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In vitro fertilisation (IVF) versus intracytoplasmic sperm injection (ICSI) in patients without severe male factor infertility: study protocol for the randomised, controlled, multicentre trial INVICSI

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In vitro fertilisation (IVF) versus intracytoplasmic sperm injection (ICSI) in patients without severe male factor infertility: study protocol for the randomised, controlled, multicentre trial INVICSI

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INVICSI: study protocol for a randomised, controlled multicentre trial

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Abstract

Introduction: Over the last decades, the use of intracytoplasmic sperm injection (ICSI) has increased, even among patients without male factor infertility. The increase has happened despite the fact that there is no evidence to support that ICSI results in higher live birth rates compared to conventional in vitro fertilisation (IVF) in cases with non-male factor infertility. The lack of robust evidence on an advantage of using ICSI over conventional IVF in these patients is problematic since ICSI is more invasive, complex and requires additional resources, time and effort. Therefore, the primary objective of the IN VItro fertilisation versus IntraCytoplasmic Sperm Injection study (INVICSI) is to determine whether ICSI is superior to standard IVF in patients without severe male factor infertility. The primary outcome measure is first live birth from fresh and frozen-thawed transfers after one stimulated cycle.

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Methods and analysis: This is a two-armed, multicentre, randomised, controlled trial. Eight hundred and twenty-four participants with infertility without severe male factor will be recruited and allocated randomly into two groups (IVF or ICSI) in a 1:1 ratio. Participants will be randomised in variable block sizes and stratified by trial site and age. The main inclusion criteria are; (i) no prior IVF/ICSI treatment (ii) male partner sperm with an expected count of minimum 2 million progressive motile spermatozoa following density gradient purification on the day of oocyte pick-up (OPU) and (iii) age of the woman between 18 and 42 years.

Ethics and dissemination: The study will be performed in accordance with the ethical principles in the Helsinki Declaration. The study is approved by the Scientific Ethical Committee of the Capital Region of Denmark and the Danish Knowledge Centre on Data Protection Compliance. Study findings will be presented, irrespectively of results at international conferences and submitted for publication in peer-reviewed journals. ClinicalTrials.gov ID: NCT04128904

Strengths and limitations of this study

- This is a randomised controlled trial with concealment of treatment allocation, stratification for age and trial site and use of variable block sizes reducing the risk of selection bias and confounding.
- The large number of subjects included, and the multicentre approach of the study increases generalisability of the results.
- The primary outcome is first live birth episode ensuring maximum clinical impact.
- Only first cycle patients are included to avoid selection bias based on the knowledge of results from previous treatment cycles.

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- The study is not blinded neither to study participants nor clinicians which could potentially introduce bias.

Introduction

Since the introduction of ICSI in the early 1990's[1], the use of ICSI has continuously increased and it is now used widely for indications other than male factor infertility. The latest reports from the European Society of Human Reproduction and Embryology (ESHRE) and The International Committee Monitoring Assisted Reproductive Technologies (ICMART) show that in Europe and globally, ICSI is used in around two-thirds of all fresh assisted reproductive technology (ART) cycles[2, 3]. The ICMART report further accentuates the significant disparities that exists in ART practices across countries. An especially high ICSI:IVF ratio is found in the Middle East where the proportion of ICSI cycles in some countries is now 100% of all fresh cycles. It is unlikely that the large disparities between countries can be explained by differences in the prevalence of male factor infertility alone. In the United States (US), a recent study, including data from 2000-2014, showed a substantial increase (52% increase) in the use of ICSI with no corresponding increase in couples treated for male factor infertility[4]. Likewise, another US study found that the largest increase in the use of ICSI between 1996-2012 (from 36% in 1996 to 76% in 2012) was observed among couples without male factor infertility (from 15% to 67%)[5]. The observed increase has happened despite the fact that the use of ICSI for non-male factor infertility remains controversial[6]. While ICSI has resulted in high success rates in couples treated for severe male factor infertility, studies have indicated that ICSI offers no advantage over conventional IVF in non-male factor infertility couples when it comes to live birth rates[7-10]. Moreover, the American Society for Reproductive Medicine (ASRM) recently published a committee opinion stating that 'in cases without male factor infertility or a history of prior fertilisation failure, the routine use of ICSI

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for all oocytes is not supported by the available evidence'[11]. In the US study from 2018, the large increase in use of ICSI was correlated with a 7.6%, (P=0.001) increase in live birth rates per cycle in women younger than 35 years. When including only data from the most recent years (2008-2014) the correlation between ICSI rates and live birth rates disappeared questioning whether the ICSI method is responsible for the increased live birth rate [4]. The increased use of ICSI without the presence of male factor infertility could be attributed to a general belief that ICSI decreases the risk of fertilisation failure in patients treated for other indications. Indeed, a systematic review and meta-analysis from 2013 reported higher fertilisation rates and a lower risk of fertilisation failure after ICSI compared with conventional IVF in sibling oocytes from patients with unexplained infertility[12]. Yet, many of the included studies did not ascertain their findings with an improvement in clinical outcome (often due to mixed transfers of embryos from IVF and ICSI). Furthermore, other studies find no difference in fertilisation rates or comparable rates of fertilisation failure between the two methods[13-16]. Overall, there is a shortage of randomised controlled trials (RCTs) comparing ICSI and conventional IVF in patients without male factor infertility and the generalisability of findings from existing studies is limited[17]. In an RCT, including 415 patients with non-male factor infertility, comparable pregnancy rates between ICSI and conventional IVF were observed as well as higher fertilisation rates in the conventional IVF group[15]. Regrettably, live birth rate was not included as an outcome. A large cohort study, including 745 women aged 40 years or older, reported similar live birth rates after ICSI and conventional IVF as well as similar rates of fertilisation and fertilisation failure[7]. Likewise, ICSI does not seem to improve reproductive outcome in women with diminished ovarian reserve (compared to conventional IVF)[18, 19]. One group that might benefit from ICSI are non-male factor infertility patients with a history of total fertilisation failure (or low fertilisation)[20].

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In conclusion, there are still significant gaps in the knowledge regarding ICSI versus conventional IVF for couples with normal and non-severe male factor infertility. Especially when including considerations of cost (either for the individual patient or for the public health care system) and complexity of the methods.

The purpose of the INVICSI study is to address this knowledge gap and to infer whether ICSI is more effective than standard IVF in patients without severe male factor infertility. The primary outcome measure is first live birth.

Methods and analysis

Hypothesis

ICSI is superior to standard IVF for obtaining live birth of a child in fertility patients without severe male factor infertility.

Study design

The INVICSI study is a multicentre, randomised, controlled trial using a parallel arm design to detect whether ICSI is superior to standard IVF in patients without severe male factor infertility. Patients will be randomised (1:1) to receive insemination of their retrieved eggs with either standard IVF or ICSI. Trial registration data are displayed in Table 1. Table 2 provides an overview of revision chronology including current protocol date and version identifier. Protocol modifications are registered continuously on Clinical Trials.gov. The SPIRIT reporting guidelines were used[21].

Setting

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The trial will be conducted in six public fertility clinics in Denmark. All clinics are part of a university hospital setting and all hospitals perform standardised treatments according to the public health care system in Denmark. The teams recruiting patients at the trial sites will include fertility doctors, nursing staff and embryologists. Patient enrolment began in November 2019 and will continue until December 2023.

Eligibility criteria

All couples/women referred for their first fertility treatment at six public fertility clinics in Denmark are screened for eligibility with the following inclusion and exclusion criteria:

Inclusion:

a. Written informed consent
b. Age of the woman 18-42 years
c. Male partner with normal or non-severely decreased sperm parameters where the semen sample (following density gradient purification) on the day of OPU is expected to contain a minimum of 2 million progressive motile spermatozoa or use of donor sperm
d. Body-mass-index (BMI) of the woman between 18-35 kg/m^2
e. First fertility treatment due to:
    i. Tubal factor
    ii. Unexplained infertility
    iii. Polycystic ovary syndrome (PCOS)
    iv. Light to moderate decreased semen quality in the male partner

Exclusion:

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a. Consent not obtained

b. Significant morbidity in the woman:

    i. Ovarian cysts >4 cm

    ii. Known liver or kidney disease

    iii. Unregulated thyroid disease

    iv. Endometriosis stage 3-4

    v. Hypogonadotropic hypogonadism

    vi. Other severe comorbidity (e.g. diabetes or cardiovascular disease)

c. Previous IVF or ICSI treatments with current partner

d. Use of donor oocytes or frozen oocytes

e. Not speaking or understanding Danish or English language

Couples using sperm from the male partner as well as couples (or single women) using donor sperm are eligible. Subsequently, randomisation and inclusion will be based on data from the female participant receiving the ovarian stimulation treatment.

The study was originally designed and performed with the additional inclusion criteria of regular menstrual cycles (21-35 days) and a diagnostic sperm sample from the male partner with a minimum of 5 mill. progressive motile spermatozoa and ≥4% morphologically normal spermatozoa (Table 2). However, an amendment was added after the inclusion of 28 participants in May 2020. In this amendment, two of the aforementioned criteria were removed (regular menstrual cycle and minimum percentage of morphological normal sperm). The criterion for sperm morphology was removed because the importance of sperm morphology and whether it should be used to predict fertilisation

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and reproductive outcome in ART has been questioned [22-26]. The criterion for regular menstrual cycle was removed as current evidence suggests that women with PCOS have similar chances of conceiving with fertility treatment compared to women without PCOS[27-29].

In September 2019, the criterion for a diagnostic semen sample with a minimum of 5 mill. progressive motile spermatozoa was also removed (after the inclusion of 88 participants). Due to differences in laboratory techniques and standard tests performed prior to IVF/ICSI on the trial sites, it was not feasible to include a criterion for a diagnostic semen sample. The criterion for number of spermatozoa in the semen sample on the day of OPU remained unchanged.

Screening, inclusion and consent

Potentially eligible patients receive verbal and written information about the study by the investigators during a consultation in the fertility clinic. Inclusion and randomisation of participants to either ICSI or conventional IVF take place after the ovulation trigger has been prescribed and before the IVF/ICSI procedure. Couples/women who wish to participate in the trial are asked to sign an informed consent form prior to enrolment. They will usually have a minimum of two days between receiving the information and deciding whether they wish to participate in the study or not. When a patient has given consent and inclusion criteria are met, randomisation is conducted in the online platform REDCap, which is also used for data collection during the study[30]. The REDCap database has a complete audit trail and is based on anonymous subject ID numbers. It is not revealed whether the patient is assigned to standard IVF or ICSI until after the patient has been recruited and baseline data has been entered in REDCap ensuring treatment allocation concealment. Participants can withdraw from the trial at any time without giving an explanation, and their fertility treatment will not be affected.

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Randomisation

An independent statistician prepared the computer-generated randomisation scheme in a I:I ratio between the two arms (IVF and ICSI). Permuted blocks of variable size between 4 and 12 were used for randomisation. The randomisation scheme was stratified by trial site and female age (three age groups: 18-25 years of age, 26-37 years of age and 38-41 years of age) to ensure that the number of participants receiving IVF and ICSI is closely balanced within each stratum. The randomisation procedure is performed online in REDCap. The allocation table was uploaded in REDCap by the independent statistician and concealed from the clinical staff performing the randomisation. The unique Danish social security number of each participant is entered initially ensuring that no participants are randomised twice.

Poor semen sample on the day of OPU

If the semen sample contains less than 2 million progressive spermatozoa in the purified sample on the day of OPU, the woman/couple will be treated with ICSI regardless of allocation.

Blinding

The study is designed with no blinding of participants, clinicians or assessors. It was decided not to blind clinicians and participants as our experience shows that patients in the Danish fertility clinics are eager to know the insemination method used in their treatment. Hence, it was deemed unrealistic to recruit participants if allocation was only revealed after the endpoints were reached.

Intervention

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The participants will receive conventional IVF or ICSI treatment as determined by randomisation. Both treatments are part of standard treatment regimens at the trial sites.

The fertility treatment:

The women have been treated in either a short gonadotropin-releasing hormone (GnRH)-antagonist protocol or a long GnRH-agonist protocol for ovarian stimulation. Both the controlled ovarian stimulation, transvaginal ultrasound examinations and the ovulation triggering are done according to the usual daily practice at the trial sites with ovulation trigger prescribed when a minimum of two to three follicles measure 17 mm or more. Women with only one mature follicle may also be prescribed the ovulation trigger. OPU is performed 36±2 hours after the ovulation trigger is administered. Oocyte insemination will be IVF or ICSI according to randomisation, using established procedures at the trial sites. However, short time insemination in the IVF arm is not allowed. Embryo culture and luteal phase support will follow the usual procedures at each trial site. Blastocyst transfer is performed on day 5. Patients with a poor ovarian reserve and few oocytes retrieved (≤4) are allowed transfer day 2 or 3 according to clinical practice. Single embryo transfers are planned. Surplus blastocysts of good quality are vitrified on day 5 or 6. Transfer and cryopreservation are done according to usual practice at each trial site. In cases with total freeze of all blastocysts due to the risk of ovarian hyperstimulation syndrome (OHSS), women are not excluded from the trial. In cases where all blastocysts or spare blastocysts are vitrified these are transferred in subsequent frozen-thawed embryo transfer (FET) cycles according to the daily practice at each trial site (i.e., natural cycles, substituted or stimulated FET cycles).

Urine pregnancy test or a serum pregnancy test is done 11-16 days after embryo transfer. If pregnancy is achieved, a transvaginal ultrasound scan is performed at pregnancy week 7-9 to confirm an ongoing and intrauterine pregnancy.

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Women will be asked to inform the clinic of the result of the pregnancy as is the usual procedure in the clinic.

Study outcomes

Primary endpoint:

The primary endpoint for the INVICSI trial is the first live birth episode following the study cycle in each of the two groups (IVF and ICSI). This is defined as the first live birth from the oocyte collection and includes transfer of fresh embryos and frozen-thawed embryos. The minimum follow-up time will be one year after inclusion. Live birth is defined as the delivery of one or more living infants ≥22 weeks gestation. When the primary endpoint is achieved, further live births from the oocyte collection will not be included in the primary outcome analysis. Subsequent live births from any FET cycles with embryos from the first fresh cycle are included as a secondary outcome (all live birth episodes). The secondary outcomes are summarised in Table 3.

Data collection methods

Before treatment is initiated all fertility patients in the clinics fill out a standard form including data on fertility and medical history, ethnicity, medications, smoking, alcohol, height, weight etc. These data are routinely entered into electronic medical files of the fertility clinics by fertility doctors prior to the patients first consultation in the clinic. This is part of standard practice for all fertility patients. For the INVICSI study, baseline data will be gathered by the investigators from the electronic files after written informed consent has been given (age, weight, height, ethnicity, antral follicle count (AFC), anti-müllerian hormone (AMH) concentration, years of infertility, primary or secondary infertility, infertility diagnosis, stimulation protocol, sperm characteristics). Data will then be entered

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into REDCap after which the randomisation and allocation to either standard IVF or ICSI will occur. Data on treatment outcome including fertilisation, embryo development, pregnancy and pregnancy loss (secondary outcomes, Table 3) will be collected and entered in REDCap. The couple/woman is asked to consent to data being obtained from the child's file in case the fertility treatment results in the birth of a living child.

To ensure data collection, an investigator will follow-up on all participants that obtains pregnancy. Follow-up will take place one year after the ultrasound scan (week 7-9). If the participant has informed the fertility clinic on birth and child, an investigator will contact the participant via a phone call or retrieve all information from the electronic patient record.

Statistical considerations

Proposed sample size:

The rate of first live births after transfer of up to all of the transferable embryos from the first OPU is set to 45% in the conventional IVF group and 55 % in the ICSI group. This is a superiority trial with a power of 80% and a 2-sided p-value of 5%. The sample size is estimated to be 392 patients in each group. Post-randomization exclusion is expected to be 5%, resulting in a total of 824 patients.

Data analysis:

ITT analysis and per-protocol analysis will be performed. Baseline characteristics and outcomes will be compared using t-test, Mann-Whitney U test or chi-square tests for continuous and categorical variables or logistic regression analysis, controlling for possible confounding effects where appropriate. P-values of <0.05 will be considered statistically significant. Statistical analyses will be performed by an investigator together with statistical experts. The primary RCT analysis will be performed by an independent statistician blinded to group allocation.

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Patient and Public Involvement

There has been no patient or public involvement in the development of study design, recruitment or research question.

Ethics and dissemination

Data security and ethical aspects

Data to describe the study population and the outcomes will be collected in a single database including all participants with an identification code, which makes every participant anonymous in the database.

The study is approved by the Scientific Ethical Committee of the Capital Region of Denmark (H-19022201) and the Danish Knowledge Centre on Data Protection Compliance. The study will be performed according to the Danish Law and Ethical principles in the Helsinki Declaration. Each participant will receive oral and written information about the study and will have opportunity for time and reflection. They can also discuss their participation with a third person. The collected oocytes of the participants will be fertilised with IVF or ICSI according to randomisation. Some couples/women may experience no fertilisation after either IVF or ICSI in the study. This risk is not considered significantly higher compared to women who do not participate in the study. The study is registered with the National Institute of Health's ClinicalTrials.gov (NCT04128904).

Dissemination

The findings of the study will be presented at national and international fertility conferences, such as the European Society of Human Reproduction and Embryology (ESHRE) annual meeting. In

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addition, the findings will be published in peer reviewed scientific journals. Public dissemination will be in the lay press.

Data-sharing

Data from the trial will be shared according to the ICJME guidelines. On request, data can be shared with parties presenting relevant aims for the use of data. Purposes and financial aspects of the other party must be approved by the steering committee of the "INVICSI" research team. No data will be shared until three months after the publication of papers reporting the primary and secondary outcomes of the trial. Any new research project must be approved by Danish authorities. The requesting party cover the costs for data sharing.

Discussion

Worldwide, the rate of treatment cycles where oocytes are fertilised with ICSI is increasing, also in patients without severe male factor infertility. Currently there is no evidence to support that ICSI results in a higher live birth rate compared to standard IVF in these patients. If the INVICSI study finds that ICSI is superior to standard IVF in cases without severe male factor infertility, the increasing use of ICSI is justified and may then be recommended. However, if the INVICSI study fails to show superiority of ICSI, standard IVF should be recommended as the preferred first choice method of fertilisation in patients without severe male factor infertility. This could potentially lead to significant cost savings and a higher use of standard IVF which is less invasive, closer to natural fertilisation and less expensive.

Authors´ contributions:

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SB and NCF were responsible for the conception, design and execution of the study protocol. SB, NCF and AP contributed to the initial revision and editing of the manuscript. AZ was consulted concerning the laboratory details of the study design. SB, NCF, AP, AZ, ALME, UBK, MRP, LFA, BN, HSN, LP, and MLG contributed to the critical revision of the manuscript as well as the approval of the final version for submission in BMJ Open.

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Competing interests:

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References:

1. Palermo G, Joris H, Devroey P, et al. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*. 1992;340(8810):17-8.

2. De Geyter C, Calhaz-Jorge C, Kupka MS, et al. ART in Europe, 2015: results generated from European registries by ESHRE. *Hum Reprod Open*. 2020;2020(1):hoz038.

3. de Mouzon J, Chambers GM, Zegers-Hochschild F, et al. International Committee for Monitoring Assisted Reproductive Technologies world report: assisted reproductive technology 2012dagger. *Hum Reprod*. 2020;35(8):1900-13.
4. Zagadailov P, Hsu A, Stern JE, et al. Temporal Differences in Utilization of Intracytoplasmic Sperm Injection Among U.S. Regions. *Obstet Gynecol*. 2018;132(2):310-20.
5. Boulet SL, Mehta A, Kissin DM, et al. Trends in use of and reproductive outcomes associated with intracytoplasmic sperm injection. *JAMA*. 2015;313(3):255-63.
6. Evers JL. Santa Claus in the fertility clinic. *Hum Reprod*. 2016;31(7):1381-2.
7. Tannus S, Son WY, Gilman A, et al. The role of intracytoplasmic sperm injection in non-male factor infertility in advanced maternal age. *Hum Reprod*. 2017;32(1):119-24.
8. Gennarelli G, Carosso A, Canosa S, et al. ICSI Versus Conventional IVF in Women Aged 40 Years or More and Unexplained Infertility: A Retrospective Evaluation of 685 Cycles with Propensity Score Model. *J Clin Med*. 2019;8(10).
9. Sfontouris IA, Kolibianakis EM, Lainas GT, et al. Live birth rates using conventional in vitro fertilization compared to intracytoplasmic sperm injection in Bologna poor responders with a single oocyte retrieved. *J Assist Reprod Genet*. 2015;32(5):691-7.
10. Li Z, Wang AY, Bowman M, et al. ICSI does not increase the cumulative live birth rate in non-male factor infertility. *Hum Reprod*. 2018;33(7):1322-30.
11. Practice Committees of the American Society for Reproductive M, the Society for Assisted Reproductive Technology. Electronic address aao. Intracytoplasmic sperm injection (ICSI) for non-male factor indications: a committee opinion. *Fertil Steril*. 2020;114(2):239-45.
12. Johnson LN, Sasson IE, Sammel MD, et al. Does intracytoplasmic sperm injection improve the fertilization rate and decrease the total fertilization failure rate in couples with well-defined unexplained infertility? A systematic review and meta-analysis. *Fertil Steril*. 2013;100(3):704-11.
13. Vitek WS, Galarraga O, Klatsky PC, et al. Management of the first in vitro fertilization cycle for unexplained infertility: a cost-effectiveness analysis of split in vitro fertilization-intracytoplasmic sperm injection. *Fertil Steril*. 2013;100(5):1381-8.
14. Kim HH, Bundorf MK, Behr B, et al. Use and outcomes of intracytoplasmic sperm injection for non-male factor infertility. *Fertil Steril*. 2007;88(3):622-8.
15. Bhattacharya S, Hamilton MP, Shaaban M, et al. Conventional in-vitro fertilisation versus intracytoplasmic sperm injection for the treatment of non-male-factor infertility: a randomised controlled trial. *Lancet*. 2001;357(9274):2075-9.
16. Ruiz A, Remohi J, Minguez Y, et al. The role of in vitro fertilization and intracytoplasmic sperm injection in couples with unexplained infertility after failed intrauterine insemination. *Fertil Steril*. 1997;68(1):171-3.
17. van Rumste MM, Evers JL, Farquhar CM. Intra-cytoplasmic sperm injection versus conventional techniques for oocyte insemination during in vitro fertilisation in patients with non-male subfertility. *Cochrane Database Syst Rev*. 2003(2):CD001301.
18. Butts SF, Owen C, Mainigi M, et al. Assisted hatching and intracytoplasmic sperm injection are not associated with improved outcomes in assisted reproduction cycles for diminished ovarian reserve: an analysis of cycles in the United States from 2004 to 2011. *Fertil Steril*. 2014;102(4):1041-7 e1.
19. Luna M, Bigelow C, Duke M, et al. Should ICSI be recommended routinely in patients with four or fewer oocytes retrieved? *J Assist Reprod Genet*. 2011;28(10):911-5.
20. van der Westerlaken L, Helmerhorst F, Dieben S, et al. Intracytoplasmic sperm injection as a treatment for unexplained total fertilization failure or low fertilization after conventional in vitro fertilization. *Fertil Steril*. 2005;83(3):612-7.
21. Chan AW, Tetzlaff JM, Gotzsche PC, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ*. 2013;346:e7586.

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22. Kohn TP, Kohn JR, Ramasamy R. Effect of Sperm Morphology on Pregnancy Success via Intrauterine Insemination: A Systematic Review and Meta-Analysis. *J Urol*. 2018;199(3):812-22.
23. Kohn TP, Kohn JR, Lamb DJ. Role of Sperm Morphology in Deciding Between Various Assisted Reproduction Technologies. *Eur Urol Focus*. 2018;4(3):311-3.
24. Lemmens L, Kos S, Beijer C, et al. Predictive value of sperm morphology and progressively motile sperm count for pregnancy outcomes in intrauterine insemination. *Fertil Steril*. 2016;105(6):1462-8.
25. Deveneau NE, Sinno O, Krause M, et al. Impact of sperm morphology on the likelihood of pregnancy after intrauterine insemination. *Fertil Steril*. 2014;102(6):1584-90 e2.
26. Hotaling JM, Smith JF, Rosen M, et al. The relationship between isolated teratozoospermia and clinical pregnancy after in vitro fertilization with or without intracytoplasmic sperm injection: a systematic review and meta-analysis. *Fertil Steril*. 2011;95(3):1141-5.
27. Sha T, Wang X, Cheng W, et al. A meta-analysis of pregnancy-related outcomes and complications in women with polycystic ovary syndrome undergoing IVF. *Reprod Biomed Online*. 2019;39(2):281-93.
28. Sigala J, Sifer C, Dewailly D, et al. Is polycystic ovarian morphology related to a poor oocyte quality after controlled ovarian hyperstimulation for intracytoplasmic sperm injection? Results from a prospective, comparative study. *Fertil Steril*. 2015;103(1):112-8.
29. Heijnen EM, Eijkemans MJ, Hughes EG, et al. A meta-analysis of outcomes of conventional IVF in women with polycystic ovary syndrome. *Hum Reprod Update*. 2006;12(1):13-21.
30. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-81.

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INVICSI: study protocol for a randomised, controlled multicentre trial

Version: 4.0

Date: March 10, 2021

Table 1. Trial registration data

Data category	Information
Primary registry and trial identifying number	ClinicalTrials.gov ID: NCT04128904, Protocol ID: INVICSI2019
Date of registration in primary registry	July 10, 2019
Secondary identifying numbers	H-19022201
Source(s) of monetary or material support	Capital Region of Denmark Gedeon Richter
Primary sponsor	Copenhagen University Hospital Hvidovre
Secondary sponsor(s)	None
Contact for public queries	SB (sineberntsen@gmail.com)
Contact for scientific queries	SB, NCF Department of Obstetrics and Gynaecology The Fertility Clinic, Hvidovre Copenhagen University Hospital Hvidovre
Public title	INVICSI – IVF versus ICSI in patients without severe male factor infertility
Scientific title	In vitro fertilisation (IVF) versus intracytoplasmic sperm injection (ICSI) in patients without severe male factor infertility (INVICSI): a randomised, controlled, multicentre trial
Countries of recruitment	Denmark
Health condition(s) or problem(s) studied	Methods of insemination (ICSI vs. conventional IVF), Infertility without severe male factor
Intervention(s)	Active comparator: Insemination with ICSI
	Active comparator: Insemination with conventional IVF
Key inclusion and exclusion criteria	Inclusion: Age of the woman 18-42 years, BMI of the woman between 18-35 kg/m2, Male partner with normal or non-severely decreased sperm parameters or use of donor sperm
	Exclusion: Previous IVF or ICSI treatments with current partner, Use of donor oocytes or frozen oocytes, Ovarian cysts >4 cm, Known liver or kidney disease, Unregulated thyroid disease, Endometriosis stage 3-4, Hypogonadotropic hypogonadism, Other severe comorbidity (e.g. diabetes or cardiovascular disease)
Study type	Randomised controlled multicenter trial using a parallel arm design. Randomisation 1:1 to receive insemination with ICSI or conventional IVF
Date of first enrolment	November 29, 2019
Target sample size	824
Recruitment status	Recruiting

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Primary outcome(s)	First live birth rate: the number of first live birth episodes from the study oocyte collections including transfer of fresh- and frozen-thawed embryos
Key secondary outcomes	Cycles with total fertilisation failure, fertilisation rate, embryo quality, positive pregnancy test rate, ongoing pregnancy rate, pregnancy loss rate, all live birth episodes, preterm delivery, birth weight and congenital anomalies

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Table 2. Protocol, Revision chronology

Version	Date of approval	Primary reasons for amendment
Original	August 8, 2019	
Amendment 1	January 28, 2020	New trial site added (The Fertility Clinic, Regional Hospital Horsens)
Amendment 2	March 20, 2020	Removed inclusion criteria: (i) Regular menstrual cycles (21-35 days). (ii) Diagnostic sperm sample from the male partner with ≥4% morphologically normal spermatozoa Added section: Handling of poor semen sample on the day of OPU
Amendment 3	September 2, 2020	New trial site added (The Fertility Clinic, Zealand University Hospital)

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Amendment 4 (Current version)	September 16, 2020	Removed inclusion criteria: Treatment with donor sperm or male partner sperm with a minimum concentration of 5 million progressive motile spermatozoa in a (purified) *diagnostic* semen sample. Added inclusion criteria: Male partner with normal or non-severely decreased sperm parameters where the sperm sample (purified) on the day of oocyte pick up is expected to contain a minimum of 2 million progressive spermatozoa.

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Table 3. Secondary outcomes

Outcome	Assessment
Fertilisation	**Fertilisation rate** per aspirated oocyte retrieved (16-20 hours after IVF/ICSI) defined as the appearance of 2 pronuclei (PN).
	Cycles with total **fertilisation failure.**
Embryo data	**Embryo quality** (i.e. good quality blastocysts according to Gardner classification).
	Embryo time-lapse kinetics including cleavage patterns.
	Embryo utilisation rate (number of transferred + cryopreserved embryos per number of 2 PN zygotes).
Freeze	Number of **frozen blastocysts** (time frame: up to six days after oocyte pick-up (OPU)).
Pregnancy	**Positive pregnancy test** (positive urine or serum hCG 11-21 days after embryo transfer).
	Multiple pregnancy (period: up to 12 weeks after embryo transfer). Number of intrauterine gestations.
	Ongoing pregnancy per transfer (fetal heartbeat on ultrasound in gestational week 7-8).
Miscarriage	**Pregnancy loss rate** (period: up to 12 weeks after embryo transfer).

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	Biochemical pregnancies (positive urine or serum hCG 11-21 days after embryo transfer without any clinical signs of intra- or extrauterine pregnancy).
	Ectopic pregnancy/pregnancy of unknown location **(PUL)**
Birth/offspring	**All live birth episodes** (all live births from the study oocyte collection (including second and further live births)
	Preterm delivery (delivery at gestational week 22-36+6).
	Birth weight/weight for gestational age.
	Congenital anomaly diagnosed at birth.

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered,	2

		name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	14
Protocol version	#3	Date and version identifier	15
Funding	#4	Sources and types of financial, material, and other support	11
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1, 10
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	14
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	11
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n/a

Introduction

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Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	2-4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	2-4
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg,	4-5

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		surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7-8
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	7
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	n/a
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8,16
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6

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Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	n/a
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	6
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6

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Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n/a
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	8-9
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	n/a
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values).	8-9

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		Reference to where details of data management procedures can be found, if not in the protocol	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	9
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	n/a
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	n/a
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial	n/a

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		conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	9-10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	4
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	4
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	9
Declaration of	#28	Financial and other competing interests for principal	11

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interests		investigators for the overall trial and each study site	
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	10
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	n/a
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

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BMJ Open

In vitro fertilisation (IVF) versus intracytoplasmic sperm injection (ICSI) in patients without severe male factor infertility: study protocol for the randomised, controlled, multicentre trial INVICSI

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Primary Subject Heading:	Reproductive medicine
Secondary Subject Heading:	Obstetrics and gynaecology

Keywords:	Subfertility < GYNAECOLOGY, Male infertility < UROLOGY, Reproductive medicine < GYNAECOLOGY, SEXUAL MEDICINE

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Version: 5.0
Date: May 06, 2021

In vitro fertilisation (IVF) versus intracytoplasmic sperm injection (ICSI) in patients without severe male factor infertility: study protocol for the randomised, controlled, multicentre trial INVICSI

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INVICSI: study protocol for a randomised, controlled multicentre trial

Version: 5.0

Date: May 06, 2021

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Abstract

Introduction: Over the last decades, the use of intracytoplasmic sperm injection (ICSI) has increased, even among patients without male factor infertility. The increase has happened even though there is no evidence to support that ICSI results in higher live birth rates compared to conventional in vitro fertilisation (IVF) in cases with non-male factor infertility. The lack of robust evidence on an advantage of using ICSI over conventional IVF in these patients is problematic since ICSI is more invasive, complex and requires additional resources, time and effort. Therefore, the primary objective of the IN VItro fertilisation versus IntraCytoplasmic Sperm Injection study (INVICSI) is to determine whether ICSI is superior to standard IVF in patients without severe male factor infertility. The primary outcome measure is first live birth from fresh and frozen-thawed

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transfers after one stimulated cycle. Secondary outcomes include fertilisation rate, ongoing pregnancy rate, birthweight and congenital anomalies.

Methods and analysis: This is a two-armed, multicentre, randomised, controlled trial. In total, 824 with infertility without severe male factor will be recruited and allocated randomly into two groups (IVF or ICSI) in a 1:1 ratio. Participants will be randomised in variable block sizes and stratified by trial site and age. The main inclusion criteria are; (i) no prior IVF/ICSI treatment (ii) male partner sperm with an expected count of minimum 2 million progressive motile spermatozoa following density gradient purification on the day of oocyte pick-up (OPU) and (iii) age of the woman between 18 and 42 years.

Ethics and dissemination: The study will be performed in accordance with the ethical principles in the Helsinki Declaration. The study is approved by the Scientific Ethical Committee of the Capital Region of Denmark. Study findings will be presented, irrespectively of results at international conferences and submitted for publication in peer-reviewed journals. ClinicalTrials.gov ID: NCT04128904

Strengths and limitations of this study

- This is a randomised controlled trial with concealment of treatment allocation, stratification for age and trial site and use of variable block sizes reducing the risk of selection bias and confounding.
- The large number of subjects included, and the multicentre approach of the study increases generalisability of the results.
- The primary outcome is first live birth episode ensuring maximum clinical impact.

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- Only first cycle patients are included to avoid selection bias based on the knowledge of results from previous treatment cycles.
- The study is not blinded neither to study participants nor clinicians which could potentially introduce bias.

Introduction

Since the introduction of ICSI in the early 1990's[1], the use of ICSI has continuously increased and it is now used widely for indications other than male factor infertility. The latest reports from the European Society of Human Reproduction and Embryology (ESHRE) and The International Committee Monitoring Assisted Reproductive Technologies (ICMART) show that in Europe and globally, ICSI is used in around two-thirds of all fresh assisted reproductive technology (ART) cycles[2, 3]. The ICMART report further accentuates the significant disparities that exists in ART practices across countries. An especially high ICSI:IVF ratio is found in the Middle East where the proportion of ICSI cycles in some countries is now 100% of all fresh cycles. It is unlikely that the large disparities between countries can be explained by differences in the prevalence of male factor infertility alone. In the United States (US), a recent study, including data from 2000-2014, showed a substantial increase (52% increase) in the use of ICSI with no corresponding increase in couples treated for male factor infertility[4]. Likewise, another US study found that the largest increase in the use of ICSI between 1996-2012 (from 36% in 1996 to 76% in 2012) was observed among couples without male factor infertility (from 15% to 67%)[5]. The observed increase has happened despite the fact that the use of ICSI for non-male factor infertility remains controversial[6]. While ICSI has resulted in high success rates in couples treated for severe male factor infertility, studies have indicated that ICSI offers no advantage over conventional IVF in non-

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male factor infertility couples when it comes to live birth rates[7-11]. Moreover, the American Society for Reproductive Medicine (ASRM) recently published a committee opinion stating that 'in cases without male factor infertility or a history of prior fertilisation failure, the routine use of ICSI for all oocytes is not supported by the available evidence'[12]. In the US study from 2018, the large increase in use of ICSI was correlated with a 7.6%, (P=0.001) increase in live birth rates per cycle in women younger than 35 years. When including only data from the most recent years (2008-2014) the correlation between ICSI rates and live birth rates disappeared questioning whether the ICSI method is responsible for the increased live birth rate [4]. The increased use of ICSI without the presence of male factor infertility could be attributed to a general belief that ICSI decreases the risk of fertilisation failure in patients treated for other indications. Indeed, a systematic review and meta-analysis from 2013 reported higher fertilisation rates and a lower risk of fertilisation failure after ICSI compared with conventional IVF in sibling oocytes from patients with unexplained infertility[13]. Yet, many of the included studies did not ascertain their findings with an improvement in clinical outcome (often due to mixed transfers of embryos from IVF and ICSI). Furthermore, other studies find no difference in fertilisation rates or comparable rates of fertilisation failure between the two methods[14-18]. Overall, there is a shortage of randomised controlled trials (RCTs) comparing ICSI and conventional IVF in patients without male factor infertility and the generalisability of findings from existing studies is limited[19]. In an RCT, including 415 patients with non-male factor infertility, comparable pregnancy rates between ICSI and conventional IVF were observed as well as higher fertilisation rates in the conventional IVF group[16]. Regrettably, live birth rate was not included as an outcome. A large cohort study, including 745 women aged 40 years or older, reported similar live birth rates after ICSI and conventional IVF as well as similar rates of fertilisation and fertilisation failure[7]. Likewise, ICSI does not seem to improve

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reproductive outcome in women with diminished ovarian reserve (compared to conventional IVF)[20, 21]. One group that might benefit from ICSI are non-male factor infertility patients with a history of total fertilisation failure (or low fertilisation)[22].

In conclusion, there are still significant gaps in the knowledge regarding ICSI versus conventional IVF for couples with normal and non-severe male factor infertility. Especially when including considerations of cost (either for the individual patient or for the public health care system) and complexity of the methods.

The purpose of the INVICSI study is to address this knowledge gap and to infer whether ICSI is more effective than standard IVF in patients without severe male factor infertility. The primary outcome measure is first live birth.

Methods and analysis

Hypothesis

ICSI is superior to standard IVF for obtaining live birth of a child in fertility patients without severe male factor infertility.

Study design

The INVICSI study is a multicentre, randomised, controlled trial using a parallel arm design to detect whether ICSI is superior to standard IVF in patients without severe male factor infertility. Patients will be randomised (1:1) to receive insemination of their retrieved eggs with either standard IVF or ICSI. Trial registration data are displayed in Table 1. Table 2 provides an overview of revision chronology including current protocol date and version identifier. Protocol modifications are registered continuously on Clinical Trials.gov. The SPIRIT reporting guidelines were used[23].

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Setting

The trial will be conducted in six public fertility clinics in Denmark. All clinics are part of a university hospital setting and all hospitals perform standardised treatments according to the public health care system in Denmark. The teams recruiting patients at the trial sites will include fertility doctors, nursing staff and embryologists. Patient enrolment began in November 2019 and will continue until December 2023.

Eligibility criteria

All couples/women referred for their first fertility treatment at six public fertility clinics in Denmark are screened for eligibility with the following inclusion and exclusion criteria:

Inclusion:

a. Written informed consent

b. Age of the woman 18-42 years

c.

   i. Male partner with normal or non-severely decreased sperm parameters where the semen sample (following density gradient purification) on the day of OPU is expected to contain a minimum of 2 million progressive motile spermatozoa

   ii. Couples/singles using donor sperm

d. Body-mass-index (BMI) of the woman between 18-35 kg/m^2

e. First fertility treatment due to:

   i. Tubal factor

   ii. Unexplained infertility

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iii. Polycystic ovary syndrome (PCOS)

iv. Light to moderate decreased semen quality in the male partner

Exclusion:

a. Consent not obtained

b. Significant morbidity in the woman:

i. Ovarian cysts >4 cm

ii. Known liver or kidney disease

iii. Unregulated thyroid disease

iv. Endometriosis stage 3-4

v. Hypogonadotropic hypogonadism

vi. Other severe comorbidity (e.g. diabetes or cardiovascular disease)

c. Previous IVF or ICSI treatments with current partner

d. Use of donor oocytes or frozen oocytes

e. Not speaking or understanding Danish or English language

Couples using sperm from the male partner as well as couples (or single women) using donor sperm are eligible. Subsequently, randomisation and inclusion will be based on data from the female participant receiving the ovarian stimulation treatment.

The study was originally designed and performed with the additional inclusion criteria of regular menstrual cycles (21-35 days) and a diagnostic sperm sample from the male partner with a minimum of 5 mill. progressive motile spermatozoa and ≥4% morphologically normal spermatozoa (Table 2).

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However, an amendment was added after the inclusion of 28 participants in May 2020. In this amendment, two of the aforementioned criteria were removed (regular menstrual cycle and minimum percentage of morphological normal sperm). The criterion for sperm morphology was removed because the importance of sperm morphology and whether it should be used to predict fertilisation and reproductive outcome in ART has been questioned [24-28]. The criterion for regular menstrual cycle was removed as current evidence suggests that women with PCOS have similar chances of conceiving with fertility treatment compared to women without PCOS[29-31].

In September 2019, the criterion for a diagnostic semen sample with a minimum of 5 mill. progressive motile spermatozoa was also removed (after the inclusion of 88 participants). Due to differences in laboratory techniques and standard tests performed prior to IVF/ICSI on the trial sites, it was not feasible to include a criterion for a diagnostic semen sample. The criterion for number of spermatozoa in the semen sample on the day of OPU remained unchanged.

Screening, inclusion and consent

Potentially eligible patients receive verbal and written information about the study by the investigators during a consultation in the fertility clinic. Inclusion and randomisation of participants to either ICSI or conventional IVF take place after the ovulation trigger has been prescribed and before the oocyte collection. This is to avoid the risk of the allocation group (IVF or ICSI) affecting the clinicians' choice when deciding the dose of the follicle stimulating hormone as well as the timing (or cancellation) of oocyte collection. Also, this ensures that the decision for inclusion is not based on the number of oocytes collected. Couples/women who wish to participate in the trial are asked to sign an informed consent form prior to enrolment. They will usually have a minimum of two days between receiving the information and deciding whether they wish to participate in the

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study or not. When a patient has given consent and inclusion criteria are met, randomisation is conducted in the online platform REDCap, which is also used for data collection during the study[32]. The REDCap database has a complete audit trail and is based on anonymous subject ID numbers. It is not revealed whether the patient is assigned to standard IVF or ICSI until after the patient has been recruited and baseline data has been entered in REDCap ensuring treatment allocation concealment. Participants can withdraw from the trial at any time without giving an explanation, and their fertility treatment will not be affected.

Randomisation

An independent statistician prepared the computer-generated randomisation scheme in a I:I ratio between the two arms (IVF and ICSI). Permuted blocks of variable size between 4 and 12 were used for randomisation. The randomisation scheme was stratified by trial site and female age (three age groups: 18-25 years of age, 26-37 years of age and 38-41 years of age) to ensure that the number of participants receiving IVF and ICSI is closely balanced within each stratum. The randomisation procedure is performed online in REDCap. The allocation table was uploaded in REDCap by the independent statistician and concealed from the clinical staff performing the randomisation. The unique Danish social security number of each participant is entered initially ensuring that no participants are randomised twice.

Poor semen sample on the day of OPU

If the purified semen sample contains less than 2 million progressive spermatozoa on the day of OPU, the woman/couple will be treated with ICSI regardless of allocation.

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Blinding

The study is designed with no blinding of participants, clinicians or assessors. It was decided not to blind clinicians and participants as our experience shows that patients in the Danish fertility clinics are eager to know the insemination method used in their treatment. Hence, it was deemed unrealistic to recruit participants if allocation was only revealed after the endpoints were reached.

Intervention

The participants will receive conventional IVF or ICSI treatment as determined by randomisation. Both treatments are part of standard treatment regimens at the trial sites.

The fertility treatment:

The women have been treated in either a short gonadotropin-releasing hormone (GnRH)-antagonist protocol or a long GnRH-agonist protocol for ovarian stimulation. Both the controlled ovarian stimulation, transvaginal ultrasound examinations and the ovulation triggering are done according to the usual daily practice at the trial sites with ovulation trigger prescribed when a minimum of two to three follicles measure 17 mm or more. Women with only one mature follicle may also be prescribed the ovulation trigger. OPU is performed 36±2 hours after the ovulation trigger is administered. On the day of OPU the concentrations of all spermatozoa and progressive motile spermatozoa are assessed in the ejaculate. Following density gradient purification, wash steps and resuspension in 1 mL media the number of all spermatozoa as well as the number of progressive motile spermatozoa are assessed again. In cases with a high concentration of spermatozoa in the ejaculate it is allowed to purify only part of the sample. In this case, a theoretical (after purification) total yield is calculated.

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Oocyte insemination will be IVF or ICSI according to randomisation, using established procedures at the trial sites. However, short time insemination in the IVF arm is not allowed. In case of total fertilisation failure, rescue ICSI is not performed. Embryo culture and luteal phase support will follow the usual procedures at each trial site. Blastocyst transfer is performed on day 5. Patients with a poor ovarian reserve and few oocytes retrieved (≤4) are allowed transfer day 2 or 3 according to clinical practice. Single embryo transfers are planned. Surplus blastocysts of good quality are vitrified on day 5 or 6. Transfer and cryopreservation are done according to usual practice at each trial site. In cases with total freeze of all blastocysts due to the risk of ovarian hyperstimulation syndrome (OHSS), women are not excluded from the trial. In cases where all blastocysts or spare blastocysts are vitrified these are transferred in subsequent frozen-thawed embryo transfer (FET) cycles according to the daily practice at each trial site (i.e., natural cycles, substituted or stimulated FET cycles).

Urine pregnancy test or a serum pregnancy test is done 11-16 days after embryo transfer. If pregnancy is achieved, a transvaginal ultrasound scan is performed at pregnancy week 7-9 to confirm an ongoing and intrauterine pregnancy.

Women will be asked to inform the clinic of the result of the pregnancy as is the usual procedure in the clinic.

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Study outcomes

Primary endpoint:

The primary endpoint for the INVICSI trial is the first live birth episode following the study cycle in each of the two groups (IVF and ICSI). This is defined as the first live birth from the oocyte collection and includes transfer of fresh embryos and frozen-thawed embryos. The minimum

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follow-up time will be one year after inclusion. Live birth is defined as the delivery of one or more living infants ≥22 weeks gestation. When the primary endpoint is achieved, further live births from the oocyte collection will not be included in the primary outcome analysis. Subsequent live births from any FET cycles with embryos from the first fresh cycle are included as a secondary outcome (all live birth episodes). The secondary outcomes are summarised in Table 3.

Data collection methods

Before treatment is initiated all fertility patients in the clinics fill out a standard form including data on fertility and medical history, ethnicity, medications, smoking, alcohol, height, weight etc. These data are routinely entered into electronic medical files of the fertility clinics by fertility doctors prior to the patients first consultation in the clinic. This is part of standard practice for all fertility patients. For the INVICSI study, baseline data will be gathered by the investigators from the electronic files after written informed consent has been given (age, weight, height, ethnicity, antral follicle count (AFC), anti-müllerian hormone (AMH) concentration, years of infertility, primary or secondary infertility, infertility diagnosis, stimulation protocol, sperm characteristics). Data will then be entered into REDCap after which the randomisation and allocation to either standard IVF or ICSI will occur. Data on treatment outcome including fertilisation, embryo development, pregnancy and pregnancy loss (secondary outcomes, Table 3) will be collected and entered in REDCap. The couple/woman is asked to consent to data being obtained from the child's file in case the fertility treatment results in the birth of a living child.

To ensure data collection, an investigator will follow-up on all participants that obtains pregnancy. Follow-up will take place one year after the ultrasound scan (week 7-9). If the participant has

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informed the fertility clinic on birth and child, an investigator will contact the participant via a phone call or retrieve all information from the electronic patient record.

Statistical considerations

Proposed sample size:

The rate of first live births after transfer of up to all of the transferable embryos from the first OPU is set to 45% in the conventional IVF group and 55 % in the ICSI group. This is a superiority trial with a power of 80% and a 2-sided p-value of 5%. The sample size is estimated to be 392 patients in each group. Post-randomization exclusion is expected to be 5%, resulting in a total of 824 patients.

Data analysis:

ITT analysis and per-protocol analysis will be performed. Baseline characteristics and outcomes will be compared using t-test, Mann-Whitney U test or chi-square tests for continuous and categorical variables or logistic regression analysis, controlling for possible confounding effects where appropriate. P-values of <0.05 will be considered statistically significant. Statistical analyses will be performed by an investigator together with statistical experts. The primary RCT analysis will be performed by an independent statistician blinded to group allocation.

Patient and Public Involvement

There has been no patient or public involvement in the development of study design, recruitment or research question.

Ethics and dissemination

Data security and ethical aspects

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Data to describe the study population and the outcomes will be collected in a single database including all participants with an identification code, which makes every participant anonymous in the database.

The study is approved by the Scientific Ethical Committee of the Capital Region of Denmark (H-19022201) and the Danish Knowledge Centre on Data Protection Compliance. The study will be performed according to the Danish Law and Ethical principles in the Helsinki Declaration. Each participant will receive oral and written information about the study and will have opportunity for time and reflection. They can also discuss their participation with a third person. The collected oocytes of the participants will be fertilised with IVF or ICSI according to randomisation. Some couples/women may experience no fertilisation after either IVF or ICSI in the study. This risk is not considered significantly higher compared to women who do not participate in the study. The study is registered with the National Institute of Health's ClinicalTrials.gov (NCT04128904).

Dissemination

The findings of the study will be presented at national and international fertility conferences, such as the European Society of Human Reproduction and Embryology (ESHRE) annual meeting. In addition, the findings will be published in peer reviewed scientific journals. Public dissemination will be in the lay press.

Data-sharing

Data from the trial will be shared according to the ICJME guidelines. On request, data can be shared with parties presenting relevant aims for the use of data. Purposes and financial aspects of the other party must be approved by the steering committee of the "INVICSI" research team. No

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data will be shared until three months after the publication of papers reporting the primary and secondary outcomes of the trial. Any new research project must be approved by Danish authorities. The requesting party cover the costs for data sharing.

Discussion

Worldwide, the rate of treatment cycles where oocytes are fertilised with ICSI is increasing, also in patients without severe male factor infertility. Currently there is no evidence to support that ICSI results in a higher live birth rate compared to standard IVF in these patients. If the INVICSI study finds that ICSI is superior to standard IVF in cases without severe male factor infertility, the increasing use of ICSI is justified and may then be recommended. However, if the INVICSI study fails to show superiority of ICSI, standard IVF should be recommended as the preferred first choice method of fertilisation in patients without severe male factor infertility. This could potentially lead to significant cost savings and a higher use of standard IVF which is less invasive, closer to natural fertilisation and less expensive.

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Authors´ contributions:

SB and NCF were responsible for the conception, design and execution of the study protocol. SB, NCF and AP contributed to the initial revision and editing of the manuscript. AZ was consulted concerning the laboratory details of the study design. SB, NCF, AP, AZ, ALME, UBK, MRP, LFA, BN, HSN, LP, and MLG contributed to the critical revision of the manuscript as well as the approval of the final version for submission in BMJ Open.

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Competing interests:

SB and NCF received a research grant from the Capital Region of Denmark and two unrestricted grants from Gedeon Richter to support the INVICSI study as mentioned under 'Funding'. Outside the submitted work authors have received grants/fees/funding or declare relationships with the following third parties:

SB: Gedeon Richter, the Capitol Region of Denmark. NCF: Gedeon Richter, Ferring Pharmaceuticals, Merck A/S, Head of the steering committee for the Danish Fertility Guidelines made by members of the Danish Fertility Society (no payment), Guerbet, Advisory Board (personal fee). AP: Gedeon Richter, Ferring Pharmaceuticals, Merck A/S, Theramex. UBK: IBSA, Ferring Pharmaceuticals, Merck A/S. LFA: Gedeon Richter. BN: Gedeon Richter, IBSA, Merck A/S. HSN: Ferring Pharmaceuticals, Merck A/S, AstraZeneca, Cook Medical, Freya Biosciences ApS, Ferring Pharmaceuticals, BioInnovation Institute, Danish Ministry of Education. MLG: Gedeon Richter, Merck A/S. LP: Gedeon Richter, Merck A/S.

The authors do not report any potential conflict of interest.

References:

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1. Palermo G, Joris H, Devroey P, et al. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*. 1992;340(8810):17-8.
2. De Geyter C, Calhaz-Jorge C, Kupka MS, et al. ART in Europe, 2015: results generated from European registries by ESHRE. *Hum Reprod Open*. 2020;2020(1):hoz038.
3. de Mouzon J, Chambers GM, Zegers-Hochschild F, et al. International Committee for Monitoring Assisted Reproductive Technologies world report: assisted reproductive technology 2012dagger. *Hum Reprod*. 2020;35(8):1900-13.
4. Zagadailov P, Hsu A, Stern JE, et al. Temporal Differences in Utilization of Intracytoplasmic Sperm Injection Among U.S. Regions. *Obstet Gynecol*. 2018;132(2):310-20.
5. Boulet SL, Mehta A, Kissin DM, et al. Trends in use of and reproductive outcomes associated with intracytoplasmic sperm injection. *JAMA*. 2015;313(3):255-63.
6. Evers JL. Santa Claus in the fertility clinic. *Hum Reprod*. 2016;31(7):1381-2.
7. Tannus S, Son WY, Gilman A, et al. The role of intracytoplasmic sperm injection in non-male factor infertility in advanced maternal age. *Hum Reprod*. 2017;32(1):119-24.
8. Gennarelli G, Carosso A, Canosa S, et al. ICSI Versus Conventional IVF in Women Aged 40 Years or More and Unexplained Infertility: A Retrospective Evaluation of 685 Cycles with Propensity Score Model. *J Clin Med*. 2019;8(10).
9. Sfontouris IA, Kolibianakis EM, Lainas GT, et al. Live birth rates using conventional in vitro fertilization compared to intracytoplasmic sperm injection in Bologna poor responders with a single oocyte retrieved. *J Assist Reprod Genet*. 2015;32(5):691-7.
10. Li Z, Wang AY, Bowman M, et al. ICSI does not increase the cumulative live birth rate in non-male factor infertility. *Hum Reprod*. 2018;33(7):1322-30.
11. Foong SC, Fleetham JA, O'Keane JA, et al. A prospective randomized trial of conventional in vitro fertilization versus intracytoplasmic sperm injection in unexplained infertility. *J Assist Reprod Genet*. 2006;23(3):137-40.
12. Practice Committees of the American Society for Reproductive M, the Society for Assisted Reproductive Technology. Electronic address aao. Intracytoplasmic sperm injection (ICSI) for non-male factor indications: a committee opinion. *Fertil Steril*. 2020;114(2):239-45.
13. Johnson LN, Sasson IE, Sammel MD, et al. Does intracytoplasmic sperm injection improve the fertilization rate and decrease the total fertilization failure rate in couples with well-defined unexplained infertility? A systematic review and meta-analysis. *Fertil Steril*. 2013;100(3):704-11.
14. Vitek WS, Galarraga O, Klatsky PC, et al. Management of the first in vitro fertilization cycle for unexplained infertility: a cost-effectiveness analysis of split in vitro fertilization-intracytoplasmic sperm injection. *Fertil Steril*. 2013;100(5):1381-8.
15. Kim HH, Bundorf MK, Behr B, et al. Use and outcomes of intracytoplasmic sperm injection for non-male factor infertility. *Fertil Steril*. 2007;88(3):622-8.
16. Bhattacharya S, Hamilton MP, Shaaban M, et al. Conventional in-vitro fertilisation versus intracytoplasmic sperm injection for the treatment of non-male-factor infertility: a randomised controlled trial. *Lancet*. 2001;357(9274):2075-9.
17. Ruiz A, Remohi J, Minguez Y, et al. The role of in vitro fertilization and intracytoplasmic sperm injection in couples with unexplained infertility after failed intrauterine insemination. *Fertil Steril*. 1997;68(1):171-3.
18. Isikoglu M, Avci A, Kendirci Ceviren A, et al. Conventional IVF revisited: Is ICSI better for non-male factor infertility? Randomized controlled double blind study. *J Gynecol Obstet Hum Reprod*. 2020;50(7):101990.
19. van Rumste MM, Evers JL, Farquhar CM. Intra-cytoplasmic sperm injection versus conventional techniques for oocyte insemination during in vitro fertilisation in patients with non-male subfertility. *Cochrane Database Syst Rev*. 2003(2):CD001301.

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20. Butts SF, Owen C, Mainigi M, et al. Assisted hatching and intracytoplasmic sperm injection are not associated with improved outcomes in assisted reproduction cycles for diminished ovarian reserve: an analysis of cycles in the United States from 2004 to 2011. *Fertil Steril*. 2014;102(4):1041-7 e1.

21. Luna M, Bigelow C, Duke M, et al. Should ICSI be recommended routinely in patients with four or fewer oocytes retrieved? *J Assist Reprod Genet*. 2011;28(10):911-5.

22. van der Westerlaken L, Helmerhorst F, Dieben S, et al. Intracytoplasmic sperm injection as a treatment for unexplained total fertilization failure or low fertilization after conventional in vitro fertilization. *Fertil Steril*. 2005;83(3):612-7.

23. Chan AW, Tetzlaff JM, Gotzsche PC, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ*. 2013;346:e7586.

24. Kohn TP, Kohn JR, Ramasamy R. Effect of Sperm Morphology on Pregnancy Success via Intrauterine Insemination: A Systematic Review and Meta-Analysis. *J Urol*. 2018;199(3):812-22.

25. Kohn TP, Kohn JR, Lamb DJ. Role of Sperm Morphology in Deciding Between Various Assisted Reproduction Technologies. *Eur Urol Focus*. 2018;4(3):311-3.

26. Lemmens L, Kos S, Beijer C, et al. Predictive value of sperm morphology and progressively motile sperm count for pregnancy outcomes in intrauterine insemination. *Fertil Steril*. 2016;105(6):1462-8.

27. Deveneau NE, Sinno O, Krause M, et al. Impact of sperm morphology on the likelihood of pregnancy after intrauterine insemination. *Fertil Steril*. 2014;102(6):1584-90 e2.

28. Hotaling JM, Smith JF, Rosen M, et al. The relationship between isolated teratozoospermia and clinical pregnancy after in vitro fertilization with or without intracytoplasmic sperm injection: a systematic review and meta-analysis. *Fertil Steril*. 2011;95(3):1141-5.

29. Sha T, Wang X, Cheng W, et al. A meta-analysis of pregnancy-related outcomes and complications in women with polycystic ovary syndrome undergoing IVF. *Reprod Biomed Online*. 2019;39(2):281-93.

30. Sigala J, Sifer C, Dewailly D, et al. Is polycystic ovarian morphology related to a poor oocyte quality after controlled ovarian hyperstimulation for intracytoplasmic sperm injection? Results from a prospective, comparative study. *Fertil Steril*. 2015;103(1):112-8.

31. Heijnen EM, Eijkemans MJ, Hughes EG, et al. A meta-analysis of outcomes of conventional IVF in women with polycystic ovary syndrome. *Hum Reprod Update*. 2006;12(1):13-21.

32. Harris PA, Taylor R, Thielke R, et al. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. 2009;42(2):377-81.

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INVICSI: study protocol for a randomised, controlled multicentre trial

Version: 5.0

Date: May 06, 2021

Table 1. Trial registration data

Data category	Information
Primary registry and trial identifying number	ClinicalTrials.gov ID: NCT04128904, Protocol ID: INVICSI2019
Date of registration in primary registry	July 10, 2019
Secondary identifying numbers	H-19022201
Source(s) of monetary or material support	Capital Region of Denmark Gedeon Richter
Primary sponsor	Copenhagen University Hospital Hvidovre
Secondary sponsor(s)	None
Contact for public queries	SB (sineberntsen@gmail.com)
Contact for scientific queries	SB, NCF Department of Obstetrics and Gynaecology The Fertility Clinic, Hvidovre Copenhagen University Hospital Hvidovre
Public title	INVICSI – IVF versus ICSI in patients without severe male factor infertility
Scientific title	In vitro fertilisation (IVF) versus intracytoplasmic sperm injection (ICSI) in patients without severe male factor infertility (INVICSI): a randomised, controlled, multicentre trial
Countries of recruitment	Denmark
Health condition(s) or problem(s) studied	Methods of insemination (ICSI vs. conventional IVF), Infertility without severe male factor
Intervention(s)	Active comparator: Insemination with ICSI
	Active comparator: Insemination with conventional IVF
Key inclusion and exclusion criteria	Inclusion: Age of the woman 18-42 years, BMI of the woman between 18-35 kg/m2, Male partner with normal or non-severely decreased sperm parameters or use of donor sperm
	Exclusion: Previous IVF or ICSI treatments with current partner, Use of donor oocytes or frozen oocytes, Ovarian cysts >4 cm, Known liver or kidney disease, Unregulated thyroid disease, Endometriosis stage 3-4, Hypogonadotropic hypogonadism, Other severe comorbidity (e.g. diabetes or cardiovascular disease)
Study type	Randomised controlled multicenter trial using a parallel arm design. Randomisation 1:1 to receive insemination with ICSI or conventional IVF

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Date of first enrolment	November 29, 2019
Target sample size	824
Recruitment status	Recruiting
Primary outcome(s)	First live birth rate: the number of first live birth episodes from the study oocyte collections including transfer of fresh- and frozen-thawed embryos
Key secondary outcomes	Cycles with total fertilisation failure, fertilisation rate, embryo quality, positive pregnancy test rate, ongoing pregnancy rate, pregnancy loss rate, all live birth episodes, preterm delivery, birth weight and congenital anomalies

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Table 2. Protocol, Revision chronology

Version	Date of approval	Primary reasons for amendment
Original	August 8, 2019	
Amendment 1	January 28, 2020	New trial site added (The Fertility Clinic, Regional Hospital Horsens)
Amendment 2	March 20, 2020	Removed inclusion criteria: (i) Regular menstrual cycles (21-35 days). (ii) Diagnostic sperm sample from the male partner with ≥4% morphologically normal spermatozoa Added section: Handling of poor semen sample on the day of OPU
Amendment 3	September 2, 2020	New trial site added (The Fertility Clinic, Zealand University Hospital)

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Amendment 4 (Current version)	September 16, 2020	Removed inclusion criteria: Treatment with donor sperm or male partner sperm with a minimum concentration of 5 million progressive motile spermatozoa in a (purified) *diagnostic* semen sample. Added inclusion criteria: Male partner with normal or non-severely decreased sperm parameters where the sperm sample (purified) on the day of oocyte pick up is expected to contain a minimum of 2 million progressive spermatozoa.

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Table 3. Secondary outcomes

Outcome	Assessment
Fertilisation	**Fertilisation rate** per aspirated oocyte retrieved (16-20 hours after IVF/ICSI) defined as the appearance of 2 pronuclei (PN).
	Cycles with total **fertilisation failure.**
Embryo data	**Embryo quality** (i.e. good quality blastocysts according to Gardner classification).
	Embryo time-lapse kinetics including cleavage patterns.
	Embryo utilisation rate (number of transferred + cryopreserved embryos per number of 2 PN zygotes).
Freeze	Number of **frozen blastocysts** (time frame: up to six days after oocyte pick-up (OPU)).
Pregnancy	**Positive pregnancy test** (positive urine or serum hCG 11-21 days after embryo transfer).
	Multiple pregnancy (period: up to 12 weeks after embryo transfer). Number of intrauterine gestations.
	Ongoing pregnancy per transfer (fetal heartbeat on ultrasound in gestational week 7-8).
Miscarriage	**Pregnancy loss rate** (period: up to 12 weeks after embryo transfer).

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	Biochemical pregnancies (positive urine or serum hCG 11-21 days after embryo transfer without any clinical signs of intra- or extrauterine pregnancy).
	Ectopic pregnancy/pregnancy of unknown location **(PUL)**
Birth/offspring	**All live birth episodes** (all live births from the study oocyte collection (including second and further live births)
	Preterm delivery (delivery at gestational week 22-36+6).
	Birth weight/weight for gestational age.
	Congenital anomaly diagnosed at birth.

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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered,	2

		name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	14
Protocol version	#3	Date and version identifier	15
Funding	#4	Sources and types of financial, material, and other support	11
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1, 11
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	14
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	11
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	n/a

Introduction

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Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	3-4
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4-5
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg,	5

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		surgeons, psychotherapists)	
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7-8
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	7
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	n/a
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8,16
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	5-6

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Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	n/a
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	6-7
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	6-7
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6-7

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Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n/a
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	8-9
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	n/a
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values).	8-9

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		Reference to where details of data management procedures can be found, if not in the protocol	
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	9
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	n/a
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	n/a
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial	n/a

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		conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	n/a
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	4
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	4
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	9
Declaration of	#28	Financial and other competing interests for principal	11

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interests		investigators for the overall trial and each study site	
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	n/a
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	10
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Suppl. file
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

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