motility: an open-label, randomised controlled trial

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See **Comment** page 1521

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Summary

Background The use of intracytoplasmic sperm injection has increased substantially worldwide, primarily in couples with non-male factor infertility. However, there is a paucity of evidence from randomised trials supporting this approach compared with conventional in-vitro fertilisation (IVF). We aimed to investigate whether intracytoplasmic sperm injection would result in a higher livebirth rate compared with conventional IVF.

Methods This open-label, multicentre, randomised trial was done at two IVF centres in Ho Chi Minh City, Vietnam (IVFMD, My Duc Hospital and IVFAS, An Sinh Hospital). Eligible couples were aged at least 18 years and the male partner's sperm count and motility (progressive motility) were normal based on WHO 2010 criteria. Couples had to have undergone two or fewer previous conventional IVF or intracytoplasmic sperm injection attempts, have used an antagonist protocol for ovarian stimulation, and agree to have two or fewer embryos transferred. Couples were randomly assigned (1:1) to undergo either intracytoplasmic sperm injection or conventional IVF, using block randomisation with variable block size of 2, 4, or 8 and a telephone-based central randomisation method. The computer-generated randomisation list was prepared by an independent statistician who had no other involvement in the study. Embryologists and couples were not masked to study groups because of the type of interventions and differences in hospital fees, but clinicians performing embryo transfer were unaware of study group allocation. The primary outcome was livebirth after the first embryo transfer from the initiated cycle. Analyses were done on an intention-to-treat basis. The trial is registered with ClinicalTrials.gov, NCT03428919.

Findings Between March 16, 2018, and Aug 12, 2019, we randomly assigned 1064 couples to intracytoplasmic sperm injection (n=532) or conventional IVF (n=532). Livebirth after the first embryo transfer from the initiated cycle occurred in 184 (35%) of 532 couples randomly assigned to intracytoplasmic sperm injection and in 166 (31%) of 532 couples randomly assigned to conventional IVF (absolute difference 3·4%, 95% CI –2·4 to 9·2; risk ratio [RR] 1·11, 95% CI 0·93 to 1·32; p=0·27). 29 (5%) couples in the intracytoplasmic sperm injection group and 34 (6%) couples in the conventional IVF group had fertilisation failure (absolute difference –0·9%, –4·0 to 2·1, RR 0·85, 95% CI 0·53 to 1·38; p=0·60).

Interpretation In couples with infertility in whom the male partner has a normal total sperm count and motility, intracytoplasmic sperm injection did not improve the livebirth rate compared with conventional IVF. Our results challenge the value of the routine use of intracytoplasmic sperm injection in assisted reproduction techniques for this population.

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Introduction

Intracytoplasmic sperm injection was first applied in the 1990s to overcome low and unpredictable fertilisation rates with conventional in-vitro fertilisation (IVF) in couples with severe male factor infertility.[1] The past two decades have seen a rapid rise in the use of intracytoplasmic sperm injection, even though the rate of male infertility has remained unchanged over this time.[2] Data from European countries in 2016 showed that intracytoplasmic sperm injection was used in 72·3% of cycles, with an increase of 1·2% compared with 2015, with substantial variation in use between countries.[3] The same trend has also been reported in the USA[4] and internationally.[5] The most substantial increase in the use of intracytoplasmic sperm injection (from 15·4% to 66·9%) has occurred in couples with non-male factor infertility.[4]

The rationale for using intracytoplasmic sperm injection in couples with non-male factor infertility assumes that this technique might avoid unexpected total fertilisation failure and increase the number of embryos available, thereby improving the chance

Research in context

Evidence before this study

When the first four pregnancies after intracytoplasmic sperm injection were reported in 1992, this novel technique was mainly applied in cases of severe male factor infertility. However, the past two decades have seen a rapid rise in the use of intracytoplasmic sperm injection—to in more than 70% of cases—although the rate of male infertility has remained unchanged over this time. The largest increase in the use of intracytoplasmic sperm injection (from 15·4% to 66·9%) has occurred in couples with non-male factor infertility. Retrospective cohort studies showed a significantly lower livebirth rate when intracytoplasmic sperm injection was used versus conventional in-vitro fertilisation (IVF), whereas other studies reported similar cumulative livebirth rates for both techniques, even when stratified by different ovarian response. However, the only randomised controlled trial comparing intracytoplasmic sperm injection and conventional IVF in this population was published in 2001, using implantation as the primary endpoint. This trial had restricted power and did not report on livebirths. Therefore, data from large randomised controlled trials, with livebirth as the primary outcome, are needed.

Added value of this study

To our knowledge, this is the largest randomised trial to date and the first to provide data on livebirth after the use of intracytoplasmic sperm injection versus conventional IVF in couples with non-male factor infertility (normal total sperm count and motility). We recruited 1064 couples in whom the male partner's total sperm count and motility were normal based on WHO 2010 criteria and who had had two or less previous IVF or intracytoplasmic sperm injection attempts. Our data showed that, compared with conventional IVF, intracytoplasmic sperm injection did not improve livebirth rates after the first transfer and did not improve cumulative ongoing pregnancy resulting in livebirth at 12 months after randomisation, from the initiated cycle. However, we found significantly higher fertilisation rates per oocyte retrieved and per oocyte inseminated or injected in the intracytoplasmic sperm injection group. Other secondary outcomes, including total fertilisation failure, were similar between the two groups.

Implications of all the available evidence

Our data showed that in couples with infertility in whom the male partner has normal total sperm count and motility, intracytoplasmic sperm injection did not improve the rates of livebirth over conventional IVF. Therefore, we question the value of the routine use of intracytoplasmic sperm injection in assisted reproduction for this population.

of having a baby. However, intracytoplasmic sperm injection does not appear to reduce total fertilisation failure in couples with non-male factor infertility, even in women with a small number of oocytes due to poor ovarian response.[6] The largest randomised controlled trial to date comparing intracytoplasmic sperm injection with conventional IVF was published in 2001, but had restricted power and did not report on livebirth.[7] Some retrospective cohort studies showed a significantly lower livebirth rate when intracytoplasmic sperm injection was used compared with conventional IVF,[4,8] whereas others reported similar cumulative livebirth rates after intracytoplasmic sperm injection and conventional IVF,[9] even when stratified by ovarian response.[10,11]

Given the large amount of intracytoplasmic sperm injection procedures done worldwide and the lack of adequately powered randomised trials, we aimed to compare intracytoplasmic sperm injection versus conventional IVF in couples with infertility in whom the male partner has a normal total sperm count and motility.

Methods

Study design and participants

This multicentre, open-label, randomised trial was done at two IVF centres in Ho Chi Minh City, Vietnam (IVFMD, My Duc Hospital and IVFAS, An Sinh Hospital). These centres are in a private setting and together conduct around 7000 conventional IVF or intracytoplasmic sperm injection cycles annually. The andrology laboratories at these centres have applied WHO criteria for semen analysis since 2010,[12] and are also certified by the Ho Chi Minh City Society for Reproductive Medicine.

Potentially eligible couples were given a study information sheet. The study was discussed with them during their first consultation at least 2 weeks before the start of their menstrual cycle. Eligible couples were aged at least 18 years and the male partner's sperm count and motility (progressive motility) were normal based on WHO 2010 criteria (total sperm count $\geq 39 \times 10^6$ sperm, progressive motility ≥32%).[12] Couples had to have undergone two or fewer previous conventional IVF or intracytoplasmic sperm injection attempts, have used an antagonist protocol for ovarian stimulation, and agree to have two or fewer embryos transferred, and not simultaneously be participating in other IVF trials. Couples undergoing in-vitro maturation cycles, couples using frozen semen, or couples with poor fertilisation (≤25%)[13] in a previous cycle were excluded. We transferred no more than two embryos to minimise the risk of multiple pregnancy. This strategy was practiced in 93·4% of cycles across Europe in 2016.[3]

To be eligible for this trial, male partners in couples had to have at least two semen analyses. A full first

analysis, including morphology according to WHO 2010 crtiteria, was done during the first consultation, but this analysis was not considered as an inclusion or exclusion criterion. On the day of oocyte retrieval, the semen sample was reanalysed, except for morphology. The time elapsed between these analyses was about 4 to 6 weeks in most couples. Semen analyses were done in the andrology laboratory, located on the same floor as the IVF clinic in the hospital, by trained embryologists. To ensure the accuracy and precision of the results, we implemented a quality assurance system in which internal quality control was done every 3 months. For this trial, we also used a checklist for assessment of sperm count and motility.[14]

The protocol (version 1, November, 2017) was approved by the institutional ethics committee (IEC) at each hospital (13/17/ĐĐ-BVMĐ and 1322B-17/AS-CT, both dated on Dec 18, 2017). The primary outcome was changed from ongoing pregnancy resulting in livebirth obtained from all embryos of the started treatment cycle to ongoing pregnancy resulting in livebirth after the first embryo transfer of the started treatment cycle, and the former was changed to a secondary outcome, with a fixed time point at 12 months after randomisation (version 2, August, 2018). This change allowed assessment of the outcome sooner than with the original primary outcome, including cumulative outcomes. To increase the study generalisability, women with polycystic ovary syndrome or with oocyte maturation triggered by gonadotropin-releasing hormone agonist were included. These amendments were approved by the IEC of My Duc Hospital (Sept 13, 2018) and An Sinh Hospital (Sept 12, 2018). Genetic and epigenetic analysis of neonates and cost-effectiveness analyses were added to the protocol version 3 (February, 2019). This amendment was approved by the IEC of My Duc Hospital (March 7, 2019) and An Sinh Hopsital (March 10, 2019). These changes are summarised in the appendix (pp 13–14). The trial was done according to Good Clinical Practice and Declaration of Helsinki principles, including oversight by an independent Data Safety Monitoring Committee (DSMC). Full details of the trial protocol have been reported previously.[15] All patients provided written informed consent for the trial.

See Online for appendix

Randomisation and masking

Eligibility screening was done by treating physicians on the day of oocyte retrieval, after semen had been obtained and before oocyte retrieval was done. Participants who fulfilled the eligibility criteria and who had been counselled were formally invited to participate in the study. If an individual agreed to participate, they were asked to sign the informed consent form. Randomisation was done after semen was obtained to check for eligibility and before the oocyte retrieval. Couples were randomly assigned (1:1) to either intracytoplasmic sperm injection or conventional IVF, using block randomisation with a variable block size of 2, 4, or 8. The computer-generated randomisation list was prepared by an independent statistician who had no other involvement in the study using the blockrand package in R. To ensure allocation concealment, telephone-based central random assignment was used. Embryologists and couples were not masked to study groups because of the type of interventions and differences in hospital fees, but clinicians performing embryo transfer were unaware of study group allocation.

Procedures

All women underwent controlled ovarian hyperstimulation in a follicle-stimulating hormone and gonadotropin-releasing antagonist protocol.[16] An antagonist was routinely used from day 5 until the day oocyte maturation was triggered. Human chorionic gonadotropin triggering was initiated when at least three leading follicles had a diameter of 17 mm. In women with an excessive follicular response (≥15 follicles of ≥12 mm in diameter), 0·2 mg of a gonadotropin-releasing hormone agonist was used when there were at least two leading follicles of 17 mm for the prevention of ovarian hyperstimulation syndrome (OHSS).[17] Oocyte retrieval was done 36 h after triggering.

On the day of oocyte retrieval, all semen samples were obtained by masturbation, and allowed to liquefy for up to 60 min and then processed by centrifugation through a discontinuous density gradient. In the intracytoplasmic sperm injection group, the pellets were washed once with sperm preparation medium. In the conventional IVF group, after being washed once with sperm preparation medium, the pellets were processed by swim-up technique. The supernatants were washed again and concentrated to between 1 and 5×10^6 motile sperm per mL. In couples allocated to the intracytoplasmic sperm injection group, intracytoplasmic sperm injection was done 3–4 h after oocyte retrieval. The oocyte–cumulus complex was stripped using hyaluronidase. Only matured oocytes were injected. Oocytes at MI stage were discarded.

In couples allocated to the conventional IVF group, 2 h after retrieval, collected oocyte–cumulus complexes were inseminated for another 2 h, using a concentration of 100 000 motile sperm/mL. Inseminated oocyte-cumulus complexes were then cultured overnight in culture medium.

In both groups, a fertilisation (two pronuclei) check was done at 16–18 h after insemination. Embryo evaluation was done at a fixed time point 66 h (±2 h) after fertilisation, according to the Istanbul consensus.[18] Fresh embryo transfer was done on day 3 under ultrasound guidance. The number of embryos transferred (one to a maximum of two) was based on the couples' preference. The remaining grade 1 and grade 2 embryos were frozen at the cleavage stage.

If there were contraindications for fresh embryo transfer, a freeze-only strategy was applied. Indications for

freeze-only include risk of OHSS, premature progesterone rise (≥1·5 ng/mL), thin endometrium (<7 mm), fluid in cavity on day of embryo transfer, and previously undiscovered endometrial polyps or hydrosalpinx that had not removed before oocyte retrieval.

In the frozen embryo transfer cycle, the endometrium was prepared using a hormonal replacement treatment regimen.[16] A maximum of two embryos were thawed on the day of transfer, 3 days after the start of progesterone. 2 h after thawing, surviving embryos were transferred into the uterus under ultrasound guidance.[16]

Luteal phase support consisted of 800 mg vaginal progesterone, starting on the day of oocyte retrieval in the fresh transfer. For frozen embryo transfer, 800 mg of vaginal progesterone per day was started with the continued use of oral estradiol 8 mg per day for 3 days before embryo transfer. Both drug regimens were continued until pregnancy testing was done. In case of pregnancy, luteal phase support was continued until 7 weeks of gestation.[16] If the pregnancy test was positive, an ultrasound scan of the uterus was done at gestational weeks 7 and 12. For the remainder of the pregnancy and neonatal period, participants were followed up and managed according to routine clinical practice.[16]

Outcomes

The primary outcome was livebirth after the first embryo transfer from the initiated cycle. Livebirth was defined as the birth of at least one baby after 24 weeks of gestation that showed any sign of life (twins as a single count). Cycles in which no embryo was available for transfer were considered failures. Full definitions of all secondary outcomes are provided in the study protocol.[15] Briefly, fertility outcomes were fertilisation rate per oocyte inseminated and per oocyte retrieved, abnormal fertilisation, total fertilisation failure, number of day 3 embryos, number of good quality day 3 embryos, number of frozen day 3 embryos, positive pregnancy test, clinical pregnancy, ongoing pregnancy, implantation, cumulative ongoing pregnancy, cumulative ongoing pregnancy resulting in livebirth from the initiated cycle at 12 months after randomisation, and time to ongoing pregnancy resulting in livebirth. For maternal safety, OHSS was assessed. Pregnancy complications included ectopic pregnancy, miscarriage, multiple pregnancy, and multiple delivery. Obstetrics and perinatal outcomes were gestational age at delivery, gestational diabetes, hypertensive disorders of pregnancy, antepartum haemorrhage, preterm delivery of any indication, spontaneous preterm delivery, iatrogenic preterm delivery before weeks 24, 28, 32, or 37 of gestation, birthweight, (very) low birthweight (low birthweight: <2500 g; very low birthweight: <1500 g), (very) high birthweight (high birthweight: >4000 g; very high birthweight: >4500 g), large for gestational age, and small for gestational age. Congenital anomaly diagnosed at birth and admission to neonatal intensive care unit were used to determine neonatal complications. Pregnancy complications, obstetrics and perinatal outcomes, and congenital anomaly were assessed after the first embryo transfer and at 12 months after random assignment from the initiated cycle.

Statistical analysis

Before the study, the livebirth rate in a double embryo transfer strategy after conventional IVF or intracytoplasmic sperm injection at IVFMD and IVFAS was 31·5%. To assess whether intracytoplasmic sperm injection would increase the livebirth rate after the first transfer by 10% compared with conventional IVF, we needed to randomly assign 1064 couples (532 per study group; 90% power, two-sided α=0·05, estimated loss to follow-up 10%). An interim analysis of ongoing pregnancy was done by an independent statistician and overseen by a DSMC after enrolling the first 500 participants. At the time when 500 couples were enrolled, ongoing pregnancy results were available in 151 couples in the intracytoplasmic sperm injection group and 147 couples in the conventional IVF group. Using a two-sided significance test with the Haybittle-Peto spending function and a type 1 error rate of 5% with stopping criteria of p<0·001 (Z α=3·29), the DSMC recommended continuation of the trial as planned.

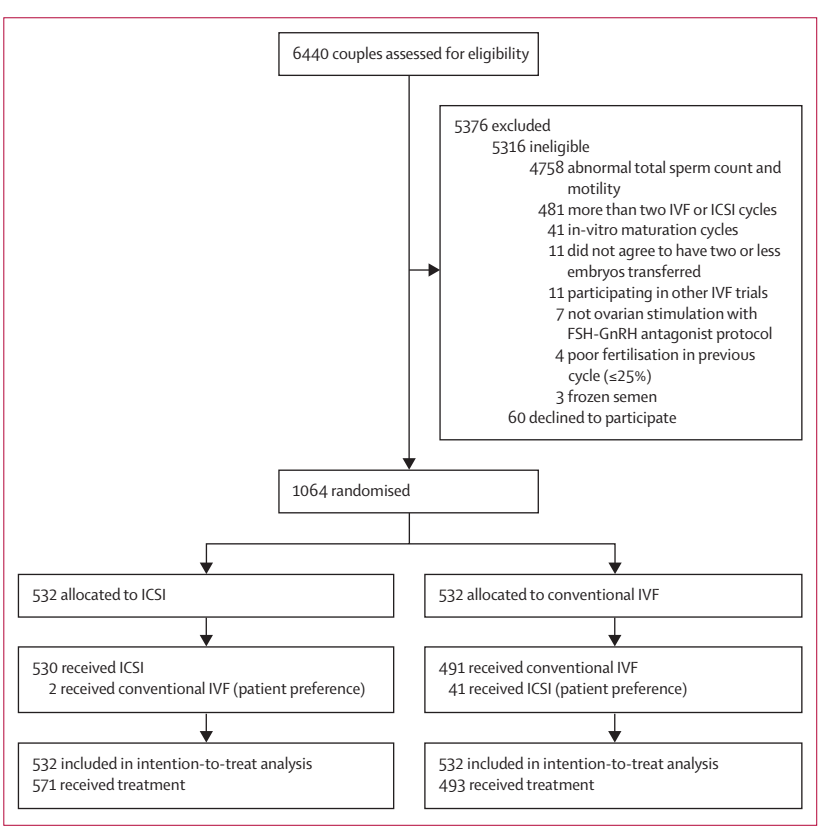

***Figure 1*: Trial profile**
FSH=follicle-stimulating hormone. GnRH=gonadotropin-releasing hormone. ICSI=intracytoplasmic sperm injection. IVF=in-vitro fertilisation.

	Intracytoplasmic sperm injection (n=532)	Conventional IVF (n=532)
Age (years)		
Women	32·7 (4·6)	32·6 (4·7)
Men	35·2 (5·2)	35·3 (5·6)
Body-mass index (kg/m²)		
Women	21·2 (2·5)	21·2 (2·4)
Female anti-Müllerian hormone (ng/mL)	2·5 (1·5–4·3)	2·6 (1·6–4·3)
Duration of infertility (years)	3·0 (2·0–5·0)	4·0 (2·0–6·0)
Number of previous IVF or intracytoplasmic sperm injection cycles		
0	480 (90%)	486 (91%)
1	52 (10%)	46 (9%)
2	0	0
Primary infertility	295 (55%)	299 (56%)
IVF indication		
Unexplained	199 (37%)	183 (34%)
Diminished ovarian reserve	121 (23%)	144 (27%)
Tubal factor	134 (25%)	120 (23%)
Ovulation disorder	58 (11%)	69 (13%)
Endometriosis	20 (4%)	16 (3%)
Duration of stimulation (days)	8·8 (1·3)	8·8 (1·3)
Total dose of follicle-stimulating hormone (IU)	2700 (2250–3075)	2700 (2100–3075)
Estradiol level on day of trigger (pg/mL)	3408·5 (1723·0–5866·3)	2943·0 (1738·0–5871·0)
Progesterone level on day of trigger (ng/mL)	0·8 (0·5–1·2)	0·8 (0·5–1·2)
Semen volume (mL)	2·0 (1·1–2·7)	2·0 (1·0–2·7)
Sperm concentration (million)	77·8 (46·8–123·4)	78·0 (51·0–143·2)
Total sperm count (million)	147·2 (91·0–230·8)	157·9 (93·0–258·5)
Sperm motility (%)	40·0 (32·0–50·3)	42·0 (34·0–51·0)
Total motile sperm count (million)	63·6 (36·5–103·5)	68·5 (38·9–117·3)
Sperm with normal morphology (%)*	3·0 (1·0–6·0)	3·0 (1·0–6·0)
Number of oocytes retrieved	11·0 (7·0–16·0)	11·0 (7·0–16·0)
Number of metaphase II oocytes	9·0 (5·0–13·0)	9·0 (5·0–14·0)

Data are mean (SD), median (IQR), or n (%). IVF=in-vitro fertilisation. *Data from samples obtained during the first consultation, before the IVF or intracytoplasmic sperm injection cycle.

Table 1: **Baseline characteristics**

The primary statistical analysis was done on an intention-to-treat basis. The livebirth rate after the first embryo transfer was compared between groups by calculating the risk difference and associated 95% CI. We calculated cumulative ongoing pregnancy resulting in livebirth from the initiated cycle at 12 months after randomisation (natural conceptions were included). Time to ongoing pregnancy resulting in livebirth was assessed using Cox proportional hazard analysis, and hazard ratios (HRs) were estimated. We constructed Kaplan-Meier curves, and analysed them with log-rank tests. Between-group differences in secondary endpoints were analysed by using parametric methods (normally distributed data), non-parametric methods (skewed data), or Fisher's exact test (categorical variables), and were reported as relative risks and 95% CIs. The absolute differences and 95% CIs of skewed data were calculated using the Hodges-Lehman method. CIs were not adjusted for multiplicity. An additional treatment-received analysis was done according to the statistical analysis plan (appendix p 20).

We did a post-hoc analysis to examine the treatment effect in different IVF indications, trial centres, trigger regimens (antogonist trigger and human chorionic gonadotrophin trigger), ovarian response categories according to the number of oocytes retrieved (1–3, 4–9, 10–15, and >15 oocytes), sperm morphology (from samples obtained during the first consultation), and four total motile sperm count quartiles on livebirth after the first embryo transfer from the initiated cycle. The number of couples without an embryo for transfer (defined by those with no embryo after failed fertilisation and those with no embryo because of embryonic block) and fetal sex (expressed as the ratio of male to female) were also calculated.

All analyses were done by using tables, epitools, survival, and survminer packages in R version 4.0. The trial is registered with ClinicalTrials.gov, NCT03428919.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, of writing of the report.

Results

Between March 16, 2018, and Aug 12, 2019, we screened 6440 couples, of whom 1124 met all eligibility criteria and were invited to participate in the study (figure 1). 60 couples declined to participate. Therefore, 1064 couples were randomly assigned to intracytoplasmic sperm injection (n=532) or conventional IVF (n=532; figure 1). The baseline characteristics of the two groups are shown in table 1. 90% of couples had no previous IVF or intracytoplasmic sperm injection attempts. Couples' socioeconomic demographics and reasons for undergoing freeze-only procedures are presented in the appendix (pp 5–6).

Livebirth after the first embryo transfer from the initiated cycle occurred in 184 (35%) of 532 couples randomly assigned to intracytoplasmic sperm injection and in 166 (31%) of 532 couples randomly assigned to conventional IVF (absolute difference 3·4%, 95% CI –2·4 to 9·2; risk ratio [RR] 1·11, 95% CI 0·93 to 1·32; p=0·27; table 2).

Fertilisation per oocyte inseminated (75·0%, 95% CI 56·9–88·9 in the intracytoplasmic sperm injection group *vs* 66·7%, 50·0–83·3 in the conventional IVF group; p<0·0001) and per oocyte retrieved (58·3%, 40·0–72·7 in the intracytoplasmic sperm injection group and 55·6%, 38·8–70·0 in the conventional IVF group; p=0·048) were significantly higher with intracytoplasmic sperm injection than with conventional IVF (table 2). The rates of abnormal fertilisation per oocyte inseminated and per oocyte retrieved were significantly lower in the intracytoplasmic sperm injection group, with absolute differences of –6·1%

	Intracytoplasmic sperm injection (n=532)	Conventional IVF (n=532)	Absolute difference (95% CI)	Risk ratio (95% CI)*	p value
Fertility outcomes					
Livebirths†	184 (35%)	166 (31%)	3·4% (–2·4 to 9·2)	1·11 (0·93 to 1·32)	0·27
Fertilisation per oocyte inseminated or injected‡	75·0% (56·9–88·9)	66·7% (50·0–83·3)	5·6% (2·2 to 8·6)	..	<0·0001
Fertilisation per oocyte retrieved	58·3% (40·0–72·7)	55·6% (38·8–70·0)	2·9% (0·0 to 5·7)	..	0·048
Abnormal fertilisation per oocyte inseminated or injected‡	1·3% (6·2)	7·4% (12·4)	–6·1% (–7·6 to –5·1)	..	<0·0001
Abnormal fertilisation per oocyte retrieved	1·1% (5·7)	6·3% (10·5)	–5·2% (–6·5 to –4·4)	..	<0·0001
Total fertilisation failure§	29 (5%)	34 (6%)	–0·9% (–4·0 to 2·1)	0·85 (0·53 to 1·38)	0·60
Couples without an embryo for transfer¶‖	8 (2%)	21 (4%)	–2·4% (–4·6 to –0·3)	0·38 (0·17 to 0·85)	0·024
Number of day 3 embryos	5 (3–8)	5 (2–8)	0 (0 to 1)	..	0·19
Number of good day 3 embryos**	4 (2–7)	3 (2–6)	0 (0 to 1)	..	0·25
Number of day 3 embryos frozen	4 (2–6)	4 (2–6)	0 (0 to 0)	..	0·37
Number of embryos transferred	1·9 (0·3)	1·9 (0·3)	..	..	..
Number of good embryos transferred	1·7 (0·6)	1·7 (0·6)	..	..	..
Type of transfer					
Fresh	213 (40%)	188 (35%)	..	..	..
Frozen-only	299 (56%)	314 (59%)	..	..	..
Positive pregnancy test	254 (48%)	236 (44%)	3·4% (–2·8 to 9·6)	1·08 (0·94 to 1·23)	0·29
Clinical pregnancy	227 (43%)	212 (40%)	2·8% (–3·3 to 8·9)	1·07 (0·93 to 1·24)	0·38
Ongoing pregnancy	190 (36%)	174 (33%)	3·0% (–2·9 to 8·9)	1·09 (0·92 to 1·29)	0·33
Implantation rate††	284/971 (29%)	278/953 (29%)	0·0% (–4·1 to 4·2)	1·00 (0·95 to 1·06)	..
Maternal safety outcomes					
Moderate or severe ovarian hyperstimulation syndrome	7 (1%)	6 (1%)	0·2% (–1·3 to 1·7)	1·17 (0·39 to 3·45)	0·99
Pregnancy complications					
Ectopic pregnancy	10 (2%)	10 (2%)	0·% (–1·6 to 1·6)	1·00 (0·42 to 2·38)	0·99
Miscarriage	27 (5%)	28 (5%)	–0·2% (–3·0 to 2·7)	0·96 (0·58 to 1·61)	0·99
Twin pregnancy	57 (11%)	66 (13%)	–1·7% (–5·9 to 2·5)	0·87 (0·62 to 1·21)	0·44
Twin delivery	50 (9%)	51 (10%)	–0·2% (–3·9 to 3·5)	0·98 (0·68 to 1·42)	0·99

Data are n (%), median (IQR), mean (SD), or n/N (%), unless otherwise indicated. IVF=in-vitro fertilisation. *Risk ratios are for the intracytoplasmic sperm injection group compared with the conventional IVF group. †Natural conceptions were included (six in the intracytoplasmic sperm injection group and three in the conventional IVF group). ‡Denominator was the number of metaphase II oocytes, calculated as the number of oocytes retrieved – number of germinal vesicle oocytes – number of metaphase I oocytes. §Defined as the absence of any zygotes with two pronuclei at 16–18 h after injection or insemination. ¶Post-hoc analysis. ‖Defined by those with no embryo after failed fertilisation and those with no embryos due to embryo block on day 2. **Embryos were rated according to the Istanbul criteria, with good defined as grade I, cell number of 7–9, even cell size, less than 10% fragmentation, and no multinucleation. ††Denominator is the total number of embryos transferred.

Table 2: **Fertility outcomes and maternal safety after the first embryo transfer (intention-to-treat)**

(95% CI –7·6 to –5·1) and –5·2% (–6·5 to –4·4), respectively (table 2). 29 (5%) couples in the intracytoplasmic sperm injection group and 34 (6%) couples in the conventional IVF group had fertilisation failure (absolute difference –0·9%, 95% CI –4·0 to 2·1; RR 0·85, 95% CI 0·53 to 1·38; p=0·60; table 2). After the first embryo transfer from the initiated cycle, positive pregnancy test, clinical pregnancy, and ongoing pregnancy rates were not significantly different between intracytoplasmic sperm injection and conventional IVF (table 2). Obstetrics and perinatal outcomes were similar between the two groups (table 3).

At 12 months after random allocation, six (1%) couples in each group had not undergone embryo transfer because of divorce (two in the intracytoplasmic sperm injection group and one in the conventional IVF group) or patient preference (four in the intracytoplasmic sperm injection group and five in the conventional IVF group). Natural conception occurred in six (1%) couples in the intracytoplasmic sperm injection group and in three (1%) couples in the conventional IVF group. The number of couples undergoing one, two, three, or four embryo transfers from the first cycle in the intracytoplasmic sperm injection group was 512, 151, 28, and eight, respectively, with corresponding figures in the conventional IVF group of 502, 161, 26, and one, respectively (appendix p 7).

There were 222 (42%) cumulative pregnancies resulting in livebirth at 12 months after random allocation in the intracytoplasmic sperm infection group versus

	Intracytoplasmic sperm injection (n=532)	Conventional IVF (n=532)	Absolute difference (95% CI)	Risk ratio (95% CI)*	p value
Gestational diabetes	25 (5%)	27 (5%)	−0·4 (−3·2 to 2·4)	0·93 (0·54 to 1·57)	0·89
Hypertensive disorders of pregnancy	1 (<1%)	1 (<1%)	0·0 (−0·5 to 0·5)	1·00 (0·06 to 15·95)	0·99
Antepartum haemorrhage	0	0	..	..	..
Gestational age at delivery (weeks)	38·0 (2·0)	37·7 (2·4)	−2·3 (−0·8 to 0·1)	..	0·13
Preterm delivery					
Delivery at <24 weeks of gestation	6 (1%)	8 (2%)	−0·4 (−1·9 to 1·2)	0·75 (0·26 to 2·15)	0·79
Delivery at <28 weeks of gestation	8 (2%)	11 (2%)	−0·6 (−2·3 to 1·2)	0·73 (0·29 to 1·79)	0·65
Delivery at <32 weeks of gestation	11 (2%)	15 (3%)	−0·8 (−2·8 to 1·3)	0·73 (0·34 to 1·58)	0·55
Delivery at <37 weeks of gestation	26 (5%)	35 (7%)	−1·7 (−4·7 to 1·3)	0·74 (0·45 to 1·22)	0·29
Spontaneous preterm birth					
Delivery at <24 weeks of gestation	6 (1%)	8 (2%)	−0·4 (−1·9 to 1·2)	0·75 (0·26 to 2·15)	0·79
Delivery at <28 weeks of gestation	8 (2%)	11 (2%)	−0·6 (−2·3 to 1·2)	0·73 (0·29 to 1·79)	0·65
Delivery at <32 weeks of gestation	11 (2%)	15 (3%)	−0·8 (−2·8 to 1·3)	0·73 (0·34 to 1·58)	0·55
Delivery at <37 weeks of gestation	22 (4%)	34 (6%)	−2·3 (−5·1 to 0·6)	0·98 (0·95 to 1·00)	0·17
Iatrogenic preterm birth					
Delivery at <24 weeks of gestation	0	0	..	..	..
Delivery at <28 weeks of gestation	0	0	..	..	..
Delivery at <32 weeks of gestation	0	0	..	..	..
Delivery at <37 weeks of gestation	4 (1%)	1 (<1%)	0·6 (−0·4 to 1·6)	4·00 (0·45 to 35·67)	0·37
Birthweight (g)					
Singleton	3132·3 (571·5)	3206·2 (496·3)	−73·9 (−213·8 to 66·1)	..	0·29
Twins	2466·1 (440·3)	2400·8 (406·3)	65·3 (−57·5 to 188·1)	..	0·29
Low birthweight	10 (2%)	6 (1%)	0·8 (−0·9 to 2·4)	1·67 (0·61 to 4·55)	0·45
Very low birthweight	3 (1%)	1 (<1%)	0·4 (−0·5 to 1·3)	3·00 (0·31 to 28·75)	0·64
High birthweight	3 (1%)	2 (<1%)	0·2 (−0·8 to 1·2)	1·50 (0·25 to 8·94)	0·99
Very high birthweight	2 (<1%)	1 (<1%)	0·2 (−0·6 to 1·0)	2·00 (0·18 to 21·99)	0·99
Large for gestational age	8 (2%)	5 (1%)	0·6 (−0·9 to 2·1)	1·60 (0·53 to 4·86)	0·58
Small for gestational age	20 (4%)	30 (6%)	−1·9 (−4·6 to 0·8)	0·67 (0·38 to 1·16)	0·19
Fetal sex (male/female [%])†	116/118 (98%)	101/116 (87%)	11·2 (−6·6 to 12·7)	1·06 (0·89 to 1·27)	0·57
Neonatal complications					
Congenital anomaly	2 (<1%)	3 (1%)	−0·2 (−1·2 to 0·8)	0·67 (0·11 to 3·97)	0·99
Admission to neonatal intensive care unit	16 (3%)	19 (4%)	−0·6 (−2·9 to 1·8)	0·84 (0·44 to 1·62)	0·73

Data are n (%) or mean (SD), unless otherwise indicated. IVF=in-vitro fertilisation. *Risk ratios are for the intracytoplasmic sperm injection group versus the conventional IVF group. †Post-hoc analysis.

Table 3: **Obstetric and perinatal outcomes after the first embryo transfer (intention-to-treat)**

217 (41%) in the conventional IVF group (RR 1·02, 95% CI 0·89–1·18; p=0·80; appendix p 7). Time to ongoing pregnancy resulting in a livebirth at 12 months after random allocation did not differ between the two groups (HR 1·06, 95% CI 0·78–1·13; p=0·51; median 178 days [IQR 160–212] *vs* 180 days [171–218]; figure 2). Data for other secondary outcomes at 12 months after random allocation, from the initiated cycle, are reported in the appendix (p 8).

In the treatment-received analysis, we compared 571 couples who underwent intracytoplasmic sperm injection and 493 couples who underwent conventional IVF. Similar results were found for livebirth after the first embryo transfer (appendix p 9) or cumulative ongoing pregnancy resulting in livebirth at 12 months after random allocation (appendix pp 4, 11). Other outcomes were also similar between the two groups (appendix pp 9–10).

In a post-hoc analysis, data showed that couples undergoing intracytoplasmic sperm injection were less likely to have no embryo for transfer (absolute difference −2·4, 95% CI −4·6 to −0·3; RR 0·38, 95% CI 0·17 to 0·85; p=0·024; table 2). After the first embryo transfer from the initiated cycle, the livebirth rate was not materially affected by the IVF indications, trial centres, trigger regimens, sperm morphology, ovarian response categories, or total motile sperm count quartiles (appendix p 12).

Discussion

In this study, we found that in infertile couples in whom the male partner has a normal total sperm count and

motility, livebirth after the first embryo transfer and cumulative ongoing pregnancy resulting in livebirth at 12 months after random allocation from the initiated cycle were similar in couples undergoing intracytoplasmic sperm injection compared with conventional IVF, despite the risk of having no embryos to transfer being significantly lower in the intracytoplasmic sperm injection group.

Over the past 20 years, intracytoplasmic sperm injection has been widely implemented in clinical practice. The rationale for the use of this technique in couples with non-male factor infertility is based on the assumption that intracytoplasmic sperm injection might avoid unexpected total fertilisation failure; increase the fertilisation rate; generate the maximum cohort of embryos; and, thus, increase the chances of having a baby. However, the small number of randomised trials done did not support its use in such cases.[19] Our study showed similar numbers of livebirths and cumulative ongoing pregnancies resulting in livebirths with intracytoplasmic sperm injection and conventional IVF, consistent with a previous smaller randomised trial published in 2001[7] and supported by more recent cohort studies.[9–11] These findings address the research gap concerning comparison between intracytoplasmic sperm injection and conventional IVF identified in the latest committee opinion of the Practice Committee of the American Society for Reproductive Medicine and Society for Assisted Reproductive Technology, which stated that data on improved livebirth outcomes of intracytoplasmic sperm injection compared with conventional IVF are scarce or absent.[20]

There are some discrepancies in the fertilisation rate after intracytoplasmic sperm injection and conventional IVF between studies. Some studies showed a significantly lower fertilisation rate after intracytoplasmic sperm injection, even when analysed per oocyte retrieved,[6,7,9] whereas others reported no difference or a significantly higher fertilisation rate after intracytoplasmic sperm injection.[10,21] In our trial, we found a significantly higher rate of fertilisation when intracytoplasmic sperm injection was used compared with conventional IVF. 5% of couples undergoing intracytoplasmic sperm injection had total fertilisation failure, which is consistent with previous studies.[7,10] In our study, couples in the intracytoplasmic sperm injection group were at a lower risk of having no embryos for transfer compared with those in the conventional IVF group. However, this did not result in a significantly higher livebirth rate after the first embryo transfer or cumulative ongoing pregnancy resulting in livebirth rate from the initiated cycle, in line with previous studies.[9–11]

The safety of intracytoplasmic sperm injection remains of concern. We found no difference between the study groups in terms of the rate of congenital anomaly at birth. Pregnancy outcomes were also similar between the two groups. However, our trial was not powered for these outcomes. Recent evidence from large cohort studies suggests that intracytoplasmic sperm injection in couples with non-male factor infertility could increase the risk of congenital abnormalities in singletons (adjusted odds ratio 1·30, 95% CI 1·16–1·45),[22] although preterm birth was not indicated as a potential problem.[23] Recent data on puberty development and reproductive hormone status during adolescence in children born after intracytoplasmic sperm injection are reassuring;[24] however, long-term outcomes remain uncertain. Intracytoplasmic sperm injection in couples with non-male factor infertility might be associated with an increased risk of autism compared with conventional IVF (adjusted HR 1·57, 95% CI 1·18–2·09), although an underlying biological mechanism through which intracytoplasmic sperm injection could be associated with autism is not known.[25] The expanded use of intracytoplasmic sperm injection in couples with non-male factor infertility clearly shows a gap between clinical practice and evidence. More studies in a randomised setting are needed to support our findings. There are several ongoing randomised trials to compare intracytoplasmic sperm injection versus conventional IVF in couples with non-severe male factor infertility (NCT04128904 and NCT03298633). Hopefully, these studies, together with our findings, will add to guidance on the use of intracytoplasmic sperm injection in different populations. Future research should also consider patient-reported outcomes including quality of life, given the highly emotive context of assisted reproductive technology and the strong participant preferences. As well as the effectiveness and safety, the choice as to whether intracytoplasmic sperm injection or conventional IVF should be used in couples without male factor infertility

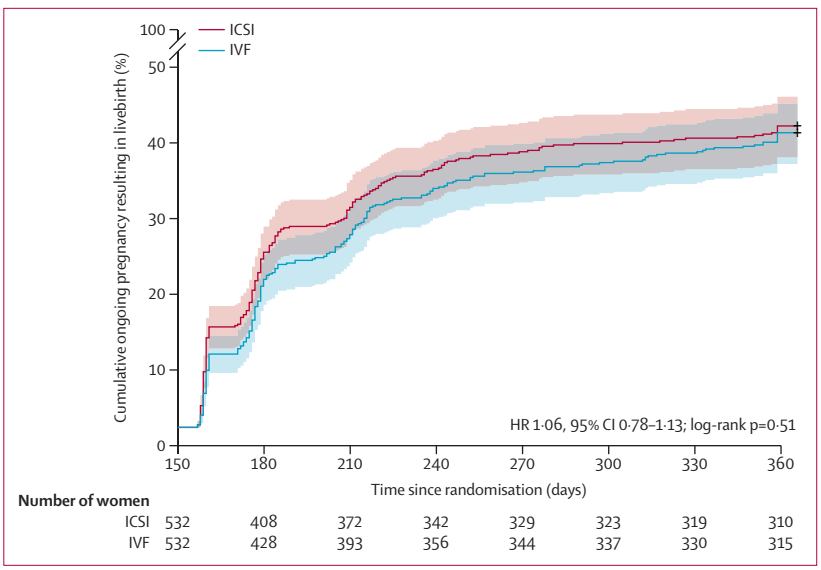

***Figure 2*: Kaplan-Meier graph of cumulative ongoing pregnancy resulting in livebirth (intention-to-treat population)**
ICSI=intracytoplasmic sperm injection. HR=hazard ratio. IVF=in-vitro fertilisation.

would also depend on costs. Expanded use of intracytoplasmic sperm injection generally increases the complexity and cost compared with conventional IVF because of the additional required laboratory experience, resources, effort, and time.[20] For example, an extra amount of at least £500 is needed for intracytoplasmic sperm injection compared with conventional IVF in the UK.[26] Moreover, intracytoplasmic sperm injection requires more resources for training of embryologists.

The strengths of our trial include its multicentre randomised design, the large sample size, and the zero loss to follow-up. However, some limitations also need to be considered. First, this trial involved infertile couples in whom the male partner has a normal total sperm count and motility, without any further male evaluation and sperm DNA fragmentation as supported by the European Association of Urology male infertility guidelines.[27] Second, most couples were young, with adequate ovarian reserves, around half had secondary infertility, and most couples were undergoing their first treatment attempt. Third, couples with a previous history of low fertilisation despite a normal semen analysis were excluded. All these issues might influence the external validity of our study. Fourth, embryo transfer was done on day 3, but there has been an increased trend for blastocyst transfer.[3] However, day 3 transfer is still practised in many parts of the world, according to the latest registry reports.[3] Further trials are needed to study whether or not the embryo stage at transfer impacts the effectiveness of intracytoplasmic sperm injection versus conventional IVF. Fifth, data on morphology of the semen samples obtained on the oocyte retrieval day were not available, although this was assessed at the first visit. However, there is evidence suggesting that sperm morphology, assessed by strict criteria, has little effect on treatment outcomes of intracytoplasmic sperm injection and conventional IVF[28,29] or intrauterine insemination.[30] Our post-hoc analysis, using morphology data obtained from the first semen sample, also showed a similar livebirth rate regardless of morphology. Sixth, crossover occurred in 41 couples randomised to conventional IVF due to patient preference. Although results from a treatment-received analysis are exploratory, our data were consistent with those obtained in the intention-to-treat analysis. The fact that only clinicians doing the embryo transfer were masked to study group allocation has the potential to introduce a source of treatment bias. Attempts to minimise this bias included performance of all interventions in the laboratory strictly adhering to standard operation procedures and similar patient management in both groups. Finally, our trial was powered to detect a 10% difference and, therefore, could not rule out a smaller difference. Nevertheless, intracytoplasmic sperm injection is unlikely to reach our hypothesised 10% increase in livebirth rate, as the upper CIs of both absolute difference and RR in this study did not exceed 10% in an absolute scale.

In conclusion, we found no significant improvement in livebirth or other pregnancy outcomes for intracytoplasmic sperm injection versus conventional IVF in couples with infertility in whom the male partner has normal total sperm count and motility. Given the additional cost and invasive nature of this technique, the routine use of intracytoplasmic sperm injection in assisted reproduction in this population should be questioned.

Contributors

The study was designed by BWM, VQD, LNV, RW, and RJN. Statistical analysis was done by TDP and QTP. The first draft of the manuscript was prepared by VQD, LNV, and BWM, who had unrestricted access to and verified the data. The manuscript was reviewed and edited by all the authors. VQD, QTP, TDP, BWM, and LNV had full access to all the data in the study. All authors had final responsibility for the decision to submit for publication and assume responsibility for the accuracy and completeness of the analyses and the fidelity of this report to the trial protocol.

Declaration of interests

LNV has received grant, speaker, and conference fees from Merck Sharp and Dohme, and grant, speaker, conference, and scientific board fees from Ferring. TMH has received speaker fees from Merck, Merck Sharp and Dohme, and Ferring. RJN receives grant funding from the National Health and Medical Research Council (NHMRC) of Australia. BWM has acted as a paid consultant to Merck, ObsEva and Guerbet, and is the recipient of money from an NHMRC Investigator Grant. All other authors declare no competing interests.

Data sharing

After publication, trial data will be made available on reasonable request to the corresponding author. A proposal with a detailed description of study objectives and a statistical analysis plan will be needed for assessment of requests. Additional materials might also be required during the process of assessment. Deidentified participant data will be provided after approval by the chief investigator and trial management group.

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