

Challenges in donor sperm treatment



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Challenges in donor sperm treatment

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CHAPTER 1

General introduction



Donor Sperm Treatment is the oldest effective fertility treatment for couples with involuntary childlessness due to male infertility. It is also the most controversial treatment, since a third party is needed which represents a fundamental shift away from traditional family building. The first insemination with donor sperm published in scientific literature was performed by William Pancoast in 1884. He treated a couple of whom the husband had post gonorrhoeic azoospermia by inseminating his wife under chloroform anesthesia with fresh sperm of one of his students. The husband was informed about the donor insemination, but was advised never to tell his wife and future child. It was the student who published his ordeal many years later (Hard 1909). From the nineteen-thirties onwards, donor sperm treatment was gradually introduced in clinical practice to assist heterosexual couples in building a family. In 1943, the results of the first survey ever on insemination with donor sperm were published, reporting on 450 children born until 1940 (Folsome 1943). Since then, donor sperm treatment became mainstream and has remained so until the present day (Gerkowicz et al. 2017, De Geyter et al. 2020).

Over the past decades, the practice of donor sperm treatment has changed dramatically. New indications for donor sperm treatment were introduced, previous indications became obsolete, anonymous sperm donation was replaced by identifiable donation, the use of frozen-thawed donor sperm became mandatory which disconnected the direct link between donation and insemination and timing of insemination changed due to the introduction of the rapid urinary luteinizing hormone test. In this introduction we briefly present these changes in the practice of donor sperm treatment.

Changes in the indications for donor sperm treatment over time

From an intervention applied in heterosexual couples with male infertility, donor sperm treatment gradually extended to an intervention also enabling lesbian couples and single women to have children (Shapiro et al. 1990, Baetens et al. 1995, Jennings 2017). Another development was that donor sperm treatment was also applied in men infected with human immunodeficiency virus, or in couples in whom one of the partners are carrier of a genetic defect (Semprini et al. 1992, Liebaers et al. 1998). This development was “counteracted” at the end of the 20th century, when donor sperm treatment in heterosexual couples was gradually replaced by new medically-assisted techniques such as intracytoplasmic sperm injection (ICSI) with or without surgical sperm extraction in men with severe male infertility or azoospermia, semen washing and insemination in men infected with human immunodeficiency virus to prevent the transmission of the virus and preimplantation genetic diagnosis in couples in whom one of the partners are carrier of a genetic defect (Palermo et al. 1992, Semprini et al. 1992, Tournaye et al. 1994, Silber et al. 1996, Liebaers et

al. 1998). With the introduction of these new reproductive techniques, Hogerzeil in the first PhD thesis from the Centre for Reproductive Medicine on donor sperm treatment predicted that donor sperm treatment for heterosexual couples would become obsolete (Hogerzeil 1995). This prediction has not become reality since over the years it has become apparent, that although these new techniques are attractive options, they are no panacea and patients make their own choices. So today, donor sperm treatment is still a treatment option after failure of surgical sperm retrieval or ICSI, after failure of sperm washing and insemination, and to prevent vertical transmission of a genetic defect. Last but not least, donor sperm treatment is a serious treatment option for couples that do not opt for these new medically-assisted reproductive techniques (Vernaev et al. 2005, NICE 2013, Hendriks et al. 2014).

The paradigm shift from anonymous donation and non-disclosure to non-anonymous sperm donation and disclosure

Concurrent with the changes in the indications for donor sperm treatment, there were major shifts in thinking about donor sperm treatment from a societal perspective. The paradigm shift from anonymous donation and non-disclosure to non-anonymous sperm donation and disclosure was a gradual process, but the new paradigm became more accepted when underpinned by the Warnock report (Warnock 1984). This influential report was instrumental in creating awareness that a donor-child should have access to identifying information on the sperm donor and represented a major shift in our beliefs about donor anonymity and parent donor-child secrecy. In 2004 the law – *Wet Donorgegevens Kunstmatige Bevruchting* – was implemented in the Netherlands which prohibited anonymous donation of sperm and oocytes and acknowledged the children's right to know their genetic origin from the age of 16 (Staatsblad 2002, Janssens et al. 2006). This legislation and similar legislation in several other European countries, New Zealand and some states of Australia can be seen as society's acceptance of the new paradigm (Edvinsson et al. 1990, Gottlieb et al. 2000, Janssens et al. 2005, Hamilton et al. 2008, Daniels et al. 2009).

As a consequence, professional counselling became an essential part of donor sperm treatment in informing couples about the unique psychosocial aspects of donor sperm treatment. The aim of counselling is to assist parents during several stages of parenthood with a focus on disclosure to a child (Hammarberg et al. 2008, Visser et al. 2012, Visser et al. 2016). Psychosocial counselling has also become important for sperm donors addressing the emotional consequences of their donation and informing them on rules and regulations. Additional counselling at the time donor-offspring actually seek contact has proven to be of great value (Greenfeld 2008, Hammarberg et al. 2008, Visser et al. 2016).

The impact of frozen-thawed donor sperm

The discovery of glycerol as a cryoprotective agent made it possible for sperm to survive freezing and thawing (Polge et al. 1949). In 1954, the first pregnancies with frozen-thawed semen were described (Bunge et al. 1953, Bunge et al. 1954). This led to the foundation of the first sperm banks and disconnected the direct link between donation and insemination (Steinberger et al. 1965). Another advantage of frozen-thawed donor sperm was that the sperm could be tested for contamination by venereal diseases and was only inseminated after a period of quarantine to rule out seroconversion (Tyler 1973, Sherman et al. 1975, Schreeder et al. 1982, Handsfield et al. 1985, Stewart et al. 1985). According to the revised guideline of 1988 of the American Fertility Society, frozen-thawed sperm became mandatory with quarantine of the frozen-thawed sperm for at least six months, to be used for insemination after confirmation of a negative test of the sperm donor for human immunodeficiency virus, hepatitis B, C and other venereal diseases (AFS 1988).

Despite all advantages of frozen-thawed donor sperm – foundation of sperm banks, constant availability of donor sperm and prevention of transmission of venereal diseases- over the years it became clear that freezing and thawing also had two major drawbacks. First, it became apparent that inseminations with frozen-thawed donor sperm resulted in lower pregnancy rates compared to inseminations with fresh semen (Subak et al. 1992). Second, freezing and thawing had an adverse effect on semen motility resulting in rejection of 85% of sperm donors due to poor semen quality (Paul et al. 2006).

Introduction of the rapid urinary luteinizing hormone test

Until 1989, intracervical insemination was performed based on basic temperature charts in combination with cervical mucus scores (Meijer et al. 1980, Robinson et al. 1992). The prediction of ovulation by the basal body temperature is correct in only 34% of the cases (Lenton et al. 1977, Vermesh et al. 1987). Women had to visit a clinic several times per cycle to check their cervical mucus score and needed several inseminations per cycle to include ovulation (Hogerzeil 1995).

In 1989 the rapid urinary Luteinizing Hormone (LH) test was introduced (Lloyd et al. 1989). The urinary LH test can detect ovulation in 60-80% of the cases when performed once a day and in 90% of the cases when performed twice a day (Lloyd and Coulam 1989). The introduction of the urinary test at home limited the number of visits to the clinic but did not lower pregnancy rates (Barratt et al. 1989, Federman et al. 1990, Odem et al. 1991, Robinson et al. 1992). After the introduction of the rapid urinary LH test one insemination per cycle was sufficient to include ovulation (Barratt et al. 1989).

In summary, over the last decades many aspects of donor sperm treatment have undergone profound changes. At one time it was thought that new reproductive techniques would replace donor sperm treatment, but this has not materialized and donor sperm treatment is still a major part of the therapeutic reproductive armamentarium.

But there is a problem and that is the discrepancy between supply and demand. The supply side of the problem is the low number of suitable sperm donors caused by the prohibition of insemination of sperm of anonymous donors, the acknowledgement of the children's right to know their genetic origin from the age of 16 and the mandatory use of frozen-thawed donor sperm. The demand side of the problem is the annual increase in the number of donor sperm treatment cycles. In view of this, all our efforts should be directed towards establishing the most (cost)- effective insemination technique, which is the topic of this thesis.

BACKGROUND OF THIS THESIS

Donor sperm treatment has been performed in the Centre for Reproductive Medicine of the Amsterdam University Medical Centre in Amsterdam since 1972. Since 1976 all women are inseminated with frozen-thawed semen which has greatly facilitated the logistics of donor sperm treatment (Hogerzeil 1995).

In 1980, the results of the first six years of donor sperm treatment were reported (Meijer and Hamerlynck 1980). They reported on inseminations with fresh donor sperm and inseminations with frozen-thawed donor sperm. Timing of insemination was based on basal temperature charts, women were inseminated daily and started intracervical insemination four to five days before their expected ovulation until a rise in their temperature was observed for a maximum of 12 cycles. Between 1972 and 1975 inseminations were done with fresh donor sperm and from 1976 onwards with frozen-thawed donor sperm. Inseminations with frozen-thawed donor sperm were performed if sperm had a total motile count of $4-6 \times 10^6$ after thawing. Ninety heterosexual couples were treated with fresh donor sperm and 105 with frozen-thawed donor sperm. After 12 cycles of intracervical insemination there were 51(57%) and 74 (70,5%) pregnancies respectively.

In contrast with this study, other studies showed that pregnancy rates were lower after intracervical insemination with frozen-thawed donor sperm compared to inseminations with fresh donor sperm (Leeton et al. 1980, Richter et al. 1984, Keel et al. 1987, Subak et al. 1992). As a consequence, studies focused on improving pregnancy rates after intracervical insemination by comparing one versus two inseminations per cycle, insemination by cervical cap versus insemination by straw and different cut off values for the number of motile

spermatozoa inseminated. One insemination versus double inseminations per cycle and the use of a cervical cap showed conflicting results; two studies showed that insemination twice every cycle and intracervical insemination by cap resulted in higher clinical pregnancy rates, while two other studies showed no evidence of a difference (Centola et al. 1990, Lincoln et al. 1995, Coulson et al. 1996, Flierman et al. 1997). The only factor possibly improving ongoing pregnancy rates in intracervical insemination was when inseminations were performed with a total motile count above eight million (Le Lannou et al. 1995). The common problem with these studies is that they are all of very low quality since they included small numbers of women, had a cross-over design or had an unclear methodology. Also, there was a risk of bias due to poor reporting of study methods and serious imprecision.

Until 1992 inseminations with donor sperm were mainly performed by intracervical insemination (Le Lannou et al. 1995). Since intracervical inseminations with frozen-thawed donor sperm were associated with lower pregnancy rates compared to inseminations with fresh sperm and modifications in technique did not improve results, the question was raised if cycle fecundity could be improved by intrauterine insemination (Patton et al. 1992, Hurd et al. 1993, Peters et al. 1993, Wainer et al. 1995, Matorras et al. 1996). The concept behind this was that intrauterine insemination brings the sperm closer to the oocyte than intracervical insemination which might overcome the negative effects of freezing and thawing (Sunde et al. 1988). A systematic review and meta-analysis that pooled data of randomised controlled trials on intracervical insemination versus intrauterine insemination showed that intrauterine insemination indeed resulted in higher clinical pregnancy rates (Besselink et al. 2008). Since the methodology of the included studies were of moderate quality and all studies were underpowered, the authors of the systematic review recommended to perform well powered randomised controlled trials.

A new development was the addition of ovarian stimulation to intrauterine insemination based on the idea that multiple follicle growth would enhance conception rates. In 1994 a study showed that the addition of ovarian stimulation to intrauterine insemination with frozen-thawed donor sperm increased pregnancy rates (23.9% versus 12.5 %) without any multiple pregnancies (Depypere et al. 1994). Two small randomised controlled trials showed that intrauterine insemination with ovarian stimulation resulted in higher clinical pregnancy rates compared to intracervical insemination with ovarian stimulation, but also in higher multiple pregnancy rates (23% vs 10%) (Wainer et al. 1995, Matorras et al. 1996). Since it is nowadays undisputed that multiple pregnancies should be prevented if at all possible and based upon these two randomised controlled trials, the guideline of The National Institute for Health and Care Excellence states that intrauterine insemination in the natural cycle

should be offered as first line treatment (O'Brien et al. 2000, NICE 2013). This is a strange recommendation since it overlooks that there is no evidence supporting intrauterine insemination with frozen-thawed donor sperm in the natural cycle. Nevertheless, for whatever reason, the recommendation to perform intrauterine insemination in the natural cycle seems not to have been followed since the European In Vitro Fertilisation Monitoring Programme reporting on 49514 cycles of intrauterine insemination with donor sperm in 2015, documents a multiple pregnancy rate of 7.3% (De Geyter et al. 2020).

In conclusion, there is - even after more than 100 years of donor sperm treatment- a lack of evidence on the most (cost)- effective insemination technique. This makes that the current practice of donor sperm treatment lacks a solid evidence base and is -possibly- inefficient.

Aims of this thesis

This thesis studies three critical knowledge gaps in women who start with insemination with frozen-thawed donor sperm in the Netherlands.

First, we aim to investigate if there is a discrepancy between the number of suitable sperm donors and the number of women who start with inseminations with donor sperm in the Netherlands. To do so, we will perform an inventory in the eight sperm banks in the Netherlands and collect data on the number of suitable sperm donors and the number of women who start with inseminations with donor sperm.

Second, we aim to investigate whether semen parameters are a determinant of success in intracervical insemination with frozen-thawed donor sperm. To do so, we will perform a retrospective cohort study and investigate if there are any thresholds for semen parameters that lead to the best possible ongoing pregnancy rates in intracervical insemination with frozen-thawed donor sperm.

Third, we aim to investigate which insemination technique we should perform with frozen-thawed donor sperm to achieve the highest possible pregnancy rates. To do so, we will evaluate intracervical insemination in the natural cycle versus intrauterine insemination in the natural cycle with frozen-thawed donor sperm with emphasis on effectiveness, safety and costs.

OUTLINE OF THE THESIS

CHAPTER 2 presents an inventory of the number of suitable sperm donors and the number of women who started inseminations with frozen-thawed donor sperm in 2010 in five of the eight sperm banks in the Netherlands. We describe the changes in donor sperm treatment care during the last decades. With this data we tried to see whether there is a discrepancy between the number of suitable sperm donors and the number of women applying for donor sperm treatment in the Netherlands.

CHAPTER 3 reports the results of a retrospective cohort study to assess the role of semen parameters in intracervical insemination with frozen-thawed donor sperm in terms of ongoing pregnancy. We acquired retrospective data from 1999 to 2015 of the Centre of Reproductive Medicine including 1136 women who underwent 7103 cycles with donor semen of 129 sperm donors. We tried to calculate thresholds for sperm parameters after freezing and thawing resulting in the best possible ongoing pregnancy rates after intracervical insemination with frozen-thawed donor sperm.

CHAPTER 4 presents an update of a systematic review, which aimed to compare the effectiveness and safety of intracervical insemination and intrauterine insemination in women who start with donor sperm treatment.

CHAPTER 5 reports the results of a retrospective cohort study on intracervical insemination compared to intrauterine insemination with frozen-thawed donor sperm in the natural cycle. We acquired data from all eight sperm banks in the Netherlands from 2009 and 2010. We included 1843 women; 1163 women underwent 4269 cycles of intrauterine insemination and 680 women underwent 2345 cycles of intracervical insemination. We compared time to ongoing pregnancy in the first six cycles of intracervical insemination and intrauterine insemination.

CHAPTER 6 reports the results of a randomised controlled non-inferiority trial comparing intracervical insemination versus intrauterine insemination with frozen-thawed donor sperm in the natural cycle in 421 women. Five clinics in the Netherlands and one in Belgium included women who started donor sperm treatment. The main outcome was conception leading to live birth within eight months after randomisation.

CHAPTER 7 provides a summary of this thesis and provides implications for clinical practice and future research. .

CHAPTER 8 Presents the Dutch translation of the summary presented in chapter 7.

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CHAPTER 2

Artificial insemination with donor
sperm in the Netherlands:
future-proof?

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Abstract

De afgelopen jaren is er veel veranderd binnen de zorg rond kunstmatige inseminatie met donorsperma (KID). Door nieuwe wet- en regelgeving is een groot aantal spermabanken gesloten en is het totale aantal spermadonoren en hun inzetbaarheid afgenomen. Lange wachttijden en het gebruik van spermadonoren van commerciële buitenlandse spermabanken kunnen duiden op een tekort. Het feit dat internet het mogelijk maakt dat vrouwen zelf donorsperma kunnen bestellen en zonder tussenkomst van een spermabank een behandeling kunnen starten, leidt ertoe dat toekomstige donorkinderen de identiteit van hun spermadonor niet kunnen opvragen zoals is vastgelegd in de Wet Donorgegevens Kunstmatige Bevruchting.

Om deze wet te kunnen handhaven is een actief wervingsbeleid voor Nederlandse spermadonoren gewenst, zodat wachttijden en behandelingen buiten de spermabanken om tegen worden gegaan. Alleen zo kan de huidige Nederlandse KID-zorg worden gewaarborgd voor de toekomst.

Introductie

De afgelopen jaren zijn er veel veranderingen opgetreden in de zorg die zich bezighoudt met kunstmatige inseminatie met donorsperma (KID). Wijzigingen in de wet- en regelgeving hebben hier een grote rol in gespeeld en hebben er mede toe geleid dat er een grote daling is opgetreden in het aantal Nederlandse spermadonoren (Janssens et al. 2005). Een nieuwe trend is het gebruikmaken van spermadonoren die worden aangeboden door buitenlandse commerciële spermabanken. Dit donorsperma kunnen vrouwen zelf bestellen via het internet (Cryos). Deze nieuwe ontwikkeling zou kunnen duiden op een tekort aan spermadonoren. Het is echter onbekend of daar momenteel werkelijk sprake van is.

In dit artikel bespreken we welke veranderingen hebben plaatsgevonden binnen de Nederlandse KID-zorg, hoe de huidige stand van zaken is en of de KID-zorg in Nederland toekomstbestendig is.

Minder spermabanken en -donoren

Sinds 1948 wordt KID uitgevoerd in Nederland (Levie 1965). Dit gebeurt bij paren van wie de man azoöspermie heeft, paren van wie de man drager is van een genetische afwijking, en bij lesbische paren of alleenstaande vrouwen (CBO 1992). Door de invoering van de Wet Veiligheid en Kwaliteit Lichaamsmateriaal in 2003 werden strengere eisen gesteld aan de spermabanken en moesten zij een erkenning als orgaanbank krijgen (WVKL 2007). Een aantal spermabanken voldeed niet aan deze nieuwe eisen en moest hun deuren sluiten. Dit kwam mede doordat er tegelijkertijd een daling van het aantal spermadonoren plaatsvond: in 1990 waren er 917 spermadonoren beschikbaar, van wie 901 anoniem, vergeleken met slechts 185 spermadonoren in 2005 (Janssens et al. 2005).

Deze daling werd waarschijnlijk veroorzaakt door de invoering van de Wet Donorgegevens Kunstmatige Bevruchting in 2004, die stelt dat donorkinderen vanaf de leeftijd van 16 jaar aanspraak kunnen maken op persoonsidentificerende gegevens van de spermadonor. Spermadonoren dienen daarom akkoord te gaan met het registreren van hun gegevens bij de Stichting Donorgegevens Kunstmatige Bevruchting (SDKB) en het verstrekken van deze gegevens aan het donorkind als hij of zij 16 jaar wordt (Staatsblad 2002). Als gevolg hiervan trokken veel spermadonoren die doneerden vanuit het principe van anonimiteit zich terug. De invoering van deze 2 wetten samen heeft erin geresulteerd dat het aantal spermabanken is teruggelopen van 21 in 1990 naar momenteel 8.

In 2010 werd het 'Standpunt infectiescreening' aangepast, waarbij screening van spermadonoren op cytomegalovirus (CMV) verplicht werd. Het huidige standpunt stelt

dat sperma van een CMV-positieve spermadonor niet voor een CMV-negatieve vrouw mag worden gebruikt (NVOG 2010). Recent heeft de Gezondheidsraad het advies uitgebracht om het maximum aantal kinderen per spermadonor op 25 te houden, maar een donor kan aangeven voor minder kinderen inzetbaar te zijn (Gezondheidsraad 2013). Door deze standpunten en adviezen neemt de inzetbaarheid van de nog beschikbare spermadonoren af en kan een tekort optreden.

Tekort aan Nederlandse spermadonoren?

Via een enquête maakten we een inventarisatie van de huidige praktijk. Deze werd ingevuld door 5 van de 8 Nederlandse spermabanken en laat zien dat er in 2010 in totaal 162 Nederlandse spermadonoren beschikbaar waren. Van deze spermadonoren was 57% CMV-negatief en dus volledig inzetbaar. In totaal startten er in dat jaar 644 vrouwen met de eerste donorinseminaties zonder ovariële stimulatie bij de 8 Nederlandse spermabanken. Van deze 644 vrouwen werden 193 (30%) doorgaand zwanger binnen 6 cycli. In 2010 hadden 5 spermabanken een wachtlijst met een gemiddelde wachttijd van 10,5 maanden (spreiding: 0-30 maanden).

Een belangrijke vraag is of er een tekort is aan Nederlandse spermadonoren. Uit de gegevens van de enquête kan een grove berekening worden gemaakt met als uitgangspunt het aantal vrouwen dat startte met een KID-behandeling. Uitgaande van een maximaal aantal van 25 kinderen per donor en een gemiddeld aantal kinderen van 2 per vrouw, zouden voor de 644 vrouwen die in 2010 startten met een KID-behandeling 52 donoren ($(2 \times 644) / 25$) voldoende zijn om aan de vraag te voldoen.

Deze berekening is puur theoretisch en kent een aantal beperkingen. Het is onbekend hoeveel kinderen de 162 beschikbare spermadonoren precies hadden op het moment van de inventarisatie. Als een donor al meerdere kinderen uit eerdere behandelingen heeft, is hij niet langer inzetbaar voor nog 25 kinderen. Tevens kan de donor hebben aangegeven voor minder dan 25 kinderen inzetbaar te willen zijn. De ervaring leert dat een spermadonor 5 jaar inzetbaar is tot het maximum van 25 kinderen is bereikt. Hierdoor is een spermadonor jaarlijks gemiddeld inzetbaar voor 5 nakomelingen. Dit zou betekenen dat er voor de 644 vrouwen 129 spermadonoren ($644 / 5$) nodig zouden zijn geweest.

Een tweede kanttekening is dat er geen rekening wordt gehouden met de CMV-status en de eventuele matching op uiterlijke kenmerken van de spermadonoren, wat de inzetbaarheid sterk verlaagt. Daarnaast is het onduidelijk hoeveel nieuwe spermadonoren zich jaarlijks aanmelden bij de spermabanken. Als dit er minder dan 52 in totaal zijn, kan dit in de komende jaren leiden tot een tekort.

Andere bronnen van donorsperma

Naast het gebruik van donorsperma van Nederlandse spermadonoren zijn er ook andere bronnen. Een gebruikelijk alternatief is om KID uit te voeren met een eigen donor. Hierbij doneert de spermadonor exclusief voor 1 vrouw. Ook kan donorsperma worden ingekocht van spermabanken in het buitenland door Nederlandse spermabanken of particulier door de vrouwen zelf. Inseminaties met sperma van buitenlandse spermadonoren kan alleen plaatsvinden als deze niet anoniem zijn en dus geregistreerd kunnen worden bij de SDKB. Eén Nederlandse kliniek maakt gebruik van het faire wederkerigheidsprincipe (NVOG 2011). Hierbij staat de vrouw eicellen af in ruil voor het gebruik van donorsperma. Het gebruik van deze andere bronnen van donorsperma kan duiden op een tekort aan spermadonoren of op een specifieke wens van de vrouw die KID ondergaat wat betreft de keuze voor haar spermadonor.

Het internet maakt het mogelijk dat vrouwen zelf donorsperma kunnen bestellen, zowel anoniem als niet-anoniem. Zo kunnen ze zonder tussenkomst van een spermabank een behandeling starten. Dit leidt ertoe dat toekomstige donorkinderen de identiteit van hun spermadonor niet kunnen opvragen, zoals vastgelegd in de Wet Donorgegevens Kunstmatige Bevruchting, omdat deze donoren niet geregistreerd worden bij de SDKB. Een actief wervingsbeleid voor Nederlandse spermadonoren is dan ook gewenst om wachttijden en behandelingen buiten de spermabanken om tegen te gaan. Alleen zo kan de huidige Nederlandse KID-zorg worden gewaarborgd voor de toekomst.

Conclusie

De afgelopen jaren is er veel veranderd binnen de zorg rond kunstmatige inseminatie met donorsperma. Door nieuwe wet- en regelgeving is in het verleden een groot aantal spermabanken gesloten en is het totale aantal spermadonoren en hun inzetbaarheid afgenomen. Sinds 2005 is het aantal spermabanken en -donoren relatief stabiel gebleven. Lange wachttijden en het gebruik van spermadonoren van commerciële buitenlandse spermabanken kan duiden op een tekort. Om de Wet Donorgegevens Kunstmatige Bevruchting te kunnen handhaven is het belangrijk voldoende aanbod van Nederlandse spermadonoren te hebben zodat behandelingen buiten de Nederlandse spermabanken om overbodig zijn.

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CHAPTER 3

Donor sperm treatment: the role of semen parameters in intracervical insemination, a retrospective cohort study.

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Abstract

Donor sperm treatment is advised to be performed with frozen-thawed donor semen. A disadvantage of frozen-thawed semen is lower pregnancy rates compared to inseminations with fresh semen. Semen parameters affect ongoing pregnancy rates in intracervical inseminations with frozen-thawed donor semen. In an attempt to translate this into clinical relevance, cohort studies have tried to find cut-off values for semen parameters after thawing for intracervical insemination, but these studies assessed only one semen parameter per study, thereby overlooking the intricate interplay between all semen parameters. We performed a retrospective cohort study and tried to calculate thresholds for all semen parameters that lead to the best possible ongoing pregnancy rates in intracervical insemination with frozen-thawed donor semen. Between April 1999 and December 2015, data from 1186 women who underwent 7103 cycles of intracervical insemination with donor semen of 129 sperm donors were available for analysis. Our results showed that total motility and total motile count (TMC) after thawing were associated with ongoing pregnancy rate. The best possible ongoing pregnancy chances after intracervical insemination were obtained at a total motility of $\geq 20\%$ and a total motile count (TMC) of $\geq 8 \times 10^6$ after thawing.

Introduction

Donor sperm treatment (DST) is the oldest intervention for infertility and was traditionally performed with fresh semen (Hard 1909, Leeton et al. 1980, Richter et al. 1984). The recognition in the late nineteen eighties that human immunodeficiency virus (HIV) could be transmitted by fresh semen, meant that guidelines prohibited the use of fresh donor semen. Frozen-thawed semen became mandatory with a quarantine of six months before use (ASRM 2013, NICE 2013).

The downside of frozen-thawed semen was that pregnancy rates were lower compared to inseminations with fresh semen due to an adverse effect of freezing and thawing on sperm motility as only 50% of motile spermatozoa survive the freeze and thawing procedure (Leeton et al. 1980, Richter et al. 1984, Keel et al. 1987).

Current guidelines on screening and selecting sperm donors recommend that the initial semen analysis should be classified according to the criteria of the World Health Organization of 2010 (WHO) (WHO 2010, ASRM 2013, HFEA 2018, Clarke et al. 2021). The minimal WHO criteria for normal semen quality are at least a volume of 1.5 ml, a concentration of 15 million/ml, a total sperm count of 39 million, a total motility of 40% and a morphology with at least 4% typical forms (WHO 2010). With this classification the guidelines completely overlook the impact of the freeze and thaw process on pregnancy rates. Cohort studies have tried to find cut-off values for semen parameters after thawing for intracervical insemination, but these studies assessed only one semen parameter per study, thereby overlooking the intricate interplay between all semen parameters (Nielsen et al. 1984, Le Lannou et al. 1995, Clarke et al. 1997).

The aim of this study was therefore to evaluate the association between all semen parameters after thawing with ongoing pregnancy and to determine any cut-off values for the associated semen parameters with frozen-thawed donor semen.

Materials and Methods

We performed a retrospective cohort study in which we included all women with an indication for DST who started intracervical insemination with frozen-thawed donor semen between April 1999 and December 2015. We chose this period since data were stored in an electronic database from 1999 onwards. Sperm donors in this cohort donated semen from 1984 to 2015. All data on sperm donors and women who started DST were collected from the sperm bank of the Center for Reproductive Medicine, Amsterdam (Amsterdam UMC, location AMC, Meibergdreef 9, Amsterdam).

Inclusion and exclusion criteria for women and sperm donors

All women with an indication for DST who started intracervical insemination were included in the study. Heterosexual couples were included if men had obstructive or non-obstructive azoospermia, if men had severely impaired semen quality but the couple did not wish to undergo intracytoplasmic sperm injection (ICSI) or had failed ICSI, or if couples wanted to prevent vertical transmission of a genetic defect or transmission of human immunodeficiency virus when semen washing was not an option. Lesbian couples or single women were also included. All women were aged between 18 and 43 years. Women before start with treatment had an assessment of their medical history by their doctor. If pregnancy did not occur after six to twelve 12 intracervical inseminations, tubal patency was assessed by a hysterosalpingography. Women with known double-sided tubal pathology or an irregular menstrual cycle were excluded.

Selection criteria for sperm donors changed over the years. Until 1992 selection procedures were based on a local protocol which was based on the guidelines of the American Fertility Society (AFS 1986, AFS 1988). These guidelines advised that sperm donors should be tested negative for sexually transmitted diseases like gonorrhoea, chlamydia, syphilis, Hepatitis B and C, Human Immunodeficiency Virus (HIV). Also, these guidelines recommended that sperm donors should not be aged over 50 years and that the donor should be generally healthy, based on a medical history and a genetic screening which included a proper family history with respect to potential hereditary disorders. In 1992, the local protocol changed after a CBO-guideline advised that sperm donors should be aged between 18-45 years and that the number of offspring of a sperm donor should be limited to 25 children per sperm donor to prevent the risk of consanguinity (CBO 1992).

In 2004 the law – Wet Donorgegevens Kunstmatige Bevruchting- was implemented in the Netherlands which prohibited anonymous donation of sperm and oocytes and acknowledged the children's right to know their genetic origin from the age of 16 (Staatsblad 2002, Janssens et al. 2006). Therefore sperm donors after 2004 could no longer be anonymous. Since then psychosocial counselling addressing the emotional consequences of their donation and informing them on rules and regulations was part of the protocol.

In 2013 the limit of children per sperm donor changed and was set at 12 families per sperm donor to allow the birth of multiple children from the same sperm donor within one family (Gezondheidsraad 2013). Sperm donors were rejected if their health status or genetic screening revealed abnormalities or if the concentration of progressive motile spermatozoa in the semen sample before freezing was below $15 \times 10^6/\text{ml}$ and after freezing and thawing if the total motile count (TMC) was below two million. Some sperm donors in our cohort are

at present older than 45. These donors started donation before the age of 45 and continued their donation for years, or specifically for second children.

Laboratory protocol

Semen samples were collected at the hospital by masturbation directly into a 50 ml sterile polyethylene jar after two to seven days of sexual abstinence. Semen samples were analysed within an hour of ejaculation. Sperm concentration was counted and total motility assessed in a Makler counting chamber or a Bürger-Türk counting chamber at a magnification of x 200.

Classification of semen parameters changed over time according to modifications in the WHO criteria. From 1984 until 1987 the WHO criteria of 1980 were used to classify semen parameters, from 1987 until 1992 the WHO criteria of 1987, from 1992 until 1999 the criteria of 1992, from 1999 until 2010 the criteria of 1999 and from 2010 onwards the criteria of 2010 (WHO 1980, WHO 1987, WHO 1992, WHO 1999, WHO 2010)

Donor sperm was frozen with a slow freeze technique during the whole study period and stored in straws of 0.3 ml with an unique code and date. From 1984 until 1998 sperm was cryopreserved in a glycerol egg yolk-citrate medium by a two-step computerized freezing protocol and stored in liquid nitrogen. In 1998 this medium was replaced by SpermFreeze medium (Fertipro, Beernem, Belgium). From 2009 onwards sperm is cryopreserved by vapor cryogenic storage. Subsequently, one straw was thawed and if TMC after thawing was more than two million the donor was accepted and started with multiple donations. Since our local protocol selects sperm donors based on a total motile count after thawing of two million, a minimal concentration of $15 \times 10^6/\text{ml}$ before freezing was needed. This minimal concentration of $15 \times 10^6/\text{ml}$ before freezing was based on a calculation: A sperm donor has a hypothetical concentration of 15 million/ml before freezing, and a hypothetical motility of 80%. Before freezing we dilute 1 ml to 1.7ml-> the concentration becomes 7.05 million/ml. In one straw of 0.3 ml there is $7.05 \times 0.3\text{ml} = 2.11$ million sperm/ ml. This calculation is before freezing. After freezing approximately 50% of motile spermatozoa survive the freeze and thawing procedure and this will result in 1.05 million/ml per straw. Nevertheless, since semen quality varies per donation, TMCs below two million per straw are occasionally present in the analysis.

Insemination protocol

During the whole study period inseminations were performed by the intracervical route in the natural cycle using one straw per insemination. Intracervical inseminations were performed with unprocessed frozen-thawed semen.

Insemination protocols changed over time. From April 1999 to July 2014 women were inseminated on the day of the positive urinary LH test and the day after the positive urinary LH test (Centola et al. 1990). The double inseminations per cycle were performed with semen of the same donor, but semen could be from different donation dates. In these cases, we used the mean of the two semen parameters for analysis. Occasionally, women were inseminated once, because their insemination was scheduled on a Sunday. At that time, no inseminations were performed on Sunday. From July 2014 to December 2015 women were inseminated once, the day after the positive urinary LH test (Lincoln et al. 1995).

Data Analysis

We analysed to a maximum of 12 insemination cycles or until pregnancy occurred within 12 insemination cycles. We used ongoing pregnancy as pregnancy outcome, because it is a valid and effective outcome measure of effectiveness (Braakhekke et al. 2014). Ongoing pregnancy was defined as a positive heartbeat at or beyond 12 weeks of gestation and was confirmed by ultrasound. For this analysis we used the WHO criteria of 2010 to classify the semen parameters (WHO 2010). Total motility and TMC were available for analysis. We evaluated associations between semen parameters and the chances of ongoing pregnancy by means of restricted cubic splines regression analysis.

Spline functions were made to express the probability of ongoing pregnancy as a flexible function of the various semen parameters. Nonlinearity of the spline function was tested with analysis of variance statistics. Based on the spline functions we defined thresholds by univariate Cox regression analysis. A multivariate Cox regression analysis was performed for confounders possibly affecting the ongoing pregnancy rate like age of the sperm donor, female age and number of inseminations. A hazard rate can be read as an estimate of the relative risk accounting for time.

Results were expressed as hazard rates (HR) with corresponding 95% intervals. All analysis were done in STATA 14.2 (StataCorp, Texas, USA).

Results

Between April 1999 and December 2015, data from 1186 women who underwent 7103 cycles of intracervical insemination with donor semen of 129 sperm donors were available for analysis. Baseline characteristics of the women, the sperm donors and the semen parameters are summarized in table 1.

Table 1. Baseline characteristics of women and sperm donors who started DST (n=1186 women, n=129 sperm donors)

Continuous variables	Mean	5th-95th percentile
Female Age (y)	35.0	21-43
Age sperm donor (y)	36.3	18-51
Categorical variables	n	%
Heterosexual couples	313	26
Lesbian couples	301	25
Single women	434	37
Indication missing	138	12
One insemination per cycle	2374	34
Two inseminations per cycle	4729	66
Ongoing pregnancy per cycle	592	8,3
Ongoing pregnancy per woman	592	50
Semen parameters	Median	5th-95th percentile
Post thawed total motility, progressive (%)	25	5-63
Post thawed TMC (10 ⁶)	5.1	0.6-34

Ongoing pregnancy was defined as a positive heartbeat at or beyond 12 weeks of gestation.

Mean female age was 35 years. There were more single women present in this cohort than heterosexual and lesbian couples. Two inseminations were performed more often than one insemination per cycle. After 12 cycles of intracervical insemination 592 ongoing pregnancies were reported. The ongoing pregnancy rate was 8.3% per cycle and 50% per woman.

After intracervical insemination the mean number of cycles to achieve an ongoing pregnancy was 3.7 cycles (95% CI: 3.51-3.94). Total motility and TMC were nonlinearly associated with ongoing pregnancy rates (Figure 1A, 1B).

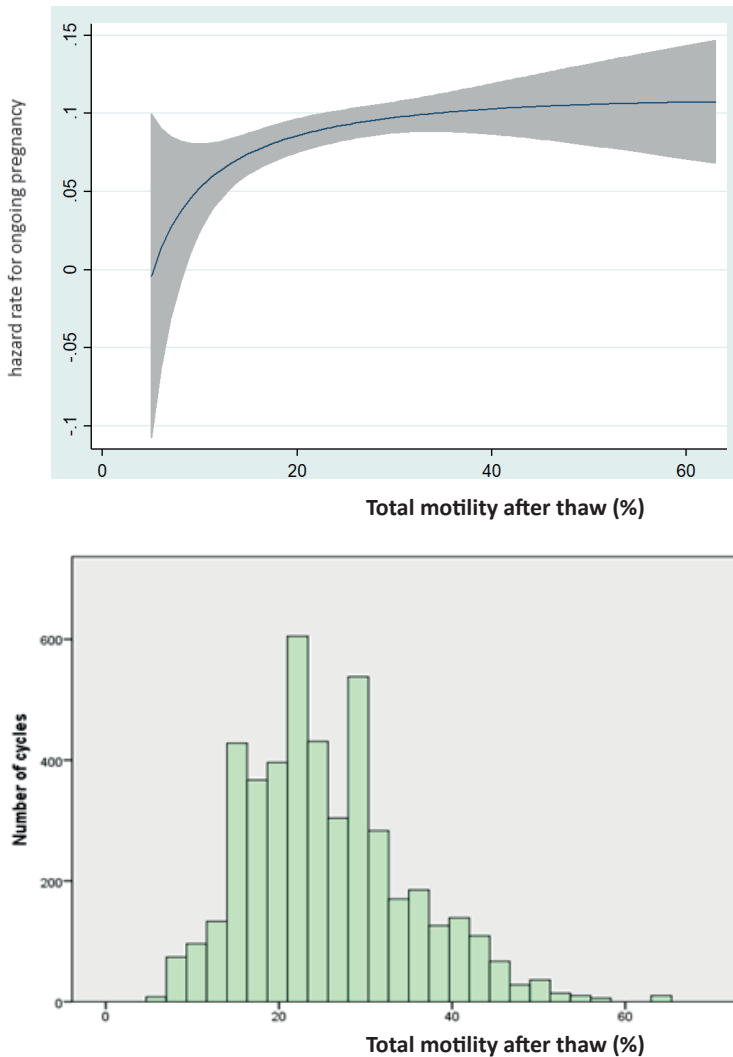
Based upon the spline function, we divided the TMC into five categories ranging from $<3 \times 10^6$ to $\geq 8 \times 10^6$ with the latter being the reference category. After adjusting for female age, two inseminations per cycle and age of the donor, chances of an ongoing pregnancy decreased with lower TMC and increased when women were younger or had two inseminations per cycle. Compared to inseminations with a TMC $\geq 8 \times 10^6$, a TMC of $3-4 \times 10^6$ and a TMC of $<3 \times 10^6$ resulted in similar ongoing pregnancy rates. Female age and age of the donor were divided in two categories < 35 years and ≥ 35 years. With women aged < 35 years as a reference, ongoing pregnancy rates were significantly lower for women aged ≥ 35 years (HR 0.76; 95% CI 0.64-0.89). There was no interaction between age of the sperm donor at time of donation and ongoing pregnancy rate (table 2).

For total motility and TMC- the semen parameters that were associated with ongoing pregnancy- we derived new dichotomous thresholds. Based upon these thresholds lower ongoing pregnancy chances were obtained at a total motility of < 20 % and a TMC $< 8 \times 10^6$.

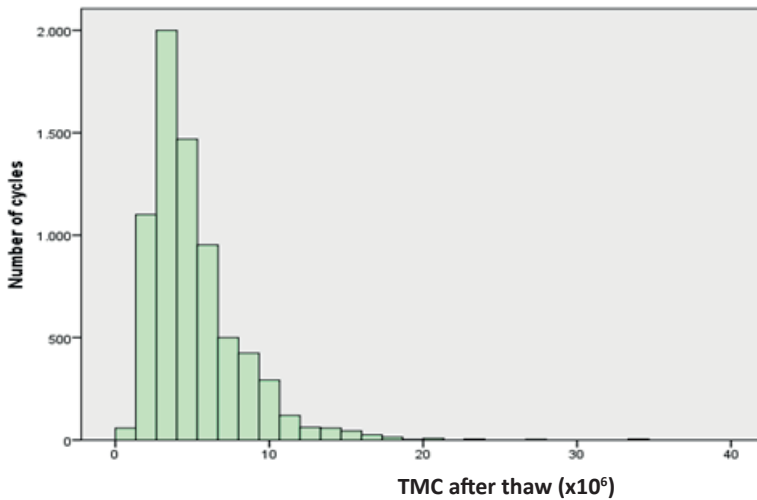
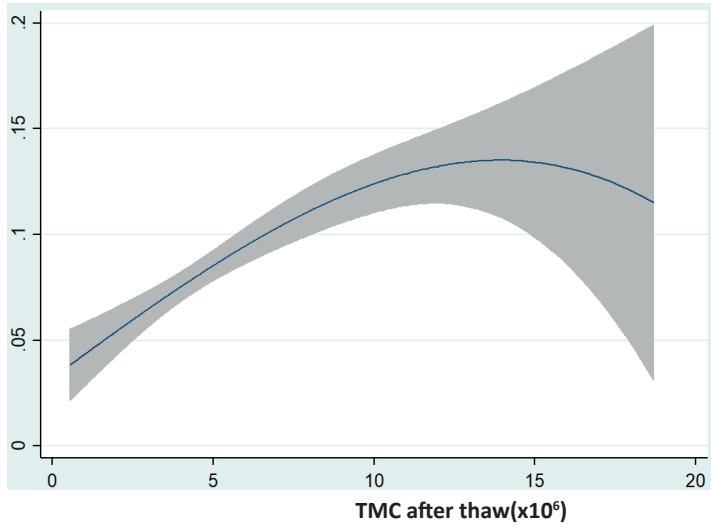
Frozen-thawed donor semen with a total motility of <20% resulted in an ongoing pregnancy rate of 7.7% per cycle, while frozen-thawed donor semen with a total motility of $\geq 20\%$ resulted in an ongoing pregnancy rate of 10% per cycle. Frozen-thawed donor semen with a TMC of $< 8 \times 10^6$ resulted in an ongoing pregnancy rate of 8% per cycle, while frozen-thawed donor semen with a TMC of $\geq 8 \times 10^6$ resulted in an ongoing pregnancy rate of 12% per cycle (Table 3).

Figure 1. Semen parameters and the probability of achieving an ongoing pregnancy.

A.



B.



Spline functions: grey area represents 95% confidence interval.

40 sperm donors had a TMC after thawing of ≥ 10 million

Only 95 cycles were performed with a TMC after thawing of ≥ 15 million

Table 2. Results of multivariate regression analysis after adjusting for factors influencing ongoing pregnancy rate

	HR adj	CI 95%
TMC $\geq 8 \times 10^6$	1.0	
TMC 6-8 $\times 10^6$	0.77	0.57-1.03
TMC 4-6 $\times 10^6$	0.67	0.52-0.86
TMC 3-4 $\times 10^6$	0.47	0.35-0.64
TMC $< 3 \times 10^6$	0.46	0.34-0.62
Female Age	0.96	0.94-0.98
Female age < 35 years	1.0	
Female age ≥ 35 years	0.76	0.64-0.89
Two inseminations per cycle	1.4	1.15-1.76
Age donor	1.0	0.99-1.02
Age donor < 35 years	1.0	
Age donor ≥ 35 years	0.9	0.74-1.10

Analysis done with and without imputation for missing indication values

Table 3. Pregnancy and semen parameters. The chance of achieving a pregnancy over time.

	N	%	HR	95% CI	OPR per cycle (%)
Semen Parameter					
Post thawed motility, progressive (%) continuous per 10% less					
< 20	1333	29	0.7	0.57-0.9	7,7
≥ 20	3190	71	1		10
Post thawed TMC (10^6) continuous per 10 $\times 10^6$ less					
< 8	6027	85	0.58	0.48-0.7	8
≥ 8	1056	15	1		12

Discussion

In view of the lower pregnancy rates with frozen-thawed donor semen compared to fresh donor semen in intracervical insemination, we searched for semen parameters that would result in the highest ongoing pregnancy chances with frozen-thawed donor semen. After thawing, a total motility of $\geq 20\%$ and a TMC $\geq 8 \times 10^6$ resulted in the best possible ongoing pregnancy chances. About 15% of the sperm donors in our cohort reached this cut off value. The strength of this study is that this large retrospective cohort provides -at this moment in time- the best evidence for the role of semen parameters for intracervical insemination with frozen-thawed donor semen. The internal validity - being a single centre study- is high, especially since selection of the sperm donors, freezing and thawing procedures and

insemination techniques did not undergo major changes over the years. Since we included all women with an indication for DST and did not limit our study to particular family types, we have ensured a reasonable generalizability of our data.

Our study also has several weaknesses. First, it is a retrospective study, which resulted in heterogeneous data due to differences in number of inseminations over time.

On the other hand, this design represents real life clinical practice. Second, data on certain basic characteristics of the women who started with DST are missing, like parity and tubal status. Nevertheless, we believe the impact of this to be minor, since main prognostic factor for pregnancy, i.e. female age, was incorporated in our analysis (Kop et al. 2018). Third, data on sperm morphology after thawing are lacking. Abnormal sperm morphology is seldom found as the only abnormality in a semen analysis, but is generally combined with a reduced total sperm count and/or a reduced semen volume and/or reduced sperm motility. Since sperm donors with an abnormal first semen analysis before freezing were rejected, it is unlikely that many sperm donors with abnormal morphology were included in this cohort. Finally, our data were not externally validated, necessary to further enhance the generalizability of our data. At present, we are performing a multi-center randomised controlled trial on intracervical insemination versus intrauterine insemination in the natural cycle with frozen-thawed donor semen (Kop et al., 2019). We will use the data of our randomised controlled trial to validate the thresholds for semen parameters in intracervical insemination from this cohort study. We aim to confirm or refute in our randomised controlled trial if intracervical inseminations with a TMC $\geq 8 \times 10^6$ resulted in higher ongoing pregnancy rates compared to intracervical inseminations performed with a TMC $< 8 \times 10^6$.

Some sperm donors in our cohort were aged over 45 years and this warrants discussion, since advanced paternal age has been suggested as a risk factor for ill health in their offspring. The correlation between advanced paternal age on adverse health outcomes like congenital abnormalities in the offspring has been observed in some studies, but is not necessarily caused by the advanced paternal age alone (Nybo Andersen et al. 2004, Yang et al. 2007, Dain et al. 2011, Harris 2019). Also, proper evidence on a cut-off for advanced paternal age is lacking. To keep the risks on congenital abnormalities to their offspring as low as possible balanced against the risk of “losing” too many potential donors, we advise in the Netherlands that sperm donors should not be aged over 45 when they start sperm donation (CBO 1992, NVOG 2018). Sperm donors in our cohort that were aged over 45 started donation before the age of 45 and continued their donation for years, specifically for second children.

Our study shows that hazard rates drop after a TMC after thawing ≥ 15 million, but with a wide confidence interval. Since only 95 inseminations (1%) were performed with a TMC ≥ 15 million, the drop may well be due to chance. Also, our study suggests that two inseminations per cycle result in more ongoing pregnancies compared to one insemination per cycle. Two randomised controlled trials on one insemination per cycle versus double inseminations per cycle showed conflicting results: one study showed that insemination twice every cycle resulted in higher ongoing pregnancy rates, whereas the other study showed no evidence of a difference (Centola et al. 1990, Lincoln et al. 1995). Therefore, double versus one intracervical insemination per cycle should be considered a topic for future research.

The clinical significance of our study is that it gives clear thresholds for semen parameters for intracervical insemination after freezing and thawing. Taking all limitations of the study design into account, we feel it justified to recommend a cutoff of the TMC $\geq 8 \times 10^6$ after thawing in donor sperm treatment with intracervical insemination.

The downside of this is that only 15% of the sperm donors in our cohort reached this cut off value, which will lead to less donor semen being available and create long waiting lists, unless one accepts somewhat compromised pregnancy rates. At the end of the day, these choices should be made after shared decision making based on women's preferences.

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CHAPTER 4

Intrauterine insemination versus intracervical insemination in donor sperm treatment

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Abstract

Background: The first-line treatment in donor sperm treatment consists of inseminations that can be done by intrauterine insemination (IUI) or by intracervical insemination (ICI).

Objectives: To compare the effectiveness and safety of intrauterine insemination (IUI) and intracervical insemination (ICI) in women who start donor sperm treatment.

Search methods: We searched the Cochrane Gynaecology and Fertility Group Trials Register, CENTRAL, MEDLINE, Embase, PsycINFO, CINAHL in October 2016, checked references of relevant studies, and contacted study authors and experts in the field to identify additional studies. We searched PubMed, Google Scholar, the Grey literature, and five trials registers on 15 December 2017.

Selection criteria: We included randomised controlled trials (RCTs) reporting on IUI versus ICI in natural cycles or with ovarian stimulation, and RCTs comparing different cointerventions in IUI and ICI. We included cross-over studies if pre-cross-over data were available.

Data collection and analysis: We used standard methodological procedures recommended by Cochrane. We collected data on primary outcomes of live birth and multiple pregnancy rates, and on secondary outcomes of clinical pregnancy, miscarriage, and cancellation rates.

Main results: We included six RCTs (708 women analysed) on ICI and IUI in donor sperm treatment. Two studies compared IUI and ICI in natural cycles, two studies compared IUI and ICI in gonadotrophin-stimulated cycles, and two studies compared timing of IUI and ICI. There was very low-quality evidence; the main limitations were risk of bias due to poor reporting of study methods, and serious imprecision. There was insufficient evidence to determine whether there was any clear difference in live birth rate between IUI and ICI in natural cycles (odds ratio (OR) 3.24, 95% confidence interval (CI) 0.12 to 87.13; 1 RCT, 26 women; very low-quality evidence). There was only one live birth in this study (in the IUI group). IUI resulted in higher clinical pregnancy rates (OR 6.18, 95% CI 1.91 to 20.03; 2 RCTs, 76 women; $I^2 = 48\%$; very low-quality evidence). No multiple pregnancies or miscarriages occurred in this study. There was insufficient evidence to determine whether there was any clear difference in live birth rate between IUI and ICI in gonadotrophin-stimulated cycles (OR 2.55, 95% CI 0.72 to 8.96; 1 RCT, 43 women; very low-quality evidence). This suggested that if the chance of a live birth following ICI in gonadotrophin-stimulated cycles was assumed to be 30%, the chance following IUI in gonadotrophin-stimulated cycles would be between 24% and 80%. IUI may result in higher clinical pregnancy rates than ICI (OR 2.83, 95% CI 1.38 to 5.78; 2 RCTs, 131 women; $I^2 = 0\%$; very low-quality evidence). IUI may be associated with

higher multiple pregnancy rates than ICI (OR 2.77, 95% CI 1.00 to 7.69; 2 RCTs, 131 women; $I^2 = 0\%$; very low-quality evidence). This suggested that if the risk of multiple pregnancy following ICI in gonadotrophin-stimulated cycles was assumed to be 10%, the risk following IUI would be between 10% and 46%. We found insufficient evidence to determine whether there was any clear difference between the groups in miscarriage rates in gonadotrophin-stimulated cycles (OR 1.97, 95% CI 0.43 to 9.04; 2 RCTs, overall 67 pregnancies; $I^2 = 50\%$; very low-quality evidence). *Timing of IUI and ICI.* We found no studies that reported on live birth rates. We found a higher clinical pregnancy rate when IUI was timed one day after a rise in blood levels of luteinising hormone (LH) compared to IUI two days after a rise in blood levels of LH (OR 2.00, 95% CI 1.14 to 3.53; 1 RCT, 351 women; low-quality evidence). We found insufficient evidence to determine whether there was any clear difference in clinical pregnancy rates between ICI timed after a rise in urinary levels of LH versus a rise in basal temperature plus cervical mucus scores (OR 1.31, 95% CI 0.42 to 4.11; 1 RCT, 56 women; very low-quality evidence). Neither of these studies reported multiple pregnancy or miscarriage rates as outcomes.

Authors' conclusions: There was insufficient evidence to determine whether there was a clear difference in live birth rates between IUI and ICI in natural or gonadotrophin-stimulated cycles in women who started with donor sperm treatment. There was insufficient evidence available for the effect of timing of IUI or ICI on live birth rates. Very low-quality data suggested that in gonadotrophin-stimulated cycles, ICI may be associated with a higher clinical pregnancy rate than IUI, but also with a higher risk of multiple pregnancy rate. We concluded that the current evidence was too limited to choose between IUI or ICI, in natural cycles or with ovarian stimulation, in donor sperm treatment.

Background

Description of the condition

Donor sperm treatment, or donor insemination, is the oldest fertility treatment for couples with involuntary childlessness due to male infertility (Seymour 1941).

Since the introduction of intracytoplasmic sperm injection (ICSI) with or without surgical sperm extraction, semen washing to prevent the transmission of human immunodeficiency virus, and preimplantation genetic diagnosis, it is possible for couples with male infertility, human immunodeficiency virus, or those who are carriers of a genetic defect to become parents of a child genetically related to both parents (Palermo et al. 1992, Semprini et al. 1992, Tournaye et al. 1994, Silber et al. 1996, Liebaers et al. 1998).

These newer types of medically-assisted reproduction have rendered donor sperm treatment a treatment option in couples after failure of surgical sperm retrieval, or ICSI. They may also be used to prevent transmission of human immunodeficiency virus after sperm washing has failed, and to prevent vertical transmission of a genetic defect. In lesbian couples or single women, it is a commonly used technique to achieve pregnancy (Vernaevae et al. 2005, NICE 2013).

Description of the intervention

Donor sperm can be introduced by intrauterine insemination (IUI) or by intracervical insemination (ICI). For both insemination techniques, the use of cryo-preserved sperm is mandatory to prevent transmission of sexually transmitted diseases, such as human immunodeficiency virus, and Hepatitis B and C, although pregnancy rates are lower compared to fresh sperm (Subak et al. 1992, ASRM 2013). The sperm can be inseminated in natural cycles or in cycles with ovarian stimulation.

In natural cycles, the timing of insemination may be determined with a basal body temperature chart in combination with cervical mucus scores (Inslar 1977). Another method to detect ovulation is by measuring urinary luteinising hormone (LH). The advantage of this test is that women can perform the urinary LH tests at home. Detection of a rise in the LH level can also be done in the clinic, with daily blood samples. Finally, transvaginal ultrasound, in combination with ovulation induction by the administration of human chorionic gonadotropin (hCG), may be used to time the insemination (Cantineau et al. 2014).

In cycles with ovarian stimulation, women receive clomiphene citrate, an anti-oestrogen, or gonadotrophins to induce the growth of up to three follicles. The timing of insemination is

determined by transvaginal ultrasound, combined with hCG-triggered ovulation.

The main difference between IUI and ICI is the processing of the sperm. In IUI, the sperm is processed. There are two preparation techniques; one is to freeze the sperm without processing, thaw the sperm when needed, and process the sperm against a density gradient centrifugation, wash it with culture medium, or both. The other is to process the sperm against a density gradient centrifugation, wash it with culture medium, or both, before freezing the sperm, and then thaw the sperm when needed. There is no evidence that one technique is superior to the other (Boomsma et al. 2007). After thawing, the sperm is inseminated into the uterine cavity.

In ICI, the sperm is cryo-preserved without processing, and thawed when needed. After thawing, the sperm is inseminated at the external cervical os, using a cap or a straw. The cervical cap acts as an ectocervical reservoir, placed in the cervical canal for a few hours (Coulson et al. 1996, Flierman et al. 1997).

How the intervention might work

In IUI, the sperm is processed and inseminated into the uterine cavity. In ICI, the sperm is not processed, and is inseminated at the external cervical os. The inseminated sperm then has to fertilize the released oocyte. Both IUI and ICI are often combined with ovarian stimulation; multiple pregnancies are a side effect, occurring in between 10% and 40% of women (Fauser et al. 2005).

Why it is important to do this review

The present review is an update and extension of a previous Cochrane review (Besselink et al. 2008). Besselink 2008 had compared IUI with ICI, and pooled the data of natural cycles with cycles with ovarian stimulation. In this update, we disentangled ovarian stimulation from the natural cycle, and added other cointerventions that might influence pregnancy rates in donor sperm treatment. It is unclear which insemination technique, IUI or ICI, is more effective in terms of live birth rate. Summarising the evidence on the effectiveness and safety of IUI and ICI in donor sperm treatment will help women and gynaecologists to make informed decisions about their choices for donor sperm treatment.

Objectives

To compare the effectiveness and safety of intrauterine insemination (IUI) and intracervical insemination (ICI) in women who start donor sperm treatment.

Methods

Criteria for considering studies for this review

Types of studies

We included randomised controlled trials. We analysed only the pre-cross-over data from cross-over trials. We excluded quasi-randomised controlled trials.

Types of participants

- women starting donor sperm treatment
- women undergoing donor sperm treatment who received treatment for anovulation

Types of interventions

- IUI or ICI in natural cycles
- IUI or ICI with ovarian stimulation
- cointerventions in IUI
- cointerventions in ICI

Types of outcome measures

Primary outcomes

1. Live birth rates - defined as delivery of a live fetus after 20 completed weeks of gestation.
2. Multiple pregnancy rates

Secondary outcomes

3. Ongoing pregnancy rates - defined as evidence of a gestational sac with fetal heart motion at 12 weeks, confirmed with ultrasound
4. Clinical pregnancy rates - defined as evidence of a gestational sac, confirmed by ultrasound
5. Miscarriage rates
6. Adverse effects, cancellation rates

If a study compared multiple cycles the cumulative outcomes were registered.

Search methods for identification of studies

We searched for all published and unpublished RCTs of IUI and ICI in donor sperm treatment, without language restriction.

We carried out all searches in consultation with the Gynaecology and Fertility Group (formerly Menstrual Disorders and Subfertility Group) Information Specialist.

Electronic searches

Marian Showell, Information Specialist, The Gynaecology and Fertility Group, developed the search strategies. See Appendix 1; Appendix 2; Appendix 3; Appendix 4; Appendix 5; Appendix 6

We searched the following electronic databases:

- The Cochrane Gynaecology and Fertility Group Trials Register, PROCITE platform (5 October 2016);
- The Cochrane Central Register of Controlled trials, via the Cochrane Register of Studies Online (CRSO Web platform) (5 October 2016);
- MEDLINE Ovid (1946 to 5 October 2016);
- Embase Ovid (1974 to 5 October 2016);
- PsycINFO Ovid (1806 to 5 October 2016);
- CINAHL EBSCO (Cumulative Index to Nursing and Allied Health Literature, 1982 to 5 October 2016).
- EU Clinical Trials Register - www.clinicaltrialsregister.eu/; World Health Organization International Trials Registry Platform search portal (WHO ICTRP; apps.who.int/trialsearch/); (5 October 2016).

On 15 December 2017, we searched the following databases and trials registers. See Appendix 7; Appendix 8; Appendix 9 for our search strategies.

Databases:

- PubMed and Google Scholar;
- Conference abstracts on the Web of Knowledge;
- Grey literature on Greylit.org;
- OpenGrey for unpublished literature for Europe at www.opengrey.eu.

Trial registers:

- U.S. National Library of Medicine - www.clinicaltrials.gov;
- EU Clinical Trials Register - www.clinicaltrialsregister.eu/;
- World Health Organization International Trials Registry Platform search portal (WHO ICTRP; apps.who.int/trialsearch/);
- BioMed Central register - www.isrctn.com/;
- Dutch trial register - www.trialregister.nl/.

Searching other resources

We handsearched reference lists of relevant trials and systematic reviews retrieved by the search, and contacted experts in the field to obtain additional data. We also handsearched relevant journals and conference abstracts that were not covered in the CGF register, in liaison with the Information Specialist.

Data collection and analysis

Selection of studies

Marian Showell conducted the initial screening of titles and abstracts retrieved by the search. We tried to retrieve the full text of all potentially eligible studies. If full texts were not available, we contacted the authors for further information. Two review authors (PK, MvW) independently examined the full text of articles for compliance with the inclusion criteria and selected eligible studies. We corresponded with study investigators as required, to clarify study eligibility. Disagreements were resolved by discussion. We planned that if any report required translation, we would describe the process used for data collection. We documented the selection process with a 'PRISMA' flow chart (Figure 1).

Data extraction and management

Two review authors (PK and MvW) independently extracted data from eligible studies using a data extraction form designed and pilot-tested by the authors. Any disagreements were resolved by discussion. We entered details of the studies into the 'Characteristics of included studies' table. We presented studies that appeared to meet the inclusion criteria but were excluded from the review in the 'Characteristics of excluded studies' table, briefly stating the reason for exclusion, but giving no further information. We corresponded with study investigators for further data on methods or results, as required.

Assessment of risk of bias in included studies

Two review authors (PK, MvW) independently extracted information regarding the risk of bias (threats to internal validity) under six domains (see the Cochrane 'Risk of Bias' assessment tool in Appendix 10 (Higgins 2011)).

1. Sequence generation. Evidence that an unpredictable random process was used.
2. Allocation concealment. Evidence that the allocation list was not available to anyone involved in the recruitment process.
3. Blinding of participants, clinicians, and outcome assessors. Evidence that knowledge of allocation was not available to those involved in subsequent treatment decisions or follow-up efforts.

4. Completeness of outcome data. Evidence that any losses to follow-up were low and comparable between groups.
5. Selective outcome reporting. Evidence that major outcomes had been reported in sufficient detail to allow analysis, independent of their apparent statistical significance. When data were obtained, but not reported in the paper, this was considered to be internal reporting bias.
6. Other potential sources. Evidence of miscellaneous errors or circumstances that might influence internal validity of trial results.

We sought missing details from the authors of the original publications. We present all details in the 'Risk of bias' table following each included study.

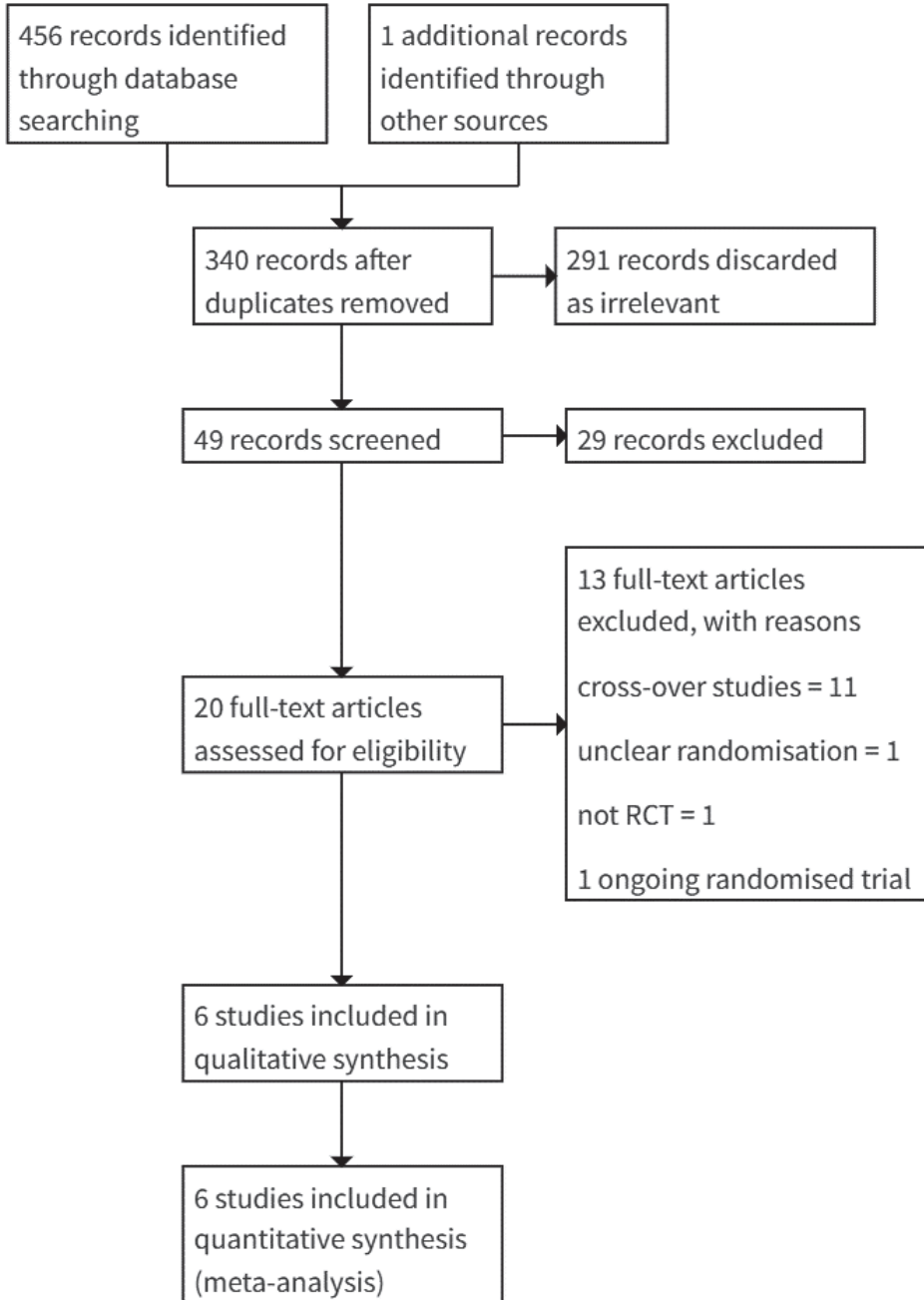
Measures of treatment effect

For dichotomous data, we used the number of events in the control and intervention groups of each study to calculate Mantel-Haenszel odds ratios (ORs). We presented 95% confidence intervals for all outcomes.

Unit of analysis issues

We expressed all outcomes per woman randomised. Where only data 'per cycle' were available without the number of women included, analysis was not possible, and we did not include the data. In the case of cross-over trials, we only included data from the first phase.

Figure 1. Study Flow diagram



Dealing with missing data

We analysed the data on an intention-to-treat basis as often as possible. When there was insufficient information in the published report, we attempted to contact the authors for clarification. If missing data became available, the data were included in the analysis. Where randomised participants were missing from outcome assessment, we first contacted the authors for additional data. If further data were not available, we assumed that missing participants had failed to achieve pregnancy, and had not experienced any of the reported adverse events. We anticipated that trials conducted over 10 years ago may not have data on live birth rates for the study participants.

Assessment of heterogeneity

We considered whether clinical and methodological characteristics of the included studies were sufficiently similar for meta-analysis to provide a clinically meaningful summary. The presence of statistical heterogeneity of treatment effect among trials was determined using the I^2 statistic (Higgins 2011). We took an I^2 measurement greater than 50% to indicate substantial heterogeneity.

Assessment of reporting biases

In view of the difficulty of detecting and correcting for publication bias and other reporting biases, we aimed to minimise their potential impact by ensuring a comprehensive search for eligible studies and by being alert for duplication of data. We had planned that if we included 10 or more studies in an analysis, we would use a funnel plot to explore the possibility of small-study effects (a tendency for estimates of the intervention effect to be more beneficial in smaller studies).

Data synthesis

When multiple studies were available on a similar comparison, we used Review Manager 5 software (Review Manager 2014) to perform the meta-analyses, using the Mantel-Haenszel method with a fixed-effect model. For reporting purposes, we translated primary outcomes to absolute risks.

Subgroup analysis and investigation of heterogeneity

We had not pre-planned subgroup analyses. If substantial heterogeneity ($I^2 > 50\%$) existed among studies, we planned to explore this informally using the clinical and design details recorded in the table 'Characteristics of included studies'.

Sensitivity analysis

We conducted sensitivity analyses for the primary outcomes to determine whether the conclusions were robust to arbitrary decisions made regarding study eligibility and analysis. These analyses included consideration of whether the review conclusions would have differed if:

- eligibility had been restricted to studies without high risk of bias;
- a random-effects model had been adopted;
- the summary effect measure had been risk ratio rather than odds ratio.

Overall quality of the body of evidence: ‘Summary of findings’ table

We prepared a ‘Summary of findings’ table using GRADEpro GDT and Cochrane methods (GRADEpro GDT; Higgins 2011). This table evaluates the overall quality of the body of evidence for the main review outcomes (live births, multiple pregnancy, clinical pregnancy, miscarriages, cancellation rate) for the main review comparison (IUI versus ICI). We prepared additional ‘Summary of findings’ tables for the main review outcomes for other important comparisons (timing of IUI, and timing of ICI). We assessed the quality of the evidence using GRADE criteria: risk of bias, consistency of effect, imprecision, indirectness, and publication bias). Two review authors independently made judgements about the quality of the evidence (high, moderate, low, or very low), resolving disagreements by discussion. They justified, documented, and incorporated judgments into reporting of results for each outcome.

Results

Description of studies

Results of the search

The search retrieved 456 references. We assessed 19 studies as potentially eligible and retrieved them in full text. We found six studies that met our inclusion criteria. We excluded 13 studies.

See Figure 1; ‘Characteristics of included studies’ table; ‘Characteristics of excluded studies’ table.

Included studies

We included six studies, all single centre randomised trials, that compared first-line treatment strategies in women who started with donor sperm treatment.

Design

All trials analysed more than one cycle per woman, with a maximum of six cycles. One study was a cross-over study, in which the cross-over was performed after one cycle. Therefore, we only used the first cycle for analysis (Hurd et al. 1993).

Only one study performed a power calculation (Blockeel et al. 2014).

Participants

Robinson 1992 and Matorras 1996 included couples with azoospermia or oligospermia (Patton et al. 1992, Matorras et al. 1996). Patton 1992 and Hurd 1993 also included single women (Patton et al. 1992, Hurd et al. 1993). Wainer 1995 included men with azoospermia, and men with a genetic defect (Wainer et al. 1995). Blockeel 2014 also included lesbian couples (Blockeel et al. 2014).

Interventions

Two studies compared intrauterine insemination (IUI) to intracervical insemination (ICI) in natural cycles, including the use of clomiphene citrate in cases of anovulation due to polycystic ovarian syndrome (PCOS) (Patton et al. 1992, Hurd et al. 1993). Two studies compared IUI to ICI in gonadotrophin-stimulated cycles (Wainer et al. 1995, Matorras et al. 1996). One study reported on the timing of ICI (Robinson et al. 1992), and one study reported on the timing of IUI (Blockeel et al. 2014).

Outcomes

Two studies reported data on live births (Hurd et al. 1993, Wainer et al. 1995) and three trials reported on multiple pregnancy rates (Hurd et al. 1993, Wainer et al. 1995, Matorras et al. 1996). Four studies reported clinical pregnancy rates as the primary outcome (Patton et al. 1992, Robinson et al. 1992, Matorras et al. 1996, Blockeel et al. 2014). Hurd 1993, Matorras 1996, and Wainer 1995 reported miscarriage rates (Hurd et al. 1993, Wainer et al. 1995, Matorras et al. 1996). Cancellation rates were not reported.

Excluded studies

We excluded thirteen trials: 11 trials because they were unable to provide pre-cross-over data (Urry et al. 1988, Byrd et al. 1990, Patton et al. 1990, Peters et al. 1993, Alexander et al. 1994, Williams et al. 1995, Coulson et al. 1996, Flierman et al. 1997, Carroll et al. 2001); two studies because they made no distinction between natural cycles and gonadotrophin-stimulated cycles (Le Lannou et al. 1989, Walker et al. 1993).

Risk of bias in included studies

We summarised the risks of bias in the included studies in Figure 2 and Figure 3.

Figure 2. ‘Risk of bias’ summary: review authors’ judgements about each risk of bias domain for each included study

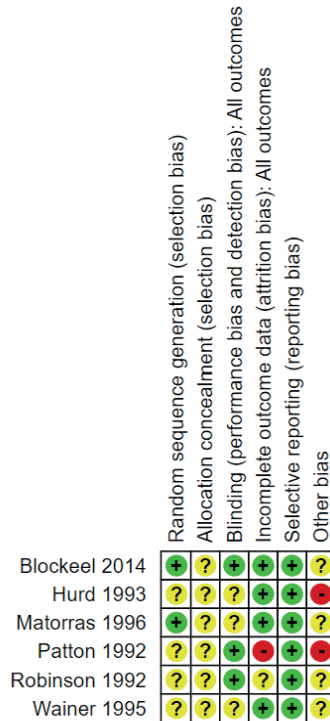
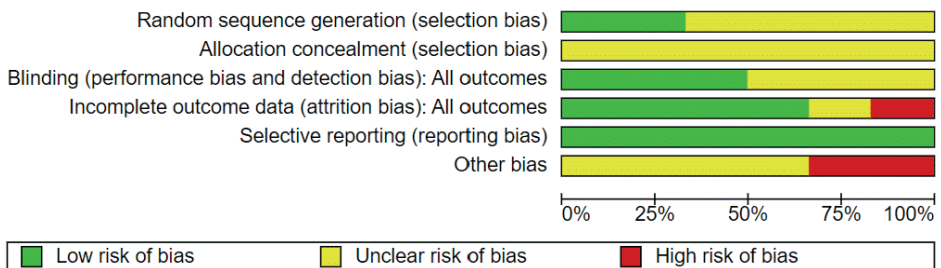


Figure 3. ‘Risk of bias’ graph: review authors’ judgements about each risk of bias domain presented as percentages across all included studies



Allocation

We rated two studies at low risk of selection bias for sequence generation, since they used computer randomisation or random number tables for sequence generation. In four studies, the method used for sequence generation was not fully described, and we rated them at unclear risk of selection bias in relation to sequence generation.

All studies failed to describe methods used for allocation concealment, and we rated these at unclear risk of bias for this domain.

Blinding

Blinding was not performed in any of the studies. We did not consider that blinding was likely to influence findings for the primary review outcomes (live birth and multiple pregnancy). Blinding might influence outcomes for other adverse events, but no studies reported relevant data for this outcome.

Incomplete outcome data

One trial had a high risk of attrition bias (Patton et al. 1992). For one trial, this was unclear (Robinson et al. 1992). The other four trials had a low risk of bias for this domain.

Selective reporting

We rated all six studies at low risk of selective reporting bias. All outcomes planned in the methods sections were reported.

Other potential sources of bias

We rated this as high for two studies, as these trials included some women with anovulation due to PCOS, who received ovulation induction with clomiphene citrate (Patton et al. 1992, Hurd et al. 1993). The number of women receiving clomiphene citrate was not described, and outcomes were not reported separately for this group, therefore, we could not perform subgroup analyses. We rated the risk of other potential biases as unclear for the other four studies. Baseline characteristics were not always provided, and were not perfectly balanced over the two treatment groups. Only one study mentioned the duration of the trial (Blockeel et al. 2014). Other studies did not report on study duration. Studies with a long duration can induce bias by creating heterogeneity in the study group.

Blockeel 2014 included women after one to six failed IUI attempts. This may have caused bias since women who became pregnant in the first cycles were not included in this study.

Effects of interventions

See: Table 1; Table 2; Table 3; Table 4

Table 1. Summary of findings for the main comparison. Intrauterine insemination versus intracervical insemination in natural cycles**Patient or population:** women starting donor sperm treatment**Settings:** outpatient clinic**Intervention:** IUI in natural cycles**Control:** ICI in natural cycles

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of evidence (GRADE)	Comments
	Assumed risk ICI	Corresponding risk IUI				
Live birth rate	Not estimable		OR 3.24 (0.12 to 87.13)	26 (1 study)	⊕⊕⊕ very low ^{1,2}	There was only one live birth in this study (in the IUI group)
Multiple pregnancy rate	No events		Not estimable	26 (1 study)	⊕⊕⊕ very low ^{1,2}	
Clinical pregnancy rate	143 per 1000	OR 3.24 (0.12 to 87.13)	26 (1 study)	⊕⊕⊕ very low ^{1,2}	There was only one live birth in this study (in the IUI group)	
Miscarriage rate	No events		Not estimable	26 (1 study)	⊕⊕⊕ very low ^{1,2}	
Cancellation rate	No available data					

*The basis for the **assumed risk** is the mean rate in the study population. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **OR:** Odds ratio;

GRADE Working Group grades of evidence

High quality: Further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: We are very uncertain about the estimate.

¹ Downgraded one level for serious risk of bias associated with poor reporting of study methods (unclear selection and other biases in the individual study)

² Downgraded two levels for very serious imprecision (effect estimate with an extremely wide confidence interval, low event rate, or both)

Table 2. Summary of findings. Intrauterine insemination versus intracervical insemination in gonadotrophin stimulated cycles.

Patient or population: women who are starting donor sperm treatment

Settings: outpatient clinic

Intervention: IUI in gonadotropin-stimulated cycles

Control: /CI in gonadotropin-stimulated cycles

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of evidence (GRADE)	Comments
	Assumed risk ICI	Corresponding risk IUI				
Live birth rate	300 per 1000	522 per 1000 (236 to 793)	OR 2.55 (0.72 to 8.96)	43 (1 study)	⊕⊕⊕⊕ very low ^{1,2}	
Multiple pregnancy rate	98 per 1000	232 per 1000 (98 to 456)	OR 2.77 (1 to 7.69)	131 (2 studies)	⊕⊕⊕⊕ very low ^{1,2}	
Clinical pregnancy rate	377 per 1000	631 per 1000 (455 to 778)	OR 2.83 (1.38 to 5.78)	131 (2 studies)	⊕⊕⊕⊕ very low ^{1,2}	
Miscarriage rate	33 per 1000	63 per 1000 (14 to 235)	OR 1.97 (0.43 to 9.04)	131 (2 studies)	⊕⊕⊕⊕ very low ^{1,2}	
Cancellation rate	No studies reported this outcome					

*The basis for the **assumed risk** is the mean rate in the study population. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; **OR:** Odds ratio;

GRADE Working Group grades of evidence

High quality: Further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: We are very uncertain about the estimate.

¹ Downgraded one level for serious risk of bias associated with poor reporting of study methods (unclear selection and other biases in the individual study)

² Downgraded two levels for very serious imprecision (effect estimate with an extremely wide confidence interval (wider than the interval (wider than the interval 0.5 to 2), or low event rate)

Table 3. Summary of findings. Timing of intrauterine insemination with donor sperm: insemination one day after the luteinising hormone (LH) surge versus insemination two days after the LH surge

Patient or population: women starting donor sperm treatment

Settings: outpatient clinic

Intervention: IUI with donor sperm one day after the LH surge (LH + 1)

Control: IUI with donor sperm two days after the LH surge (LH + 2)

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	LH+2	LH+1				
Live birth rate	No studies reported this outcome					
Multiple pregnancy rate	No studies reported this outcome					
Clinical pregnancy rate	130 per 1000	230 per 1000 (146 to 346)	OR 2 (1.14 to 3.53)	351 (1 study)	130 per 1000	
Miscarriage rate	No studies reported this outcome					
Cancellation rate	No studies reported this outcome					

*The basis for the **assumed risk** is the mean rate in the study population. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

CI: Confidence interval; OR: Odds ratio;

GRADE Working Group grades of evidence

High quality: Further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: We are very uncertain about the estimate.

¹ Downgraded one level for serious risk of bias associated with poor reporting of study methods (unclear selection and other biases in the individual study)

² Downgraded two levels for very serious imprecision (low event rate)

Table 4. Summary of findings. Timing for intrauterine insemination (ICI) with donor sperm: urinary luteinising hormone (LH) test versus temperature curve and cervical mucus score.

Patient or population: women starting donor sperm treatment
Settings: outpatient clinic
Intervention: urinary LH test
Control: temperature curve and cervical mucus score

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of participants (studies)	Quality of evidence (GRADE)	Comments
	Assumed risk temperature curve and cervical mucus score	Corresponding risk urinary LH test				
Live birth rate	No studies reported this outcome					
Multiple pregnancy rate	No studies reported this outcome					
Clinical pregnancy rate	276 per 1000	333 per 1000 (138 to 610)	OR 1.31 (0.42 to 4.11)	56 (1)	276 per 1000	
Miscarriage rate	No studies reported this outcome					
Cancellation rate	No studies reported this outcome					

*The basis for the **assumed risk** is the mean rate in the study population. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **OR:** Odds ratio;

GRADE Working Group grades of evidence

High quality: Further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.

Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.

Very low quality: We are very uncertain about the estimate.

¹Downgraded one level for serious risk of bias associated with poor reporting of study methods (unclear selection and other biases in the individual study).

²Downgraded two levels for very serious risk associated with serious imprecision and indirectness (effect estimate with wide confidence interval (wider than the interval 0.5 to 2)).

1. Intrauterine insemination (IUI) versus intracervical insemination (ICI)

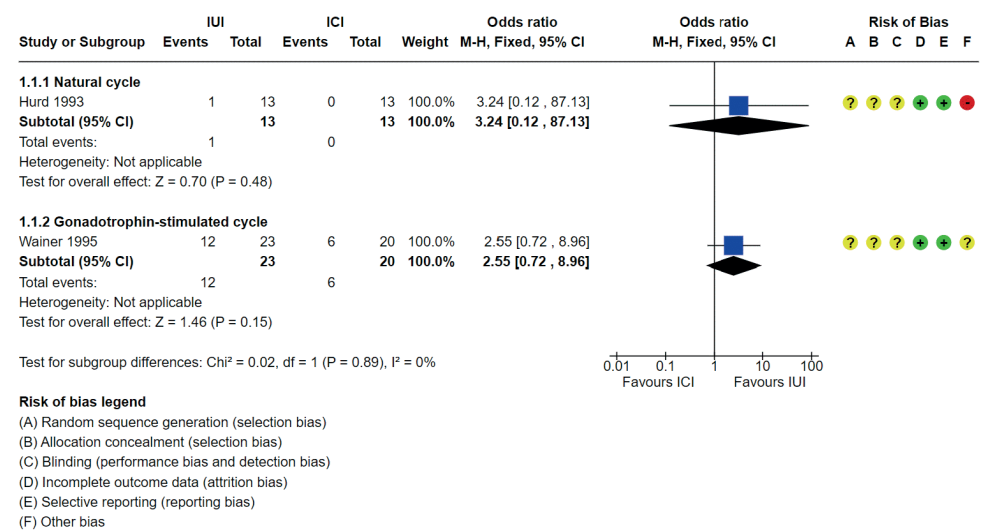
See Table 1, Table 2.

Primary outcomes

1.1 Live birth rate

(Figure 4)

Figure 4. Forest plot of comparison: 1 Intrauterine insemination (IUI) versus intracervical insemination (ICI), outcome: 1.1 Live birth rate.



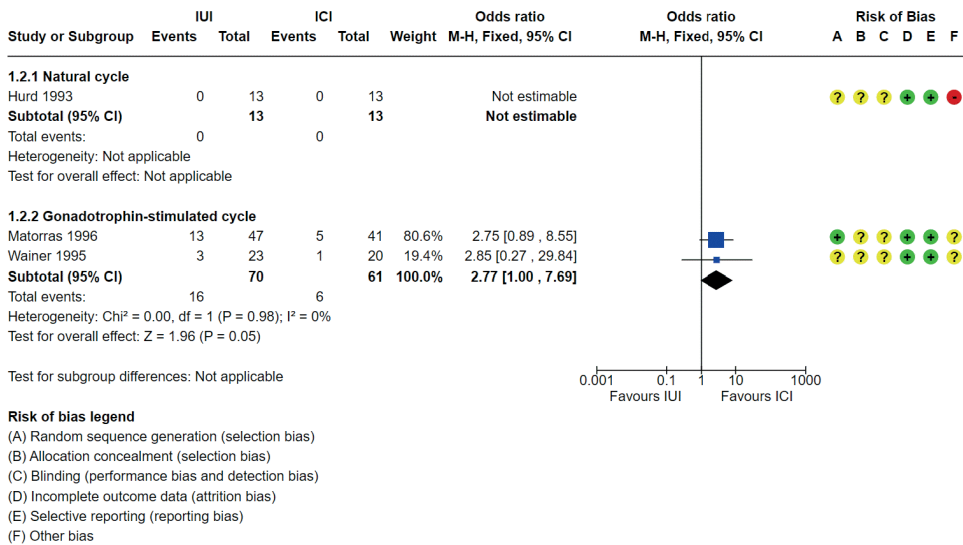
Two randomised controlled trials (RCT), including 69 women, reported on live births (Hurd et al. 1993, Wainer et al. 1995). There was insufficient evidence to determine whether there was any clear difference in live birth rates in natural cycles after IUI or ICI (OR 3.24, 95% CI 0.12 to 87.13; 1 RCT, 26 women; Analysis 1.1; very low-quality evidence (Hurd et al. 1993)). There was also insufficient evidence to determine whether there was any difference in live birth rates in gonadotrophin-stimulated cycles after IUI or ICI (OR 2.55, 95% CI 0.72 to 8.96; 1 RCT, 43 women; Analysis 1.1; very low-quality evidence (Wainer et al. 1995)). This suggested that if the chance of a live birth following ICI in gonadotrophin-stimulated cycles was assumed to be 30%, the chance following IUI in gonadotrophin-stimulated cycles would be between 24% and 80%.

Sensitivity analyses - as only one study reported on live birth the only sensitivity analysis possible was to determine the risk ratio. The RR for live birth was 1.83 (95% CI 0.86 to 3.91) for IUI versus IC.

1.2 Multiple pregnancy rate

(Figure 5)

Figure 5. Forest plot of comparison: 1 Intrauterine insemination (IUI) versus intracervical insemination (ICI), outcome: 1.2 Multiple pregnancy rate.



For IUI and ICI in natural cycles, no multiple pregnancies were reported. ICI in gonadotrophin-stimulated cycles was associated with lower multiple pregnancy rates than IUI in gonadotrophin-stimulated cycles (OR 2.77, 95% CI 1.00 to 7.69; 2 RCTs, 131 women; I² = 0%; Analysis 1.2; very low-quality evidence). This suggested that if the risk of a multiple pregnancy following ICI in gonadotrophin-stimulated cycles was assumed to be 10%, the risk following IUI gonadotrophin-stimulated cycles would be between 11% and 35%.

Sensitivity analyses - as only two studies reported on multiple pregnancy per woman we used a random effect model and determined the risk ratio. Pooling data using a random effect model resulted in exactly the same OR of 2.77 (95% CI 1.00 to 7.69). The risk ratio for multiple pregnancy per woman was 2.32 (95% CI 0.98 to 5.53) for IUI versus ICI.

Secondary outcomes

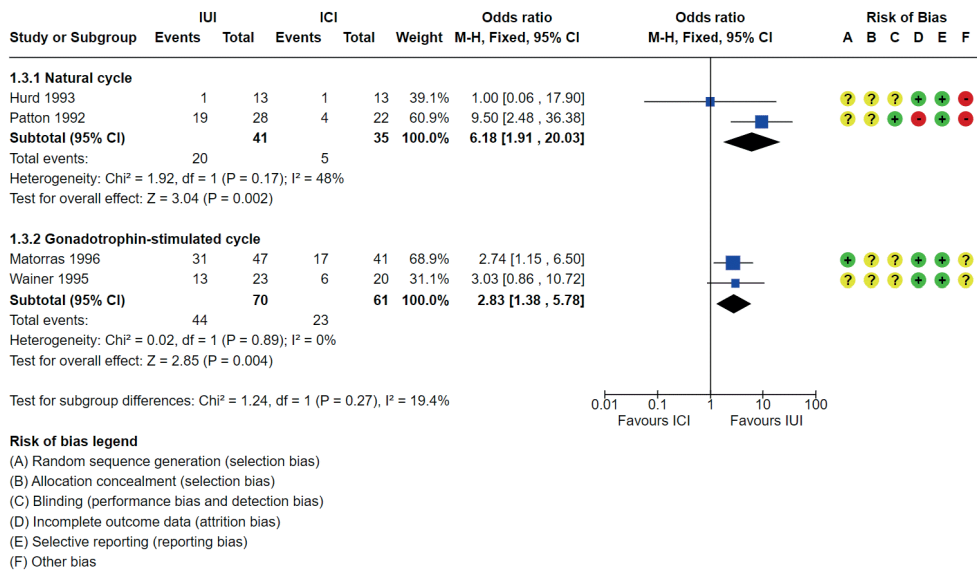
1.3 Ongoing pregnancy rate

No studies reported on ongoing pregnancy rate.

1.4. Clinical pregnancy rate

(Figure 6)

Figure 6. Forest plot of comparison: 1 Intrauterine insemination (IUI) versus intracervical insemination (ICI), outcome: 1.3 Clinical pregnancy rate



Four studies, including 207 women, reported on clinical pregnancy (Patton et al. 1992, Hurd et al. 1993, Wainer et al. 1995, Matorras et al. 1996).

There was a higher clinical pregnancy rate after IUI compared to ICI in natural cycles (OR 6.18, 95% CI 1.91 to 20.03; 2 RCTs, 76 women; I² = 48%; Analysis 1.3; very low-quality evidence (Patton et al. 1992, Hurd et al. 1993)).

There was a higher clinical pregnancy rate after IUI compared to ICI in gonadotrophin-stimulated cycles (OR 2.83, 95% CI 1.38 to 5.78; 2 RCTs, 131 women; I² = 0%; Analysis 1.3; very low-quality evidence (Wainer et al. 1995, Matorras et al. 1996)).

1.5 Miscarriage rate

Three studies, including 157 women, reported on miscarriage (Hurd et al. 1993, Wainer et al. 1995, Matorras et al. 1996).

In natural cycles, no miscarriages were reported (Hurd et al. 1993).

There was insufficient evidence to determine whether there was any clear difference in miscarriage rates after IUI compared to ICI in gonadotrophin-stimulated cycles (OR 1.97, 95% CI 0.43 to 9.04; 2 RCTs, overall 67 pregnancies; $I^2 = 50\%$; Analysis 1.4; very low-quality evidence ((Wainer et al. 1995, Matorras et al. 1996).

1.6 Adverse effects, cancellation rate

Cancellation rates were not reported.

2. Timing of intrauterine insemination (IUI)

See Table 3.

One study reported on the timing of IUI (Blockeel et al. 2014). IUI was performed one day after a rise in luteinising hormone (LH) blood levels compared to two days after a rise in LH blood levels.

Primary outcomes

2.1. Live birth rate

No studies reported on live birth rates.

2.2. Multiple pregnancy rate

No studies reported on multiple pregnancy rates.

Secondary outcomes

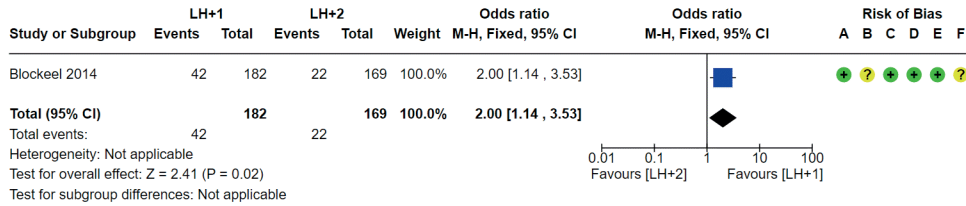
2.3. Ongoing pregnancy rate

No studies reported on ongoing pregnancy rates.

2.4. Clinical pregnancy rate

(Figure 7)

Figure 7. Forest plot of comparison: 2 Timing of intrauterine insemination (IUI) with donor sperm: luteinising hormone (LH) + 1 day versus LH + 2 days, outcome: 2.3 Clinical pregnancy rate



Risk of bias legend

- (A) Random sequence generation (selection bias)
- (B) Allocation concealment (selection bias)
- (C) Blinding (performance bias and detection bias)
- (D) Incomplete outcome data (attrition bias)
- (E) Selective reporting (reporting bias)
- (F) Other bias

One study, including 351 women, reported on clinical pregnancy rate and the timing of IUI (Blockeel et al. 2014).

We found a higher clinical pregnancy rate when IUI was performed one day after a rise in LH blood levels compared to two days after a rise in LH blood levels (OR 2.00, 95% CI 1.14 to 3.53; 1 RCT, 351 women; Analysis 2.3; low-quality evidence (Blockeel et al. 2014).

2.5. Miscarriage rate

No studies reported on miscarriage rates.

2.6 Adverse effects, cancellation rate

No studies reported these outcomes.

3. Timing of intracervical insemination (ICI)

See Table 4.

One study reported on the timing of ICI (Robinson et al. 1992). ICI performed following a rise in urinary luteinising hormone (LH) levels was compared to ICI performed after a rise in basal temperature in combination with cervical mucus scores.

Primary outcomes

3.1. Live birth rate

No studies reported on live birth rates.

3.2. Multiple pregnancy rate

No studies reported on multiple pregnancy rates.

Secondary outcomes

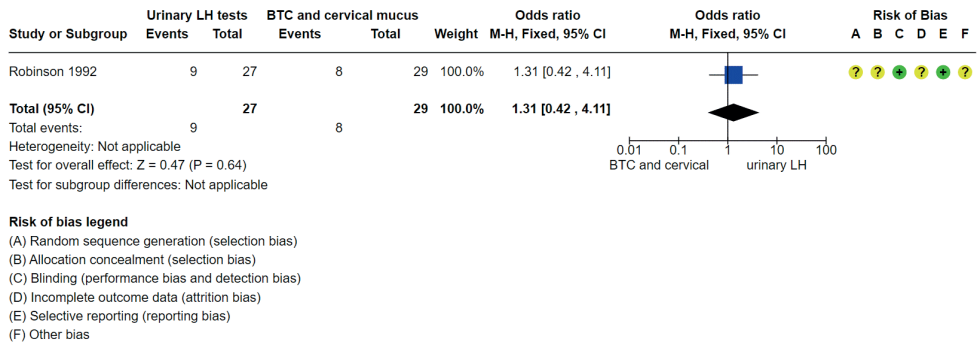
3.3. Ongoing pregnancy rate

No studies reported on ongoing pregnancy rates.

3.4. Clinical pregnancy rate

(Figure 8)

Figure 8. Forest plot of comparison: 3 Timing of intracervical insemination (ICI) with donor sperm: urinary luteinising hormone (LH) test versus temperature curve and cervical mucus score, outcome: 3.2 Clinical pregnancy rate



One study, including 56 women, reported on clinical pregnancy rate and timing of ICI (Robinson et al. 1992).

There was insufficient evidence to determine whether there was any clear difference in clinical pregnancy rate after ICI was performed following a rise in urinary LH levels compared to a rise in basal temperature in combination with cervical mucus scores (OR 1.31, 95% CI 0.42 to 4.11; 1 RCT, 56 women; Analysis 3.2; very low-quality evidence (Robinson et al. 1992)).

3.5. Miscarriage rate

No studies reported on miscarriage rates.

3.6 Adverse effects, cancellation rates

No studies reported on these outcomes.

Assessment of reporting biases

We were unable to assess the risk of reporting biases because there were insufficient data to construct a funnel plot.

Discussion

Summary of main results

In this systematic review, we selected studies that provided data on the effectiveness and safety of intrauterine insemination (IUI) and intracervical insemination (ICI) in donor sperm treatment. We found four studies that compared IUI and ICI, two of which performed IUI and ICI in natural cycles, and two performed IUI and ICI in gonadotrophin-stimulated cycles.

There was insufficient evidence to determine whether there was any real difference in live birth rates between IUI and ICI in natural cycles. There were more clinical pregnancies following IUI in natural cycles compared to ICI in natural cycles. There were no multiple pregnancies reported following IUI or ICI in natural cycles. Also, there was insufficient evidence to determine whether there was any clear difference in live birth rates between IUI and ICI in gonadotrophin-stimulated cycles. There were more clinical pregnancies following IUI than ICI in gonadotrophin stimulated cycles. IUI was associated with higher multiple pregnancy rates than ICI in gonadotrophin-stimulated cycles.

We found two studies on timing of insemination; one on timing of ICI and one on timing of IUI. None of the studies reported on the primary outcomes of live birth and multiple pregnancy rates.

Overall completeness and applicability of evidence

The studies included in this review were clinically heterogeneous. Studies differed in population, intervention, and laboratory techniques, and used different inclusion criteria. Two studies included couples with azoospermia or oligospermia, one study included couples with azoospermia and men with a genetic defect, two studies included single women, and one study included lesbian couples. This may have biased outcomes, since it is known that in heterosexual couples, partners of azoospermic men conceive more quickly after donor sperm treatment, compared to partners of men with spermatozoa in their ejaculates, suggesting that in heterosexual couples, unknown female factors also contribute to subfertility of these couples (NICE 2013).

One study included women after one to six failed IUI attempts. This may have caused bias, since women who became pregnant in the first cycles were not included in this study (Blockeel et al. 2014).

The studies also differed in treatment regimens. One study started ovarian stimulation on the second day of the menstrual cycle with 150 IU of recombinant human follicle stimulating hormone (recFSH (Matorras et al. 1996)) while one study started on the fourth or fifth day of the menstrual cycle with 75 IU of human menopausal gonadotrophin (hMG) followed by 150 IU of hMG on the sixth and seventh day (Wainer et al. 1995). Human chorionic gonadotropin (hCG) was administered on the day that two or more follicles were larger than 17 mm and oestrogen levels were higher than 400 pg/mL, and IUI was performed 36 hours after ovulation was triggered (Matorras et al. 1996). Another study administered hCG if one to three follicles were larger than 15 mm, and IUI was performed 38 hours after ovulation was triggered (Wainer et al. 1995). In ICI with ovarian stimulation, insemination was performed twice, with a cervical cap — 12 and 36 hours after ovulation was triggered. Cancellation of the cycle was mentioned in one study when more than six follicles reached 17 mm or more (Matorras et al. 1996). Studies on IUI and ICI in natural cycles performed one insemination, on the day after the urinary luteinising hormone (LH) level surge. In ICI, inseminations were performed with a straw. The number of motile sperm used for insemination differed between studies, from 1.58 million post wash to 43.7 million. It was difficult to assess if differences in sperm count influenced pregnancy outcomes, since studies on the effect of sperm count on pregnancy rates in donor sperm treatment are lacking.

One study in natural cycles, and one study in gonadotrophin-stimulated cycles did report on live births, and one study in natural cycles and two studies in gonadotrophin-stimulated cycles did report on multiple pregnancy rates. Most of these trials were published between 1992 and 1993, when clinical pregnancy rate was still an accepted endpoint. Nowadays, it is common to use live birth as a primary outcome, and multiple pregnancies as a reflection of the quality of care or safety.

Finally, studies on IUI or ICI that compared natural cycles with gonadotrophin-stimulated cycles were lacking.

In conclusion, it was uncertain which insemination technique, IUI or ICI, resulted in higher live birth rates. Differences in inclusion criteria, treatment regimens, number of motile sperm used for insemination, primary outcomes, and small study sizes made it difficult to compare studies on the effectiveness and safety of IUI and ICI in natural cycles. Moreover, studies on IUI and ICI in gonadotrophin-stimulated cycles used differences in stimulation protocols, cancellation rates for the number of follicles, and number of motile sperm used for insemination.

Quality of the evidence

Following the GRADE assessment, we found that evidence for all outcomes was of very low quality; the main limitations were risk of bias, mainly due to poor reporting of study methods, and serious imprecision. None of the studies described the method they used for allocation concealment, we judged one study to have a high attrition bias, and two to have high risk of potential bias associated with the inclusion of anovulatory women receiving clomiphene citrate.

Potential biases in the review process

Strengths of this review included a comprehensive systematic search for eligible studies, rigid inclusion criteria for RCTs, and independent data extraction and analysis by two review authors. The possibility of publication bias was minimized by searching for both published and unpublished studies, such as abstracts from meetings. We may have introduced bias in our assumptions about missing data. We assumed that if further data were not available, missing participants had failed to achieve pregnancy. This may have caused under-reporting of pregnancies and adverse events. As with any review, we cannot guarantee that we found all eligible studies.

One of our peer reviewers noted that our review did not include studies of cointerventions associated with donor sperm treatment, apart from comparisons of the timing. Potential cointerventions are luteal support, type of trigger, and single versus double insemination. These comparisons were outside the planned scope of the current review, and we were not aware of any relevant randomised evidence. However, we may consider extending the scope of the review in future updates.

Agreements and disagreements with other studies or reviews

We added RCTs on the timing of IUI and ICI to the previous Cochrane review, which focused their conclusions on clinical pregnancy (Besselink et al. 2008). Our results concerning pregnancy outcomes were comparable.

A previous non-Cochrane review and meta-analysis suggested that IUI resulted in higher clinical pregnancy rates than ICI (Goldberg et al. 1999). The authors pooled data of the four RCTs that were also included in our review with the results of three cross-over studies. As pre-cross-over data were not retrievable, these results could not validly be used.

Authors' conclusions

Implications for practice

There was insufficient evidence to determine whether there was a clear difference in live birth rates between intrauterine insemination (IUI) and intracervical insemination (ICI) in natural or gonadotrophin-stimulated cycles in women who started donor sperm treatment. There was no evidence available for the effect of timing on live birth following either IUI or ICI. Very low-quality evidence suggested that in gonadotrophin-stimulated cycles, ICI may be associated with a higher clinical pregnancy rate than IUI, but also with a higher risk of multiple pregnancy.

We concluded that the evidence was too limited to choose between IUI or ICI, in natural cycles or with ovarian stimulation, in donor sperm treatment.

Implications for research

We suggest that further research is needed. Most women requiring donor sperm treatment are fertile women. In these women, primary treatment should be aimed at facilitating conception leading to a live birth without increasing the risks of multiple pregnancy rates. Therefore, we suggest that further research should focus on (cost) effectiveness of IUI versus ICI in natural cycles in women who are starting donor sperm treatment.

Appendices

Appendix 1. Cochrane Gynaecology and Fertility specialised register search strategy

From inception to 5 October 2016

Procite platform

Keywords CONTAINS "insemination" or "insemination-artifical by donor" or "artifical insemination by donor" or "artifical insemination by partner" or "artifical insemination" or "donor insemination" or "donor semen" or "donors" or "insemination-donor" or Title CONTAINS "insemination" or "insemination-artifical by donor" or "artifical insemination by donor" or "artifical insemination by partner" or "artifical insemination" or "donor insemination" or "donor semen" or "donors" or "insemination-donor"

AND

Keywords CONTAINS "insemination-cervical cap" or "insemination, intracervical" or "insemination-pericervical" or "cervical" or "cervical cap" or "cervix" or "pericervical" or

“pericervical insemination” or “intracervical” or “intracervical insemination” or “cervical cap reservoir” or “insemination-intrauterine” or “insemination techniques” or Title CONTAINS “insemination-cervical cap” or “insemination, intracervical” or “insemination-pericervical” or “cervical” or “cervical cap” or “cervix” or “pericervical” or “pericervical insemination” or “intracervical” or “intracervical insemination” or “cervical cap reservoir” or “insemination-intrauterine” or “insemination techniques” (178 hits)

Appendix 2. Central Register of Studies Online search strategy

From inception to 5 October 2016

CRSO web platform

#1 MESH DESCRIPTOR Insemination, Artificial EXPLODE ALL TREES 324

#2 inseminat*:TI,AB,KY 985

#3 #1 OR #2 985

#4 intrauterine:TI,AB,KY 2737

#5 intra-uterine:TI,AB,KY 190

#6 intracervical:TI,AB,KY 427

#7 intra-cervical:TI,AB,KY 32

#8 pericervical:TI,AB,KY 12

#9 peri-cervical:TI,AB,KY 0

#10 (cervix or cervical):TI,AB,KY 10819

#11 (cup or cap):TI,AB,KY 2218

#12 ICI:TI,AB,KY 359

#13 straw:TI,AB,KY 74

#14 #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 16048

#15 #3 AND #14 679

#16 MESH DESCRIPTOR Insemination, Artificial, Heterologous EXPLODE ALL TREES 39

#17 (Heterologous adj5 Inseminat*):TI,AB,KY 4

#18 donor*:TI,AB,KY 5746

#19 (sperm* adj5 donat*):TI,AB,KY 5

#20 (semen adj5 donat*):TI,AB,KY 1

#21 #16 OR #17 OR #18 OR #19 OR #20 5757

#22 #15 AND #21 45

Appendix 3. MEDLINE search strategy

1946 to 5 October 2016

Ovid platform

- 1 exp Insemination, Artificial/ (10658)
- 2 inseminat\$.tw. (16412)
- 3 euteleogenesis.tw. (6)
- 4 or/1-3 (20081)
- 5 IUI.tw. (1429)
- 6 intrauterine.tw. (45130)
- 7 intra-uterine.tw. (4231)
- 8 intracervical.tw. (807)
- 9 intra-cervical.tw. (68)
- 10 pericervical.tw. (64)
- 11 peri-cervical.tw. (9)
- 12 (cervix or cervical).tw. (212205)
- 13 (cup or cap).tw. (48778)
- 14 ICI.tw. (7123)
- 15 straw.tw. (6998)
- 16 or/5-15 (321428)
- 17 4 and 16 (3340)
- 18 exp Insemination, Artificial, Heterologous/ (1506)
- 19 (Heterologous adj5 Inseminat\$).tw. (115)
- 20 donor\$.tw. (257425)
- 21 (sperm\$ adj5 donat\$).tw. (374)
- 22 (semen adj5 donat\$).tw. (124)
- 23 or/18-22 (258230)
- 24 17 and 23 (314)
- 25 randomized controlled trial.pt. (432375)
- 26 controlled clinical trial.pt. (91772)
- 27 randomized.ab. (372212)
- 28 randomised.ab. (76436)
- 29 placebo.tw. (184362)
- 30 clinical trials as topic.sh. (179773)
- 31 randomly.ab. (264844)

- 32 trial.ti. (163027)
- 33 (crossover or cross-over or cross over).tw. (71410)
- 34 or/25-33 (1124241)
- 35 exp animals/ not humans.sh. (4323394)
- 36 34 not 35 (1036540)
- 37 24 and 36 (50)

Appendix 4. Embase search strategy

1974 to 5 October 2016

Ovid platform

- 1 exp artificial insemination/ (16958)
- 2 inseminat\$.tw. (17699)
- 3 euteleogenesis.tw. (3)
- 4 or/1-3 (23413)
- 5 IUI.tw. (2460)
- 6 intrauterine.tw. (57192)
- 7 intra-uterine.tw. (5601)
- 8 intracervical.tw. (974)
- 9 intra-cervical.tw. (95)
- 10 pericervical.tw. (97)
- 11 peri-cervical.tw. (12)
- 12 (cervix or cervical).tw. (252399)
- 13 (cup or cap).tw. (61244)
- 14 ICI.tw. (13529)
- 15 straw.tw. (8052)
- 16 or/5-15 (394288)
- 17 4 and 16 (4521)
- 18 (Heterologous adj5 Inseminat\$.tw. (101)
- 19 donor\$.tw. (335280)
- 20 (sperm\$ adj5 donat\$.tw. (546)
- 21 (semen adj5 donat\$.tw. (154)
- 22 or/18-21 (335690)
- 23 17 and 22 (424)
- 24 Clinical Trial/ (970336)
- 25 Randomized Controlled Trial/ (450491)

- 26 exp randomization/ (82635)
- 27 Single Blind Procedure/ (25645)
- 28 Double Blind Procedure/ (134838)
- 29 Crossover Procedure/ (52902)
- 30 Placebo/ (317470)
- 31 Randomi?ed controlled trial\$.tw. (145345)
- 32 Rct.tw. (21721)
- 33 random allocation.tw. (1600)
- 34 randomly allocated.tw. (26146)
- 35 allocated randomly.tw. (2188)
- 36 (allocated adj2 random).tw. (837)
- 37 Single blind\$.tw. (18313)
- 38 Double blind\$.tw. (170634)
- 39 ((treble or triple) adj blind\$.tw. (619)
- 40 placebo\$.tw. (243788)
- 41 prospective study/ (376485)
- 42 or/24-41 (1734183)
- 43 case study/ (90742)
- 44 case report.tw. (317914)
- 45 abstract report/ or letter/ (978017)
- 46 or/43-45 (1377711)
- 47 42 not 46 (1684913)
- 48 23 and 47 (47)

Appendix 5. **PsycINFO search strategy**

1806 to 5 October 2016

Ovid platform

- 1 exp Reproductive Technology/ (1306)
- 2 inseminat\$.tw. (743)
- 3 euteleogenesis.tw. (1)
- 4 or/1-3 (1802)
- 5 IUI.tw. (20)
- 6 intrauterine.tw. (1383)
- 7 intrauterine.tw. (152)
- 8 intracervical.tw. (3)

- 9 (cervix or cervical).tw. (5403)
 10 (cup or cap).tw. (2521)
 11 ICI.tw. (483)
 12 straw.tw. (599)
 13 or/5-12 (10511)
 14 4 and 13 (28)
 15 random.tw. (40620)
 16 control.tw. (315260)
 17 double-blind.tw. (17867)
 18 clinical trials/ (7614)
 19 placebo/ (3778)
 20 exp Treatment/ (580009)
 21 or/15-20 (885819)
 22 14 and 21 (12)

Appendix 6. CINAHL search strategy

1982 to 5 October 2016

EBSCO platform

#	Query	Results
S23	S10 AND S22	37
S22	S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21	1,078,367
S21	TX allocat* random*	5,276
S20	(MH "Quantitative Studies")	14,878
S19	(MH "Placebos")	9,823
S18	TX placebo*	39,610
S17	TX random* allocat*	5,276
S16	(MH "Random Assignment")	41,649
S15	TX randomi* control* trial*	110,479
S14	TX ((singl* n1 blind*) or (singl* n1 mask*)) or TX ((doubl* n1 blind*) or (doubl* n1 mask*)) or TX ((tripl* n1 blind*) or (tripl* n1 mask*)) or TX ((trebl* n1 blind*) or (trebl* n1 mask*))	854,497
S13	TX clinic* n1 trial*	189,861
S12	PT Clinical trial	79,719
S11	(MH "Clinical Trials+")	203,253
S10	S3 AND S9	150
S9	S4 OR S5 OR S6 OR S7 OR S8	18,645
S8	TX semen N2 donat*	9
S7	TX sperm* N2 donat*	208
S6	TX donor*	18,535
S5	TX Heterologous N2 inseminat*	3

S4	(MM "Sperm Donation")	113
S3	S1 OR S2	643
S2	TX inseminat*	643
S1	(MM "Insemination, Artificial")	256

Appendix 7. **Trial registers search strategy**

We searched five trial registers on 15 December 2017, using the terms 'insemination and sperm' and 'insemination and donor'.

- U.S. National Library of Medicine - www.clinicaltrials.gov
- EU Clinical Trials Register - www.clinicaltrialsregister.eu/
- World Health Organization International Trials Registry Platform search portal (WHO ICTRP; apps.who.int/trialsearch/)
- BioMed Central register - www.isrctn.com/
- Dutch trial register - www.trialregister.nl/

We found one ongoing trial evaluating ICI versus IUI (METC AMC 2013_364/ NL 4733001813/ NTR 16798/ ZonMw 80-83700-98-42063)

Appendix 8. **Grey literature search strategy**

We searched the grey literature on 15 December 2017, using the terms 'insemination and donor semen'. We found no studies.

- New York Academy of Medicine - www.greylit.org/
- Grey Literature in Europe - www.opengrey.eu

Appendix 9. **PubMed and Google Scholar search strategy**

We searched PubMed and Google Scholar on 15 December 2017, using the keywords as text: insemination AND donor AND random*. We did not find any recent studies (over the last 18 months).

Appendix 10. **Cochrane's 'Risk of bias' assessment tool**

Table 8.5.a: The Cochrane Collaboration's tool for assessing risk of bias

Domain	Support for judgement	Review authors' judgement
Selection bias		
Random sequence generation	Describe the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups.	Selection bias (biased allocation to interventions) due to inadequate generation of a randomised sequence.
Allocation concealment	Describe the method used to conceal the allocation sequence in sufficient detail to determine whether intervention allocations could have been foreseen in advance of, or during, enrolment.	Selection bias (biased allocation to interventions) due to inadequate concealment of allocations prior to assignment.
Performance bias		
Blinding of participants and personnel (<i>Assessments should be made for each main outcome (or class of outcomes)</i>).	Describe all measures used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. Provide any information relating to whether the intended blinding was effective.	Performance bias due to knowledge of the allocated interventions by participants and personnel during the study.
Detection bias		
Blinding of outcome assessment (<i>Assessments should be made for each main outcome (or class of outcomes)</i>).	Describe all measures used, if any, to blind outcome assessors from knowledge of which intervention a participant received. Provide any information relating to whether the intended blinding was effective.	Detection bias due to knowledge of the allocated interventions by outcome assessors.
Attrition bias		
Incomplete outcome data (<i>Assessments should be made for each main outcome (or class of outcomes)</i>).	Describe the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. State whether attrition and exclusions were reported, the numbers in each intervention group (compared with total randomised participants), reasons for attrition or exclusions where reported, and any re-inclusions in analyses performed by the review authors.	Attrition bias due to amount, nature, or handling of incomplete outcome data.
Reporting bias		
Selective reporting	State how the possibility of selective outcome reporting was examined by the review authors, and what was found.	Reporting bias due to selective outcome reporting.
Other bias		
Other sources of bias	State any important concerns about bias not addressed in the other domains in the tool. If particular questions or entries were prespecified in the review's protocol, responses should be provided for each question or entry.	Bias due to problems not covered elsewhere in the table.

Data and analysis

Comparison 1. Intrauterine insemination (IUI) versus intracervical insemination (ICI)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
<u>1 Live birth rate</u>	2		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.1 Natural cycle	1	26	Odds Ratio (M-H, Fixed, 95% CI)	3.24 [0.12, 87.13]
1.2 Gonadotrophin-stimulated cycle	1	43	Odds Ratio (M-H, Fixed, 95% CI)	2.55 [0.72, 8.96]
<u>2 Multiple pregnancy rate</u>	3		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 Natural cycle	1	26	Odds Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
2.2 Gonadotrophin-stimulated cycle	2	131	Odds Ratio (M-H, Fixed, 95% CI)	2.77 [1.00, 7.69]
<u>3 Clinical pregnancy rate</u>	4		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
3.1 Natural cycle	2	76	Odds Ratio (M-H, Fixed, 95% CI)	6.18 [1.91, 20.03]
3.2 Gonadotrophin-stimulated cycle	2	131	Odds Ratio (M-H, Fixed, 95% CI)	2.83 [1.38, 5.78]
<u>4 Miscarriage rate</u>	3		Odds Ratio (M-H, Fixed, 95% CI)	Subtotals only
4.1 Natural cycle	1	26	Odds Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
4.2 Gonadotrophin-stimulated cycle	2	131	Odds Ratio (M-H, Fixed, 95% CI)	1.97 [0.43, 9.04]

Comparison 2. Timing of intrauterine insemination (IUI) with donor sperm: luteinising hormone (LH) + 1 day versus LH + 2 days

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
<u>1 Live birth rate</u>	1	351	Odds Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
<u>2 Multiple pregnancy rate</u>	1	64	Odds Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
<u>3 Clinical pregnancy rate</u>	1	351	Odds Ratio (M-H, Fixed, 95% CI)	2.00 [1.14, 3.53]

Comparison 3. Timing of intracervical insemination (ICI) with donor sperm: urinary luteinising hormone (LH) test versus temperature curve and cervical mucus score

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
<u>1 Multiple pregnancy rate</u>	1	64	Odds Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
<u>2 Clinical pregnancy rate</u>	1	56	Odds Ratio (M-H, Fixed, 95% CI)	1.31 [0.42, 4.11]

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CHAPTER 5

Intrauterine insemination or
intracervical insemination with
cryopreserved donor sperm in the
natural cycle: a cohort study.

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Abstract

Study Question: Does intrauterine insemination in the natural cycle lead to better pregnancy rates than intracervical insemination (ICI) in the natural cycle in women undergoing artificial insemination with cryopreserved donor sperm.

Summary answer: In a large cohort of women undergoing artificial insemination with cryopreserved donor sperm, there was no substantial beneficial effect of IUI in the natural cycle over ICI in the natural cycle.

What is known already: At present, there are no studies comparing IUI in the natural cycle versus ICI in the natural cycle in women undergoing artificial insemination with cryopreserved donor sperm.

Study design, size, duration: We performed a retrospective cohort study among all eight sperm banks in the Netherlands. We included all women who underwent artificial insemination with cryopreserved donor sperm in the natural cycle between January 2009 and December 2010. We compared time to ongoing pregnancy in the first six cycles of IUI and ICI, after which controlled ovarian stimulation was commenced. Ongoing pregnancy rates (OPRs) over time were compared using life tables. A Cox proportional hazard model was used to compare the chances of reaching an ongoing pregnancy after IUI or ICI adjusted for female age and indication.

Participants/materials, setting, methods: We included 1843 women; 1163 women underwent 4269 cycles of IUI and 680 women underwent 2345 cycles of ICI with cryopreserved donor sperm.

Main results and the role of chance: Baseline characteristics were equally distributed (mean age 34.0 years for the IUI group versus 33.8 years for the ICI group), while in the IUI group, there were more lesbian women than in the ICI group (40.6% for IUI compared with 31.8% for ICI). Cumulative OPRs up to six treatment cycles were 40.5% for IUI and 37.9% for ICI. This corresponds with a hazard rate ratio of 1.02 (95% confidence interval (CI) 0.84–1.23) after controlling for female age and indication. Increasing female age was associated with a lower OPR, in both the IUI and ICI groups with a hazard ratio for ongoing pregnancy of 0.94 per year (95% CI 0.93–0.97).

Limitations reasons for caution: This study is prone to selection bias due to its retrospective nature. As potential confounders such as parity and duration of subfertility were not registered, the effect of these potential confounders could not be evaluated.

Wider implications of the findings: In women inseminated with cryopreserved donor sperm in the natural cycle, we found no substantial benefit of IUI over ICI. A randomized controlled trial with economic analysis alongside, it is needed to allow a more definitive conclusion on the cost-effectiveness of insemination with cryopreserved donor sperm.

Introduction

Artificial insemination with donor sperm (AID) may be performed for medical reasons or to assist lesbian couples or single women to achieve pregnancy. Medical reasons include obstructive and non-obstructive azoospermia, severely impaired semen quality in couples who do not want to undergo or were not successful with ICSI, severe rhesus isoimmunization, prevention of vertical transmission of a genetic defect or prevention of transmission of human immunodeficiency virus (HIV)(NICE 2013). Although fresh sperm leads to higher pregnancy rates, cryopreserved donor sperm is inseminated to prevent transmission of sexually transmitted diseases such as HIV and Hepatitis B and C (ASRM 2013). AID can be done via the intrauterine (IUI) or the intracervical route (ICI), with or without ovarian stimulation. The guidelines of the UK-based National Institute for Health and care Excellence (NICE) recommends IUI(NICE 2013). The recommendation to perform IUI relies upon a Cochrane review in which IUI gives higher ongoing pregnancy rates (OPRs) per cycle compared with ICI (Besselink et al. 2008). The NICE guideline does not recognize that this review includes only studies in which ovarian stimulation was performed. To reduce multiple pregnancies and their attendant risks, NICE considers it reasonable to try six cycles of unstimulated donor insemination initially in regularly ovulating women, since these women are not infertile. However, they also acknowledge that there is no evidence from randomized controlled trials (RCTs) to support this recommendation. In the absence of proof of superiority of IUI over ICI in the natural cycle, it should be realized that IUI is more expensive than ICI, due to the sperm processing. Considering that IUI in the natural cycle is recommended over ICI in the natural cycle in the first six cycles, without any evidence that in the natural IUI generates higher pregnancy rates compared with ICI, and in view of the higher costs generated by processing the sperm for IUI, the aim of this retrospective study was to assess whether IUI does achieve higher OPRs compared with ICI in the natural cycle.

Materials and methods

Patients

We performed a retrospective cohort study among Dutch women who underwent AID between January 2009 and December 2010. Data were collected from all eight sperm banks in the Netherlands: Center for Reproductive Medicine Amsterdam (Academical Medical Center), Isala Fertility Centre Zwolle, MCK Fertility Centre Leiderdorp, Reinier de Graafgroep Voorburg, Rijnstate Hospital Arnhem, Stichting Geertgen Elsendorp, University Medical Center Groningen, University Medical Center Utrecht.

Indications for AID were azoospermia, severely impaired semen quality or failed TESE-ICSI. Also, couples who were at risk of vertical transmission of a genetic defect and lesbian couples or single women were admitted to the AID programs. We studied up to six treatment cycles of IUI or ICI with cryopreserved donor sperm in the natural cycle in these women. Four centres performed IUI as a routine, two centres performed ICI, while two centres performed both IUI and ICI: these centres switched during the period under study from ICI to IUI because they experienced low success rates after ICI. For this retrospective cohort, we only included therapy-naïve women who started the initial treatment strategy and did not switch to another AID method. In the ICI cycles, women were inseminated once or twice per cycle according to local protocol. One straw was thawed at room temperature and insemination took place without processing of the sperm. For insemination, sperm was deposited near the cervical canal.

Data analysis

The primary outcome was ongoing pregnancy, defined as the presence of fetal cardiac activity at transvaginal ultrasonography at a gestational age beyond 12 weeks.

We compared OPRs over time using life table analysis. On the basis of the cumulative pregnancy rates, a curve was constructed showing the time to pregnancy over multiple cycles. A number of women who started were given per cycle. The univariable and multivariable Cox regression analysis was performed for variables possibly affecting the OPR. Variables considered in the analysis were female age and indication for AID. The linearity of the association between age and ongoing pregnancy was evaluated with spline functions. Results were expressed as hazard rate (HR) with corresponding 95% intervals. Data analysis was carried out using the STATA version 11.

Results

The eight clinics had considerable practice variation. In all clinics, ovulation was detected by LH testing in urine; in two clinics, ovulation was induced by human chorionic gonadotrophin if a dominant follicle was present at ultrasonography (Pregnyl, Organon, Oss, The Netherlands). In the case of IUI, various methods of sperm processing were used. Five sperm banks froze the unprocessed sperm and performed processing after thawing and one sperm bank performed processing before freezing. One clinic performed two inseminations per cycle for ICI. All baseline characteristics of the clinics are summarized in Table 1.

Table 1. Baseline characteristics of practice variation between clinics

Clinic	1	2	3	4	5	6	7	8
Insemination technique	ICI	ICI	IUI	ICI IUI	ICI IUI	IUI	IUI	IUI
Ovulation detection	LH tests	LH test	HCG or LH test	LH test	LH test	HCG or LH test	LH test	LH test
Number of inseminations per cycle	2	1	1	1	1	1	1	1
Sperm processing*			Before freezing	After freezing	After freezing	After freezing	After freezing	After freezing

LH tests were performed in urine

*sperm processing was only performed in case of IUI

We studied 1843 women of whom 1163 underwent 4269 cycles of IUI (3.7 cycles per woman) and 680 women underwent 2345 cycles of ICI (3.4 cycles per woman). Baseline characteristics of the women are summarized in Table 2. The average age was 34.0 in the IUI group and 33.8 in the ICI group (P -value 0.55). In the IUI group, there were more lesbian women than in the ICI group: 41.0% for IUI compared with 31.8% for ICI, respectively ($P < 0.001$). In 10% of the IUI group, the indication for AID was unknown compared with none in the ICI group ($P < 0.001$).

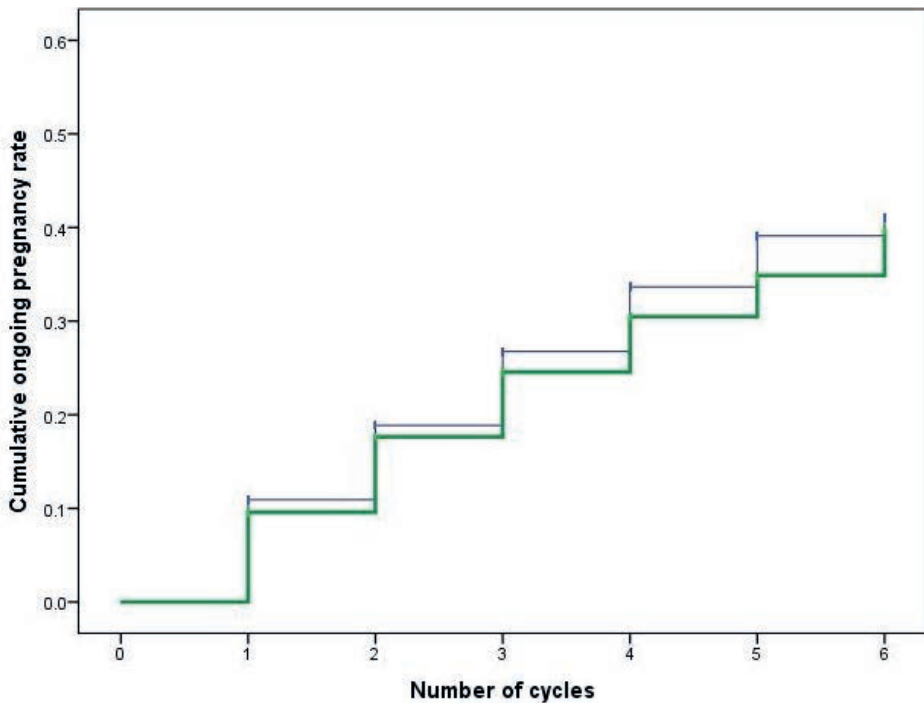
Table 2. Baseline characteristics of women undergoing AID.

	IUI (n=1163)	ICI (n=680)	P-value
Age years (mean \pm sd)	34.0 \pm 4.3	33.8 \pm 4.5	0.55
Indication for AID n (%)			<0.001
Heterosexual couples	295 (25.4)	249 (36.6)	
Lesbian couples	477 (41.0)	216 (31.8)	
Single women	273 (23.5)	215 (31.6)	
Unknown	118 (10.0)	0	

There were 361 ongoing pregnancies in the IUI group resulting in an OPR of 40.5% after six treatment cycles. In the ICI group, there were 177 ongoing pregnancies that resulted in an OPR of 37.9% after six treatment cycles (uncontrolled hazard ratio (HR) 1.2 (95% confidence interval (CI) 0.95–1.40)). The number of women who started a next cycle decreased per cycle (Fig. 1). Dropout rates were 11% for IUI and 15% for ICI after five cycles ($P = 0.013$). Note in figure 1, the first cycle includes 1159 out of 1163 women in the IUI group and 597 out of 680 women in the ICI group. Although the study included all women who underwent artificial

insemination with cryopreserved donor sperm between January 2009 and December 2010, some women started their first cycles in 2008 but did not become pregnant. For this analysis, these cycles were excluded because they were prone to bias since women who became pregnant in their first cycles in 2008 were not included. The outcome did not differ to that found when these women were included in the cohort. Therefore, these women were not excluded from our overall analysis in order to have a complete overview of all women who underwent AID in the Netherlands in the period under study

Figure 1. Kaplan Meier: Cumulative OPR's from first to sixth cycle and number at risk for ongoing pregnancy per cycle.



Number of women who started per cycle

IUI	1159	928	775	593	472	366
ICI	597	472	285	301	225	162

Kaplan–Meier: cumulative OPR's from first to sixth cycle and number at risk for ongoing pregnancy per cycle.

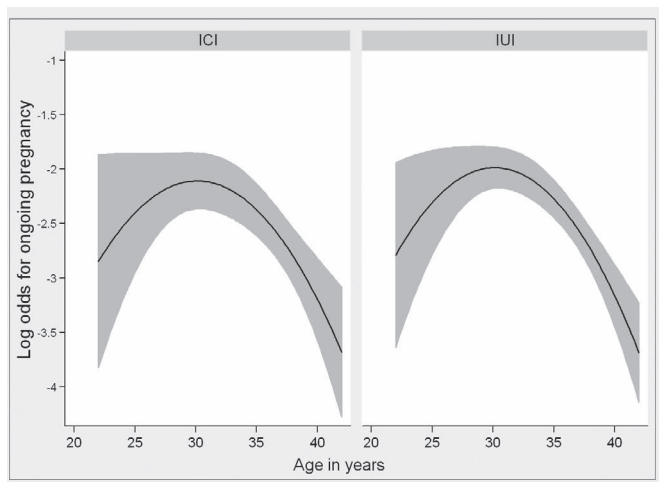
After controlling for female age and indication, the HR for IUI versus ICI was 1.02 (95% CI 0.84–1.23). Increasing female age was associated with a lower OPR, in both the IUI and ICI groups [HR for ongoing pregnancy (HR) 0.94; 95% CI 0.93–0.96] (Table 3). A spline showed that pregnancy rates increased up to the age of 32, and thereafter there was a general decline both in IUI and ICI cycles (figure 2). There was no interaction between the applied insemination technique and age. With lesbian couples as a reference for the indication for AID, OPR did not significantly differ for heterosexual couples (HR 1.2; 95% CI 0.98–1.48) and single women (HR 0.83; 95% CI 0.66–1.04). There was no interaction between female age and indication.

Table 3. results of Cox regression analysis after adjusting for factors influencing ongoing pregnancy outcome from cycle 1-6

	HRadj	95%CI	P-value
IUI versus ICI	1.02	0.84-1.23	0.85
Age	0.94	0.93-0.96	<0.001
Indication for AID*			
Lesbian couples	1.0		
Heterosexual couples	1.2	0.98-1.48	0.08
Single women	0.83	0.66-1.04	0.12

There was no interaction between indication and female age.
analysis done with and without imputation for missing indication values

Figure 2. Spline: age in relation to OPR.



Results are expressed as hazard rate (HR) with corresponding 95% intervals. The HR is the black line, the CI is the grey area around the line.

One clinic performed two inseminations per ICI treatment. Excluding this clinic from the analysis in a sensitivity analysis resulted in an HR for IUI versus ICI of 0.89 (95% CI 0.73–1.10).

Discussion

In this multicentre nationwide cohort study in women undergoing AID in the natural cycle, we found no statistically significant differences between the first six treatment cycles of IUI and ICI in terms of OPR.

An increasing female age from 32 years onward was the only factor influencing OPR negatively for both treatments. Other factors, such as applied insemination technique and indication for AID, had no effect on the OPR. This cohort is unique since it is the largest cohort study on this topic and compares IUI and ICI in the natural cycle. Furthermore, it describes heterosexual, lesbian couples and single women, while all previous studies were limited to heterosexual couples and single women (Besselink et al. 2008). One of the limitations of this retrospective cohort is that there was considerable practice variation in semen processing, single or double insemination and timing of insemination between the participating centres. For ICI, sperm processing was never used. For IUI, five sperm banks froze the donor sperm and performed processing after thawing and one sperm bank performed processing before freezing. Therefore, it was not feasible to evaluate the confounding or modifying effect of semen processing on the ongoing pregnancy chances following IUI and ICI. The only evidence that semen processing does not affect pregnancy chances comes from a study that combined retrospective data from 209 women and prospective data from 39 women (Wolf et al. 2001). In the case of ICI, one clinic performed two inseminations per cycle. Excluding this clinic from the analysis did not result in evidence of a difference between IUI and ICI. Also the timing of insemination was performed in different ways; some clinics used urine LH tests and some ovulation induction by human chorionic gonadotrophin (Pregnyl). Guidelines do not report on timing of insemination in the case of AID. Because of these variations, it is impossible to subscribe any effect of IUI or ICI on OPR on the insemination technique only.

A second limitation is that data on the medical history including previous pregnancies and duration of subfertility were not obtained. These factors may influence pregnancy rates (van der Steeg et al. 2008). Nevertheless, the main prognostic factor to predict conception, e.g. age, is incorporated in the analysis. From a theoretical point of view, we do not expect that duration of infertility and previous obstetric history results will add any additional information, since most women who opt for AID are not subfertile at all. Evidence of whether medical history does influence pregnancy outcome in this population is lacking.

Thirdly, in heterosexual couples, it is known that partners of azoospermic men conceive more quickly with AID than partners of men with spermatozoa in their ejaculates, suggesting that in the latter, unknown female factors also contribute to the subfertility of the couples (NICE 2013). In this cohort, we did not differentiate between the indications for AID in heterosexual couples, because the data were not available. This could have resulted in lower OPRs in heterosexual women. Finally, the number of women who started inseminations dropped after the first cycle, which makes calculation of OPRs less reliable. OPRs dropped after the first cycle, but cycles up to the sixth cycle still gave ongoing pregnancies. Several findings in our study warrant further discussion. First, in our cohort, the cumulative OPRs were lower compared than expected for a normal fertile population. This may be explained by a study that compared the use of fresh sperm with cryopreserved sperm and found that pregnancy rates after 3 months were 48% for fresh sperm versus 22% for cryopreserved sperm (Subak et al. 1992). We assume that the lower OPR in this cohort is due to the use of cryopreserved donor sperm.

Second, after every cycle, there were non-pregnant women who stopped treatment before the six cycles were completed. Dropout rates were higher in the ICI group. Fear of failure is a well-known and important factor in fertility treatment from the point of view of the patient, but also from the perspective of the doctor, and this may have led to the number of dropouts (Campana et al. 1996, Olivius et al. 2004). Reasons for discontinuing inseminations are numerous; for a couple with repeated failed attempts, continuing AID may become a frustrating experience; from the clinician's perspective, repeating AID cycles can be time-consuming and it may seem easier to offer alternative options than to motivate patients who have lost confidence. In view of this, we have to realize that women applying for AID are not proven to be subfertile. Furthermore, our data show that continuation of treatment after several failed attempts may be rewarding. Appropriate counselling should help the couples to understand the principle of the treatment without ovarian stimulation and their pregnancy chances.

Current guidelines recommend IUI in the natural cycle, since women who start with AID are not subfertile and multiple pregnancy rates should be prevented, but this advice is based upon inferences drawn from the studies applying IUI and ICI with ovarian stimulation (NICE 2013). Our study provides for the first time data questioning the use of IUI and at the same time underpinning the recommendation to inseminate in the natural cycle and thus not to add ovarian stimulation. The costs for IUI have been estimated to be four times higher than ICI, mostly because of the additional sperm preparation required. In the Netherlands, costs for ICI are estimated to be 150 Euros per cycle versus 650 Euros per cycle for IUI (NZU 2011). Assuming pregnancy rates

of 20.1 and 22.4% for ICI and IUI after three cycles, the costs per ongoing pregnancy will be 1768 Euros over three ICI cycles, versus 7012.5 Euros per three IUI cycles. In the absence of a significant difference, ICI should therefore be the preferred treatment. Even if IUI would be 2.5% more effective, this would implicate a cost of 6841.5 Euros to establish one additional ongoing pregnancy, while even at a 5% increase (corresponding with the upper level of our 95% CI), these costs would be 6678.6 Euros. Obviously, it is more efficient to invest in additional cycles of ICI, even if the ICI cost 300 Euros.

In conclusion, this retrospective cohort study showed no substantial benefit of IUI in the natural cycle above ICI in the natural cycle for insemination with cryopreserved donor sperm. An RCT with an economic analysis alongside it is highly recommended to provide definitive evidence on the most cost-effective insemination technique.

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CHAPTER 6

Intracervical insemination versus
intrauterine insemination with
cryopreserved donor sperm in the
natural cycle: a randomised controlled
trial.

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Abstract

Study question: Is intracervical insemination (ICI) non-inferior to intrauterine insemination (IUI) with cryopreserved donor sperm in the natural cycle in terms of live birth?

Summary answer: ICI with cryopreserved donor sperm in the natural cycle was inferior to IUI in terms of live birth.

What is already known: Both ICI and IUI in the natural cycle are performed as first line treatments in women who are eligible for donor sperm treatment. High quality data on the effectiveness of ICI versus IUI with cryopreserved donor sperm in the natural cycle in terms of live birth is lacking.

Study design, size, duration: We performed an open-label multicentre randomised non-inferiority trial in the Netherlands and Belgium (NTR 4462).

Participants/materials, setting, methods: We randomly allocated women who were eligible for donor sperm treatment with cryopreserved donor semen to six cycles of ICI in the natural cycle or six cycles of IUI in the natural cycle. The primary outcome was conception within eight months after randomisation leading to a live birth. Secondary outcomes were ongoing pregnancy, multiple pregnancy, clinical pregnancy, miscarriage and time to conception leading to live birth. We calculated relative risks (RR) and risk differences (RD) with 95% confidence intervals. Non-inferiority would be shown if the lower limit of the 95% RD confidence interval was less than minus 12%.

Main results and the role of chance: Between June 2014 and February 2019, we included 421 women, of whom 211 women were randomly allocated to ICI and 210 to IUI. Of the 211 women allocated to ICI, two women were excluded, 126 women completed treatment according to protocol and 75 women did not complete six treatment cycles. Of the 210 women allocated to IUI, three women were excluded, 140 women completed treatment according to protocol and 62 women did not complete six treatment cycles. Mean female age was 34 years (SD \pm 4) in both interventions. Conception leading to live birth occurred in 51 women (24%) allocated to ICI and in 81 women (39%) allocated to IUI (RR 0.63, 95% CI: 0.47 to 0.84). This corresponds to an absolute risk difference of minus 15%; 95% CI: -24% to -6.9%, suggesting inferiority of ICI. ICI also resulted in a lower live birth rate over time (HR 0.58, 95% CI: 0.41-0.82). Our per protocol analysis showed that within the eight months treatment horizon 48 women (38%) had live births after ICI and 79 women (56%) had live births after IUI (RR 0.68, 95% CI: 0.52-0.88; RD minus 18%, 95% CI: -30% to -6%).

Limitations, reasons for caution: The study was non-blinded due to the nature of the interventions. We consider it unlikely that this has introduced performance bias, since pregnancy outcomes are objective outcome measures.

Wider implications of the findings: Since ICI in the natural cycle was inferior to IUI in the natural cycle with cryopreserved donor sperm in terms of live birth rate, IUI is the preferred treatment.

Study funding/ competing interest: This trial received funding from the Dutch Organization for Health Research and Development (ZonMw project number 837002407). The authors declare that they have no competing interests.

Trial registration number: NTR4462

Trial registration date: 11-03-2014

Date of first patient's enrolment: 03-06-2014

Key words: Intrauterine insemination, intracervical insemination, donor sperm, donor sperm treatment, natural cycle, cryopreserved donor sperm

Introduction

Inseminations with cryopreserved donor sperm are widely performed. In Europe approximately 49,000 cycles were reported by the European Society of Human Reproduction and Embryology in 2015 (ESHRE)(De Geyter et al. 2020). The National Perinatal Epidemiology and Statistics Unit (NPESU) reported 3262 cycles with donor sperm in Australia and New Zealand in 2018 (Newman et al. 2020). Data on other continents are lacking.

To prevent transmission of sexually transmitted diseases, the use of cryopreserved donor semen which is quarantined until the donor is tested negative for human immunodeficiency virus, hepatitis B, C and other venereal diseases, is mandatory (European Union 2004, ASRM 2013, NICE 2013). The downside of cryopreserved semen is that pregnancy rates are lower compared to inseminations with fresh semen due to an adverse effect of freezing and thawing on sperm motility (Leeton et al. 1980, Richter et al. 1984, Keel et al. 1987).

Donor sperm treatment can be carried out by intracervical inseminations (ICI) or by intrauterine inseminations (IUI). The only guideline on the practice of donor sperm treatment, published by the National Institute for Health and Care Excellence (NICE) recommends IUI in the natural cycle for six cycles (NICE 2013). The evidence upon which this guideline is based is weak and of low quality. Only two small randomised controlled trials of low quality compared ICI and IUI in the natural cycle, with only one trial reporting live birth as an outcome in just 26 women (Patton et al. 1992, Hurd et al. 1993).

In our Cochrane review we subsequently pooled the data of these two studies and reported that there is insufficient evidence of a difference in live birth between ICI and IUI in the natural cycle (one study, 26 women, OR 3.24, 95% CI: 0.12 to 87.13), though IUI might result in higher clinical pregnancy rates compared to ICI (two studies, 76 women, OR 6.18, 95% CI : 1.91 to 20.03)(Kop et al 2018). The only available large retrospective cohort study suggests similar cumulative ongoing pregnancy rates after six cycles of ICI and IUI in the natural cycle (1843 women, Hazard Ratio 1.02 95% CI: 0.84 to 1.23). Live birth rate was not reported (Kop et al. 2015).

In view of this lack of evidence, we aimed to study the effectiveness of ICI compared to IUI in the natural cycle in women who started inseminations with cryopreserved donor sperm.

Materials and Methods

Study design

This study was an open-label multicenter, randomised controlled non-inferiority trial among five fertility clinics in the Netherlands and one in Belgium. We recruited women between

June 2014 and February 2019. The Medical Ethical Committee of the Academic Medical Centre and the Dutch Central Committee on Research involving Human Subjects approved this study (CCMO NL 47330-018-13) and the board of directors of each participating site approved local execution. The trial was registered at the Dutch trial register (NTR 4462). The protocol (see Supplementary material) was published previously (Kop et al. 2019).

Study population

All women with an indication for donor sperm treatment were eligible for the study. Indications for donor sperm treatment were obstructive and non-obstructive azoospermia in heterosexual couples, severely impaired semen quality in couples who did not wish to undergo or had not been successful with intracytoplasmic sperm injection (ICSI), prevention of vertical transmission of a genetic defect or prevention of transmission of human immunodeficiency virus in couples who did not wish to try natural conception or semen washing. In addition, lesbian couples or single women who applied for donor sperm treatment were eligible to participate.

Women had to be between 18 and 43 years of age with a regular menstrual cycle, or ovulatory after ovulation induction in women with normogonadotrophic anovulation. Women with normogonadotrophic anovulation started ovulation induction according to local protocol with clomiphene citrate or letrozole. After ovulation was detected by a basal temperature chart or ultrasound monitoring women could start insemination with donor sperm in the next cycle.

Women with known double-sided tubal pathology, irregular menstrual cycles, in vitro fertilization (IVF) or IUI in their history were not eligible. Heterosexual couples after failed ICSI or ICSI with TESE were excluded from the study if there was a history of female factors, such as low ovarian response during ICSI treatment.

Sperm donors

In the Netherlands all sperm donors were non-anonymous according to national legislation (Staatsblad 2002). In Belgium sperm donors could be non-anonymous or anonymous (Senaat 2005-2006). The screening and selection procedure was performed according to local protocols, adapted from the EU tissue directive (EU 2004/23/EC).

Interventions

We treated couples for a maximum of six cycles within a time horizon of eight months. Ovulation was detected by urinary or serum LH tests or transvaginal sonography, depending on the local protocol of the fertility clinic. In case of monitoring with urinary LH tests, women

tested their urine once per day, starting on an individually calculated cycle day based on their basal body temperature chart of the previous cycle. Insemination followed within 24 hours after LH detection in urine. In case of monitoring with serum LH measurements, women tested from cycle day 11 onwards and insemination followed within 24 hours after the serum LH rise. In case of monitoring by transvaginal sonography, women were followed until a dominant follicle was present with a diameter of at least 16mm. Insemination followed after ovulation triggering by human chorionic gonadotropin (Pregnyl, Organon, Oss, the Netherlands) 36 to 40 hours thereafter.

In the ICI cycles, inseminations were performed with unprocessed cryopreserved semen by cap or by straw. In the IUI cycles, inseminations were performed with processed semen. The processing could be done in one of two ways depending on the local laboratory protocol. The first technique was that the unprocessed semen was first cryopreserved and thawed, and then processed against a density gradient centrifugation and/or a washing step with culture medium. The second technique was that semen was first processed against a density gradient centrifugation and/or a washing step with culture medium and then cryopreserved. All inseminations were performed in the clinics.

Women were treated for a maximum of six cycles or until pregnancy occurred within a time horizon of eight months. Clinical and ongoing pregnancies were confirmed by ultrasound.

Outcome measures

The primary outcome was conception leading to live birth per woman, defined as any baby born alive with a gestational age beyond 24 weeks. Pregnancies that occurred within the first eight months after randomisation counted for assessment of the primary outcome.

Secondary outcomes were clinical pregnancy defined as any registered heartbeat on ultrasound, ongoing pregnancy defined as a positive heartbeat at or beyond 12 weeks of gestation, miscarriage defined as registered heartbeat before 12 weeks of gestation, multiple pregnancy defined as registered heartbeat of at least two foetuses at 12 weeks of gestation, ectopic pregnancy, congenital anomalies defined as structural or functional anomalies that occur intrauterine and can be identified prenatally or at birth and time from randomisation leading to the birth of a live child.

Sample size calculation

We designed the study as a non-inferiority trial. We assumed live birth rates of 40% after six cycles ICI and IUI, based on our retrospective cohort (Kop et al. 2015). To exclude a non-

inferiority margin of 12% to the detriment of ICI – by a one-sided Z test (unpooled) with an 80% power and 5% alpha-, we needed to recruit 208 women per treatment.

Randomisation and masking

Eligible women were informed about the study by their doctor or by a research nurse. After written informed consent women were randomised using a central password protected Internet-based randomisation programme.

The randomisation list had been prepared by an independent statistician with a variable block size with randomly selected block sizes that varied between two, four and six. There was no stratification. Neither the recruiters nor the trial project group could access the randomisation sequence.

Statistical analysis

We analysed all outcomes on an intention to treat basis. For live birth we tested non-inferiority on basis of the absolute risk difference with the absolute left boundary margin of 12%. We also expressed differences as absolute risk differences and relative risks with 95% CI and used Chi square test for formal analysis, and did the same for secondary endpoints. To account for time to conception leading to live birth, we constructed cumulative hazard curves using the log rank test to compare the interventions and calculated the corresponding hazard rates with 95% CI. We constructed a hazard curve showing time to conception leading to live birth over six cycles and a hazard curve showing time to conception leading to live birth over eight months. For the primary outcome, we also performed a per protocol analysis, which was not pre-planned. The per protocol analysis was limited to women who were treated according to the assigned intervention, who did not switch treatment and who had completed six inseminations in case of treatment failure. We performed an analysis on pre-specified subgroups possibly affecting our primary outcome. Variables considered in the analysis were women aged under 35 or over 35 years and women being nulliparous or multiparous. Results were expressed as Relative Risks (RR) with corresponding 95% intervals.”

Data Safety Monitoring Board

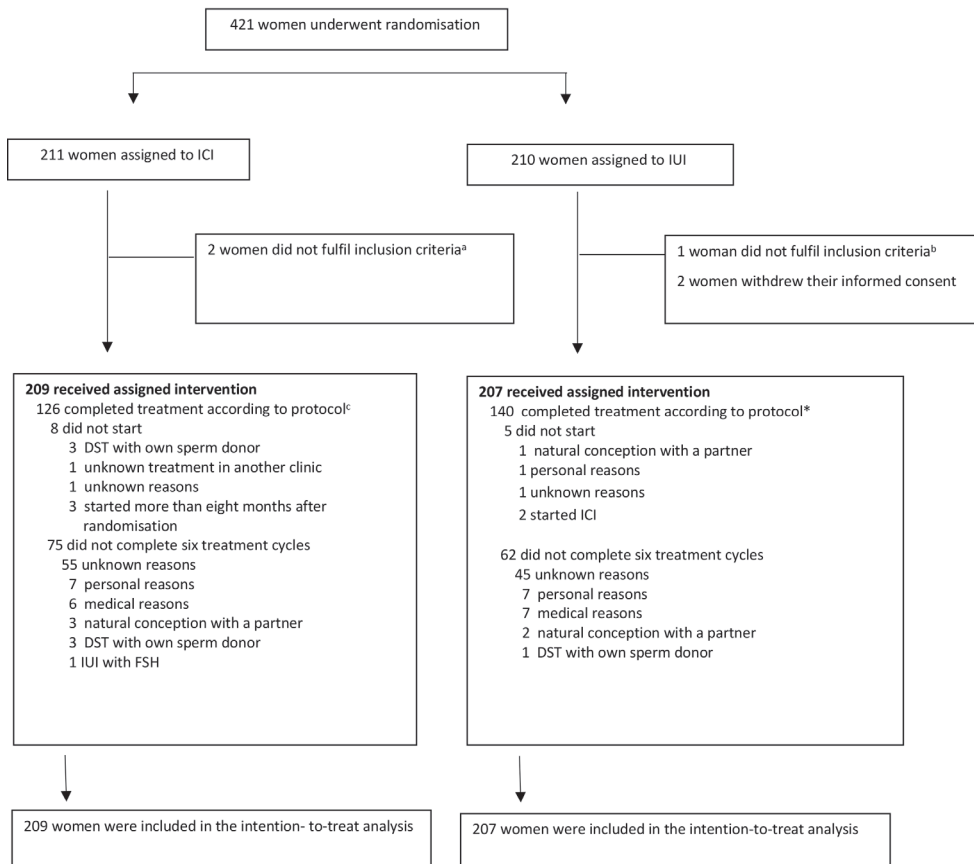
A pre-planned independent interim analysis was performed by the Data Safety Monitoring board of the Dutch Consortium for Women’s Health Research when 200 women were included to exclude large differences in conception leading to live birth (Haybittle–Peto with $p \leq 0.001$). The Data Safety Monitoring Board advised to proceed with the trial as planned.

Results

Between June 2014 and February 2019, we recruited 421 women of whom 211 women were allocated to ICI and 210 women to IUI. We excluded five women after randomisation but before starting treatment; three women because they did not fulfil the inclusion criteria and two women because they withdrew their informed consent.

For the intention to treat analysis we could include 209 women allocated to ICI and 207 women allocated to IUI (Figure 1).

Figure 1. Trial Profile



^a One woman had bilateral tubal pathology, one woman was randomised a second time after a live birth after the assigned intervention

^b One woman was randomised a second time after a live birth after the assigned intervention.

^c treatment according to protocol: women who did not switch treatment and who had completed six inseminations in case of treatment failure.

The baseline characteristics were well balanced between women, except for nulliparity that was present in 77% of women allocated to ICI versus 87% of women allocated to IUI (Table 1). One hundred ninety six women (94%) allocated to ICI and 196 women (95%) allocated to IUI were inseminated with donor sperm of a Dutch sperm bank.

Table 1. Baseline characteristics

Characteristics	ICI (n= 209)	IUI (n=207)
Women		
Mean Female age (years)	34.4 ± 3.8	34.4 ± 3.9
Nulliparous	161 (77)	180 (87)
Indication for donor sperm treatment		
Heterosexual couples	47 (23)	48 (24)
Azoospermia, did not opt for TESE	4	8
No sperm after TESE	28	24
Partner had vasectomy	0	1
No pregnancy after ICSI-TESE	8	5
No pregnancy after ICSI	2	3
Partner carrier genetic defect	4	6
Poor embryo quality after ICSI	0	1
Unknown	1	0
Lesbian couples	69 (33)	58 (28)
Single women	93 (44)	101 (48)
Current smoking status	24 (11)	34 (16)
Normogonadotropic anovulation	1 (0.5)	1 (0.5)
Mean body mass index in kg/m ³	25 (17-36)	24 (16-43)
Mean total motile count (x10 ⁶)	5.9 (1.05-24.3)	5.7 (0.52-26.7)

Data are n (%), mean (SD). The only significant difference being more women allocated to IUI were nulliparous (P < 0.05).

In ICI, inseminations were performed with donor sperm with a median total motile sperm count of 5.9×10^6 (quartiles: $1.05-24.3 \times 10^6$) per sample and in IUI with donor sperm with a median total motile sperm count of 5.7×10^6 (quartiles: $0.52-26.7 \times 10^6$) per sample.

Pregnancy outcomes are summarized in Table II. Within the eight months treatment horizon, there were 51 live births in 209 women (24%) after ICI and 81 live births in 207 women (39%) after IUI (RR 0.62, 95% CI: 0.47-0.84). This corresponds to an absolute risk difference of minus 15% (95% CI: -24% to -6.9%) and to a 90% left boundary of minus 22%, thus crossing the pre-set absolute difference of 12%.

The conception rate leading to live birth within eight months and over six treatment cycles after ICI and IUI was lower following ICI (log rank score 10.64, p= 0.001 (cycles), long rank score (months) 9.04 p=0.003) (Figure 2a+b).

Table 2. Pregnancy outcomes per woman randomised

	ICI (n= 209)	IUI (n=207)	Relative Risk (95% CI)
Live birth	51 (24)	81 (39)	0.62 (0.47-0.84)
Ongoing pregnancy	52 (25)	82 (39)	0.62 (0.46-0.82)
Clinical pregnancy	61 (29)	95 (45)	0.64 (0.49-0.82)
Multiple pregnancy	1 (0.5)	1 (0.5)	1.00 (0.06-15.8)
Miscarriage	8 (4)	12 (6)	0.66 (0.28-1.58)
Ectopic pregnancy	0	1 (0.5)	0.33 (0.01-8.06)
Congenital anomalies¹	2 (1)	8 (4)	0.25 (0.05-1.15)

Data are n (%)

After ICI: one case of sickle cell anemia and albinism and one case of trisomy 21.

After IUI: one case of albinism, one case of hypospadias, one case of polydactyly, one case of pelvic ureteric stenosis, one case of anorectal malformation, one case of Turner's syndrome, one case of West syndrome, one case of Tetralogy of Fallot.

Cycle data are summarized in table III. In both treatments there were live births until the fifth cycle, to drop strongly in the 6th cycle.

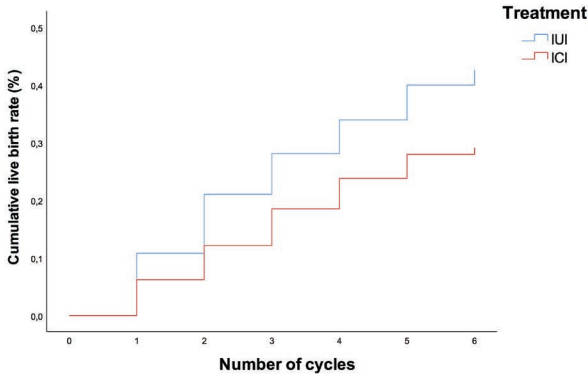
The corresponding HR for conception leading to live birth over six treatment cycles was 0.58 (95% CI: 0.41 to 0.82) for ICI versus IUI.

Table 3. Overview per cycle with logistic regression analysis per cycle

ICI	Total number of women	Inseminated (n%)	Not inseminated (n%)	Did not continue treatment (n%)	Live birth (%)	OR (95% CI)
Cycle 1	202	184 (91)	18 (9)	19 (10)	14(8)	1.0
Cycle 2	171	163 (95)	8 (5)	6 (4)	8 (6)	0.66 (0.27-1.61)
Cycle 3	156	140 (90)	16 (10)	6 (4)	12 (9)	1.12 (0.50-2.50)
Cycle 4	131	116 (88)	15 (12)	1 (1)	9 (8)	0.99 (0.41-2.34)
Cycle 5	112	103 (92)	9 (8)	1 (1)	7 (7)	0.91 (0.35-2.31)
Cycle 6	85	77 (91)	8 (9)	0 (0)	1 (1)	0.16 (0.021-1.25)
IUI						
Cycle 1	206	187 (91)	19 (7)	14 (7)	24(13)	1.0
Cycle 2	164	149 (91)	15 (9)	3 (2)	28(19)	1.50 (0.83-2.67)
Cycle 3	126	114 (91)	12 (9)	3 (2)	13(11)	0.82 (0.41-1.66)
Cycle 4	108	104 (96)	4 (4)	2 (2)	5(5)	0.41 (0.16-1.04)
Cycle 5	90	79 (88)	11 (12)	3 (3)	9(10)	0.59 (0.25-1.40)
Cycle 6	72	62 (86)	10 (12)	0 (0)	2(3)	0.20 (0.045-0.85)

Data are n (%), percentages were calculated per women. Percentages for live birth were calculated per insemination. Logistic regression with cycle one as reference

Figure 2a. Time to conception leading to live birth (per cycle)



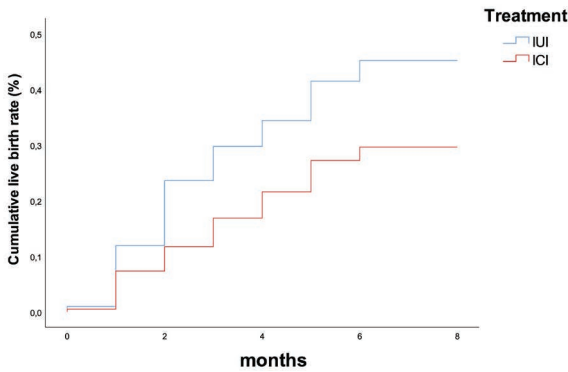
ICI= intracervical insemination, IUI= intrauterine insemination

*corresponding Hazard rate for conception leading to live birth 0.58 (95% CI: 0.41 to 0.82)

Numbers at risk

	0	1	2	3	4	5	6
ICI	209	184	163	140	116	103	77
IUI	207	187	149	114	104	79	62

Figure 2b. Time to conception leading to live birth (months)



ICI= intracervical insemination, IUI= intrauterine insemination

corresponding Hazard rate for conception leading to live birth 0.59 (95% CI: 0.41 to 0.84)

Numbers at risk

	0	1	2	3	4	5	6	7	8
ICI	209	163	138	123	121	73	36	9	7
IUI	207	137	112	110	92	84	49	5	1

Analysis of our pre-specified subgroups showed that our primary outcome did not change according to age or parity (Table 4).

Table 4. Live birth rate in pre-specified subgroups

	n/N ICI	n/N IUI	RR	CI
< 35 years	29/97	46/96	0.62	0.43-0.90
≥35 years	22/106	33/109	0.69	0.43-1.10
Nulliparous	36/161	69/180	0.58	0.41-0.82
Multiparous	15/48	12/28	0.73	0.40-1.33

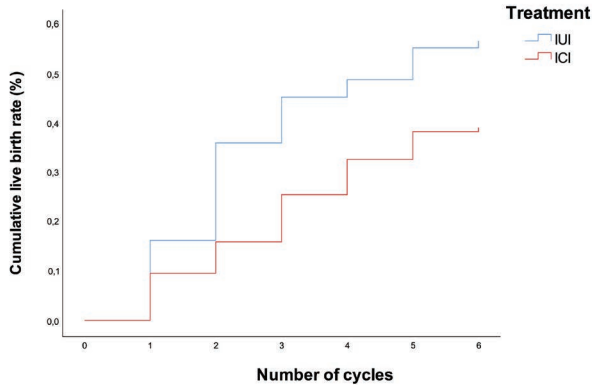
The per protocol population consisted of 126 women allocated to ICI and 140 women allocated to IUI that completed six insemination cycles in their allocated treatment arm or had a live birth. All per protocol pregnancy outcomes are summarized in table V. Within the eight months treatment horizon 48 women had live births (38%) after ICI and 79 women had live births (56%) after IUI (RR 0.68, 95% CI: 0.52-0.88; RD minus 18%, 95% CI: -30% to -6%). The conception rate leading to live birth within the eight months treatment horizon after ICI and IUI over six treatment cycles was lower following ICI (log rank score 9.66 , p= 0.002). The corresponding HR for conception leading to live birth was 0.61 (95% CI: 0.43 to 0.87)(Figure 3).

Table 5. Pregnancy outcomes per woman randomised - per protocol analysis

	ICI (n= 126)	IUI (n=140)	Relative Risk (95% CI)
Live birth	48 (38)	79 (56)	0.68 (0.52-0.88)
Ongoing pregnancy	50 (40)	80 (57)	0.69 (0.54-0.91)
Clinical pregnancy	54 (43)	84 (60)	0.71 (0.56-0.83)
Multiple pregnancy	1 (0.8)	1 (0.7)	1.00 (0.06-15.8)
Miscarriage	3 (2)	3 (2)	1.11 (0.23-5.40)
Congenital anomalies	1 (1)	7 (5)	0.16 (0.02-1.27)

Data are n (%)

Two women after ICI and one woman after IUI conceived naturally and were excluded in this analysis

Figure 3. Time to conception leading to live birth - per protocol analysis

ICI= intracervical insemination, IUI= intrauterine insemination

corresponding Hazard rate for conception leading to live birth 0.61 (95% CI: 0.43 to 0.87).

Numbers at risk

	0	1	2	3	4	5	6
ICI	126	126	114	105	93	83	76
IUI	140	140	117	88	75	69	61

Discussion

In this multicenter, non-blinded, randomised controlled non-inferiority trial in women who were eligible for donor sperm treatment, over six cycles ICI was inferior compared to IUI in terms of live birth within the treatment horizon of eight months.

Our randomised clinical trial has several strengths. First, we included all women with an indication for donor sperm treatment and did not limit our study to any particular family type, thus enhancing the generalizability of our data. Second, we based our power analysis on our large retrospective cohort study and not on outdated results of small randomised controlled trials, ensuring robustness of the data (Kop et al., 2015). Third, our per protocol analysis limited to women that received the allocated treatment and that completed six treatment cycles did not alter our results, suggesting that treatment switches and not completing six treatment cycles did not have an effect on our primary outcome.

One of the limitations in our study is that due to the nature of the interventions we were not able to blind this study. Also, in our sample size calculation we did not take into account

that women would not start with the assigned intervention or would not complete the six insemination cycles offered. Another concern is that fewer women assigned to ICI were nulliparous compared to women assigned to IUI. Since the chances to become pregnant are lower for nulliparous women, the possible bias caused by these differences in parity would work in the opposite direction of our results (van der Steeg et al. 2008). Finally there was considerable practice variation in semen processing for IUI and timing of insemination. While this represents daily practice, this enhances the generalizability of our data.

The results of our trial are in line with the only two existing underpowered randomised controlled trials, had clinical pregnancy as primary outcome and were performed almost 30 years ago and therefore perhaps not representative of current practice (Patton et al. 1992, Hurd et al. 1993). In contrast with our retrospective cohort study with a hazard rate of 1.0, our randomised controlled trial showed a hazard rate of 0.58 after ICI versus IUI. This randomised design probably represents the best estimate of the relative effectiveness.

Our trial shows a strong drop in live birth rate in the sixth cycle after ICI and IUI. Since only a small number of women are present in this analysis, the drop may be due to chance. How many cycles of intrauterine insemination we should perform before we continue with a second-line treatment could be topic of future research. Subsequently it is unknown what the second-line treatment should be if women do not conceive after intrauterine insemination in the natural cycle; this could also be a topic for future research.”.

Thirteen women (3%) decided not to start with the assigned intervention and 137 women (32%) did not complete 6 treatment cycles within the eight months’ time horizon. This was mainly due to the fact that women found their own sperm donor, tried to conceive with a partner or for medical or personal reasons. This underpins that women, even though they had decided to start donor sperm treatment, can make other decisions later on how they fulfill their wish for a child or abstain as they go along. Life is not predictable.

Why ICI is inferior to IUI in these women who - by definition – have no known fertility problems- remains a moot point. Intrauterine insemination brings the sperm closer to the oocyte than intracervical insemination and this might compensate for decreased sperm motility after freezing and thawing (Keel et al. 1987, Sunde et al. 1988).

Next to effectiveness, treatment costs play an increasingly large role in clinical decision making (Reinhardt et al. 2004). ICI is cheaper, while processing of donor sperm is not needed and for IUI processing of donor sperm is needed. In view of the large differences in conception leading to live birth between ICI and IUI, it is unlikely that the additional costs for IUI will impact decision making.

In summary, our study shows that ICI is inferior to IUI in the natural cycle in women undergoing inseminations with cryopreserved donor sperm in terms of live birth rate. Therefore, IUI in the natural cycle should be the preferred first line treatment in inseminations with cryopreserved donor sperm.

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CHAPTER 7

Summary and implications for
clinical practice and future research



In 1948, donor sperm treatment was introduced in the Netherlands (Levie 1965). Donor sperm treatment has been controversial from the outset, since a third party is needed which represents a fundamental shift away from traditional family building. In 1957, the 'Rapport van de Generale Synode van de Hervormde Kerk' rejected insemination with donor sperm (Levie 1959). Churches were not the only institutions rejecting donor sperm treatment; the director of the 'Geneeskundige en Gezondheidsdienst' of the Department of Justice stressed the immorality of denying a child a 'natural' father (Tromp 1949). Also the 'Gezondheidsraad' reported ethical objections against donor sperm treatment (Gezondheidsraad 1965). As a consequence, donor sperm treatment was for decades practiced in secrecy and donor anonymity was recommended (Kleegman 1954, Levie 1965).

Another issue is also typical for donor sperm treatment and that is the discrepancy between supply and demand. Initially, the supply side of the problem was the unwillingness to perform donor sperm treatment; approximately 6% of Dutch gynecologists were prepared to perform donor sperm treatment (van Unen 1971). The demand side of the problem was the growing number of heterosexual couples applying for donor sperm treatment, partly due to a diminished number of Dutch children given up for adoption (Kremer 1982).

Nowadays, many aspects in donor sperm treatment have undergone profound changes, but still there is a discrepancy between supply and demand. The supply side of the problem is the low number of suitable sperm donors caused by the prohibition of insemination of sperm of anonymous donors, the acknowledgement of the children's right to know their genetic origin from the age of 16 and the mandatory use of frozen- thawed donor sperm (AFS 1986, AFS 1988, Staatsblad 2002, NVOG 2015, NVOG 2018). The demand side of the problem is the annual increase in the number of women applying for donor sperm treatment caused by the social acceptance of donor sperm treatment, which enables also lesbian couples and single women to have children next to heterosexual couples (Shapiro et al. 1990, Baetens et al. 1995, Jennings 2017, De Geyter et al. 2020). Therefore, it is crucial to use the most (cost)-effective insemination technique with frozen-thawed donor sperm.

Traditionally, intracervical insemination in the natural cycle was the first line treatment in donor sperm treatment (Meijer et al. 1980, Le Lannou et al. 1989). After the use of frozen-thawed donor sperm became mandatory to prevent transmission of sexually transmitted diseases in insemination with donor sperm, pregnancy rates declined (Richter et al. 1984, Keel et al. 1987, AFS 1988, Subak et al. 1992). As a consequence, studies focused on improving pregnancy rates after intracervical insemination with modifications like one versus two inseminations per cycle, insemination by cervical cap versus insemination by straw and

different cut off values for the number of motile spermatozoa inseminated (Centola et al. 1990, Lincoln et al. 1995, Coulson et al. 1996, Flierman et al. 1997). From all these factors the only factor possibly improving ongoing pregnancy rates in intracervical insemination was when inseminations were performed with a total motile count above eight million (Le Lannou et al. 1995).

Meanwhile, intrauterine insemination was widely implemented worldwide as a fertility enhancing treatment in case of male subfertility and later on also in unexplained subfertility (Kerin et al. 1984, Chung et al. 1995). To enhance pregnancy rates with frozen-thawed donor sperm, it was thought that intrauterine insemination could improve pregnancy rates compared to intracervical insemination with frozen-thawed donor sperm (Patton et al. 1992, Hurd et al. 1993, Peters et al. 1993, Wainer et al. 1995, Matorras et al. 1996).

A systematic review and meta-analysis that pooled data of four randomised controlled trials on intracervical insemination versus intrauterine insemination showed that intrauterine insemination indeed resulted in higher clinical pregnancy rates (Besselink et al. 2008). Since this meta-analysis pooled natural cycles with cycles with ovarian stimulation and all included studies were underpowered, the results were not unequivocally in favor of one or the other technique. Still, from then on, intrauterine insemination was widely implemented, and is now the first line treatment in insemination with donor sperm (NICE 2013, De Geyter et al. 2020).

In view of this, we addressed several issues to try and provide an evidence base for donor sperm treatment with the ultimate aim to establish a cost-effective donor sperm treatment strategy. First we explored if there still is a discrepancy between the number of suitable sperm donors and the number of women applying for donor sperm treatment in the Netherlands, since the last evaluation on this topic was performed in 2006 (Janssens et al. 2006). Second, we evaluated if semen parameters affected pregnancy rates in intracervical insemination. Finally, we compared intracervical insemination with intrauterine insemination in the natural cycle with frozen-thawed donor sperm regarding effectiveness and costs.

In **chapter 1** we provide a general introduction of this thesis and describe the objectives of this thesis.

In **chapter 2** we assessed whether there is a discrepancy between the number of suitable sperm donors and the number of women applying for donor sperm treatment in the Netherlands. Eight sperm banks in the Netherlands were asked to complete a questionnaire about the number of suitable sperm donors and the number of women applying for donor sperm treatment in

2010. Five sperm banks completed the questionnaire. In 2010, 162 suitable Dutch sperm donors were available for 644 women. Three sperm banks also used donor sperm from sperm banks abroad. There were 193 (30%) ongoing pregnancies within six insemination cycles. The average waiting list was 15 months (average 0-30 months). A calculation showed that with a maximum of 25 children per sperm donor and an average of two children per woman, 52 sperm donors $((2 \times 644) / 25)$ would be sufficient to start donor sperm treatment for all 644 women that applied in 2010. The results of this calculation should be interpreted with caution, as there are several limitations. First, the number of children is set at maximum of 25 per sperm donor, although a sperm donor can obviously choose to donate for less than 25 children. Of the 162 sperm donors it was unknown how many children per sperm donor were born at the time of the questionnaire, implying that not all sperm donors were available for 25 children. Second, we did not take the results of the cytomegaly virus screening into account and matching on physical characteristics, which strongly reduces availability of a sperm donor. Finally, it was unclear how many new sperm donors are enrolled each year. The results of our study demonstrated that in 2010 it was unclear if there was a discrepancy between the number of sperm donors and the number of women who started with donor sperm treatment. Since long waiting lists existed and sperm banks also used sperm donors from abroad this indirect evidence suggests a shortage of Dutch sperm donors.

In **chapter 3** we investigated whether semen parameters are a determinant of success in intracervical insemination with frozen-thawed donor sperm. We performed a retrospective cohort study and included all women who started intracervical insemination with frozen-thawed donor sperm between April 1999 and December 2015. Sperm donors in this cohort donated sperm from 1984 to 2015. We analysed the sperm parameters after thawing and the chances on ongoing pregnancy by means of restricted cubic splines regression analyses. Based on the spline functions we defined thresholds. We included 1186 women that underwent 7103 cycles of intracervical insemination with frozen-thawed donor sperm of 129 sperm donors. Our results showed that total motility and total motile count (TMC) after thawing were associated with ongoing pregnancy rate. The best possible ongoing pregnancy chances after intracervical insemination were obtained at a total motility of $\geq 20\%$ and a total motile count (TMC) of $\geq 8 \times 10^6$ after thawing.

In **chapter 4** we present an update of a systematic review which aimed to assess the effectiveness of intracervical insemination compared to intrauterine insemination with frozen-thawed donor sperm. We searched for randomised controlled trials comparing intracervical insemination with intrauterine insemination in natural cycles and cycles with ovarian stimulation, and randomised controlled trials comparing different co-interventions in intracervical insemination and intrauterine insemination. Two review authors screened

456 titles and 19 full text articles that were identified by the conducted searches. We included six randomised controlled trials and made four comparisons (708 women analysed). The first comparison studied intracervical insemination and intrauterine insemination in natural cycles. There was insufficient evidence to determine whether there was any clear difference in live birth rate between intrauterine insemination and intracervical insemination in natural cycles (odds ratio (OR) 3.24, 95% confidence interval (CI) 0.12 to 87.13; 1 RCT, 26 women; very low-quality evidence). Intrauterine insemination resulted in higher clinical pregnancy rates (OR 6.18, 95% CI 1.91 to 20.03; 2 RCTs, 76 women; $I^2 = 48\%$; very low-quality evidence). There were no multiple pregnancies. The second comparison studied intracervical insemination and intrauterine insemination in gonadotrophin stimulated cycles. There was insufficient evidence to determine whether there was any clear difference in live birth rate between intrauterine insemination and intracervical insemination in gonadotrophin-stimulated cycles (OR 2.55, 95% CI 0.72 to 8.96; 1 RCT, 43 women; very low-quality evidence). Intrauterine insemination may be associated with higher multiple pregnancy rates than intracervical insemination (OR 2.77, 95% CI 1.00 to 7.69; 2 RCTs, 131 women; $I^2 = 0\%$; very low-quality evidence).

The third comparison studied timing of intracervical insemination. We found insufficient evidence to determine whether there was any clear difference in clinical pregnancy rates between intracervical insemination timed after a rise in urinary levels of LH versus a rise in basal temperature plus cervical mucus scores (OR 1.31, 95% CI 0.42 to 4.11; 1 RCT, 56 women; very low-quality evidence). The fourth comparison studied timing of intrauterine insemination. There was a higher clinical pregnancy rate when intrauterine insemination was timed one day after a rise in blood levels of luteinising hormone (LH) compared to intrauterine insemination two days after a rise in blood levels of LH (OR 2.00, 95% CI 1.14 to 3.53; 1 RCT, 351 women; low-quality evidence). Overall, there was a very low-quality of evidence; the main limitations were risk of bias due to poor reporting of study methods and serious imprecision. Therefore, the current evidence was insufficient to prefer intracervical insemination or intrauterine insemination, in natural cycles or with ovarian stimulation in donor sperm treatment.

In **chapter 5** we report a retrospective cohort study among all eight sperm banks in the Netherlands in which we assessed the effectiveness of intracervical insemination versus intrauterine insemination in the natural cycle with frozen-thawed donor sperm. We included all women who underwent insemination with frozen-thawed donor sperm in the natural cycle between January 2009 and December 2010. We compared time to pregnancy in the first six cycles of intracervical insemination and intrauterine insemination. We included

1843 women; 680 women underwent 2345 cycles of intracervical insemination and 1163 women underwent 4269 cycles of intrauterine insemination. Cumulative ongoing pregnancy rates up to six treatment cycles were 40.5% after intrauterine insemination and 37.9% after intracervical insemination. This corresponds with a hazard ratio of 1.02 [95% confidence interval (CI) 0.84-1.23] after controlling for female age and indication. Increasing female age was associated with a lower ongoing pregnancy rate, after both intrauterine insemination and intracervical insemination with a hazard ratio for ongoing pregnancy of 0.94 per year (95% CI 0.93-0.97). This led to the conclusion that there was no substantial beneficial effect of intracervical insemination in the natural cycle over intrauterine insemination in the natural cycle with frozen-thawed donor sperm.

In **chapter 6** we compared the effectiveness of intracervical insemination with intrauterine insemination in the natural cycle with frozen-thawed donor sperm in terms of live birth rate. We performed a multicenter open-label randomised controlled non-inferiority trial in five centres in the Netherlands and one centre in Belgium. We randomly allocated women who were eligible for donor sperm treatment with frozen-thawed donor semen to six cycles of intracervical insemination in the natural cycle or six cycles of intrauterine insemination in the natural cycle. The primary outcome was conception within eight months after randomisation leading to a live birth. Secondary outcomes were ongoing pregnancy, multiple pregnancy, clinical pregnancy, miscarriage and time to conception leading to live birth. Between June 2014 and February 2019, 421 women were randomly assigned, with 211 women allocated to intracervical insemination and 210 to intrauterine insemination. Conception leading to live birth occurred in 51 women (24%) allocated to intracervical insemination and in 81 women (39%) allocated to intrauterine insemination (RR 0.63, 95% CI: 0.47 to 0.84). This corresponds to an absolute risk difference of minus 15%; 95% CI: -24% to -6.9%, suggesting inferiority of intracervical insemination. Intracervical insemination also resulted in a lower live birth rate over time (HR 0.58, 95% CI: 0.41-0.82). Our per protocol analysis showed that within the eight months treatment horizon 48 women (39%) had live births after ICI and 79 women (57%) had live births after IUI (RR 0.68, 95% CI: 0.52-0.88; RD minus 18%, 95% CI: -30% to -6%).

The results of our study demonstrate that intrauterine insemination in the natural cycle should be the first line treatment in insemination with frozen-thawed donor sperm.

Implications for practice

We recommend intrauterine insemination in the natural cycle as first line treatment in women who start with donor sperm treatment, since it is more effective than intracervical insemination in the natural cycle.

We highly recommend to develop a national guideline on donor sperm treatment with attention not only for the demand side, but also the supply side. Without such a national guideline we feel it is no longer acceptable to practice DST

Implications for future research

It is a well-known pitfall that clinicians are usually so excited about new innovative treatments, that they forget to evaluate existing treatments. During the last decades research focused mainly on 'preventing' donor sperm treatment in heterosexual couples by focusing on new medically-assisted techniques such as intracytoplasmic sperm injection (ICSI) with or without surgical sperm extraction in men with severe male infertility or azoospermia, semen washing and insemination in men infected with human immunodeficiency virus to prevent transmission of the virus and preimplantation genetic diagnosis in couples in whom one of the partners are carrier of a genetic defect (Palermo et al. 1992, Semprini et al. 1992, Tournaye et al. 1994, Silber et al. 1996, Liebaers et al. 1998). Hereby clinicians completely overlooked that donor sperm treatment is still a treatment option after failure of surgical sperm retrieval or ICSI, after failure of sperm washing and insemination, after failed preimplantation genetic diagnosis, for couples that do not opt for these new medically-assisted reproductive techniques, lesbian couples and single women. As a consequence, we have inseminated women for decades with frozen-thawed donor sperm without evidence on the most effective insemination technique.

In this thesis we provided evidence on the first line treatment for women who start with donor sperm treatment, but evidence on how many cycles of intrauterine insemination we should perform before we switch to a second line treatment is unknown. Although our randomised controlled trial seems to indicate that pregnancy rates decline after the fifth cycle of intrauterine insemination, the number of women is too small to draw any conclusions. To investigate this further, a retrospective cohort study should be performed in the Netherlands to evaluate how many cycles of intrauterine insemination we should perform before we continue with a second line treatment.

Another critical knowledge gap in donor sperm treatment is that it is unknown which treatment should be second line treatment after intrauterine insemination in the natural

cycle has failed. Nowadays, when women do not conceive after six insemination cycles in the natural cycle, treatment is continued with intrauterine insemination with ovarian stimulation for six cycles. When women do not conceive after six cycles of intrauterine insemination with ovarian stimulation three cycles of in vitro fertilization are offered. Whether this empirical scheme is the most cost effective and safe strategy is unknown, and we recommend a randomised controlled clinical trial. The design of this randomised controlled trial could be to compare six cycles of intrauterine insemination with ovarian stimulation with three cycles of in vitro fertilization with single embryo transfer and focus on multiple pregnancy rates and costs.

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CHAPTER 8

Samenvatting



In 1948 werden behandelingen met donorsperma geïntroduceerd in Nederland (Levie 1965). De behandelingen met donorsperma waren vanaf het begin af aan controversieel omdat er een derde persoon nodig is om zich voort te planten en dit zorgt voor een fundamenteel andere gezinssamenstelling dan het gekende traditionele gezin. In 1957 werden behandelingen met donorsperma afgekeurd in een 'Rapport van de Generale Synode van de Hervormde Kerk' (Levie 1959). Kerken waren niet de enige instituten die de behandeling met donorsperma afkeurden, de directeur van de 'Geneeskundige en Gezondheidsdienst' van het Departement van Justitie vond het immoreel dat een kind zijn 'biologische vader' werd ontzegd (Tromp 1949). Ook de Gezondheidsraad rapporteerde ethische bezwaren tegen behandelingen met donorsperma (Gezondheidsraad 1965). Het gevolg was dat behandelingen met donorsperma lange tijd werden uitgevoerd in het geheim en het anoniem houden van de spermadonor werd aanbevolen (Kleegman 1954, Levie 1965).

Een ander probleem binnen de behandelingen met donorsperma is de discrepantie tussen vraag en aanbod. Initieel was het aanbodprobleem de onbereidheid om behandelingen met donorsperma uit te voeren, ongeveer 6% van de Nederlandse gynaecologen was bereid om behandelingen met donorsperma uit te voeren (van Unen 1971). Het probleem met betrekking tot de vraag was dat het aantal heteroseksuele koppels dat gebruik wilden maken van behandelingen met donorsperma steeg, doordat het aantal kinderen dat werd afgestaan voor adoptie afnam (Kremer 1982).

Tegenwoordig zijn er veel aspecten veranderd binnen de behandelingen met donorsperma, maar nog steeds is er een discrepantie tussen vraag en aanbod. Het aanbodprobleem is een beperkt aantal beschikbare Nederlandse spermadonoren veroorzaakt door het opheffen van de anonimiteit. Hierdoor werd het gebruik maken van anonieme spermadonoren verboden en kinderen werden in staat gesteld om de persoonsidentificerende gegevens van de spermadonor op te vragen vanaf een leeftijd van 16 jaar. Daarnaast werd het verplicht gebruik te maken van gecryopreserveerd donorsperma (AFS 1986, AFS 1988, Staatsblad 2002, NVOG 2015, NVOG 2018). Het aanbodprobleem is de jaarlijkse groei van het aantal vrouwen dat wil starten met de behandeling met donorsperma veroorzaakt door de sociale acceptatie van de behandeling met donorsperma, hierdoor werd het naast heteroseksuele koppels ook mogelijk voor lesbische koppels en alleenstaande vrouwen om zwanger te worden met donorsperma. (Shapiro et al. 1990, Baetens et al. 1995, Jennings 2017, De Geyter et al. 2020). Daarom is het belangrijk om de meest (kosten)- effectieve inseminatietechniek te gebruiken met gecryopreserveerd donorsperma.

Van oudsher is intra-cervicale inseminatie in de natuurlijke cyclus de eerstelijns behandeling met donorsperma (Meijer et al. 1980, Le Lannou et al. 1989). Nadat het gebruik van gecryopreserveerd donorsperma verplicht werd om transmissie van seksueel overdraagbare aandoeningen te voorkomen, daalde het aantal zwangerschappen na inseminatie met donorsperma (Richter et al. 1984, Keel et al. 1987, AFS 1988, Subak et al. 1992). Hierdoor gingen studies zich concentreren op het verbeteren van het aantal zwangerschappen na intra-cervicale inseminatie met donorsperma. Voorbeelden hiervan zijn één versus twee inseminaties per cyclus, inseminatie met een cupje versus inseminatie met een rietje en verschillende afkapwaarden voor het aantal motiele spermatozoa die werden geïnsemineerd (Centola et al. 1990, Lincoln et al. 1995, Coulson et al. 1996, Flierman et al. 1997). De enige factor die mogelijk het aantal zwangerschappen na intra-cervicale inseminatie verbetert is als er geïnsemineerd wordt met een total motile count boven de acht miljoen (Le Lannou et al. 1995).

Ondertussen werd intra-uteriene inseminatie wereldwijd geïntroduceerd als een fertiliteit bevorderende behandeling voor milde mannelijke factor en onverklaarbare subfertiliteit (Kerin et al. 1984, Chung et al. 1995). Er werd gedacht dat intra-uteriene inseminatie met gecryopreserveerd donorsperma de kans op een zwangerschap zou kunnen vergroten vergeleken met intra-cervicale inseminatie met gecryopreserveerd donorsperma (Patton et al. 1992, Hurd et al. 1993, Peters et al. 1993, Wainer et al. 1995, Matorras et al. 1996).

Een systematische review en meta-analyse voegde vier gerandomiseerde gecontroleerde trials over intra-cervicale inseminatie versus intra-uteriene inseminatie samen en liet zien dat intra-uteriene inseminatie inderdaad resulteerde in een hoger aantal klinische zwangerschappen (Besselink et al. 2008). Aangezien deze meta-analyse resultaten van cycli in de natuurlijke cyclus samen heeft gevoegd met cycli met ovariële stimulatie en omdat alle geïncludeerde studies zeer weinig patiënten hadden geïncludeerd, leidden de resultaten niet tot de voorkeur voor de ene of de andere techniek. Desondanks is intra-uteriene inseminatie geïmplementeerd en is het nu de eerstelijnsbehandeling in inseminaties met donorsperma. (NICE 2013, De Geyter et al. 2020). Dit in acht nemend, hebben we een aantal issues aangestipt en zo getracht bewijskracht te vinden voor de behandelingen met donorsperma met als ultieme doel een kosteneffectieve behandelstrategie. Eerst hebben we onderzocht of er nog steeds een discrepantie is tussen het aantal beschikbare spermadonoren en het aantal vrouwen dat in aanmerking komt voor de behandeling met donorsperma in Nederland, aangezien de laatste evaluatie met betrekking tot dit onderwerp in 2006 is uitgevoerd (Janssens et al. 2006). Als tweede hebben we geëvalueerd of semen parameters de kans op een doorgaande zwangerschap beïnvloeden na intra-cervicale

inseminatie. Tenslotte hebben we intra-cervicale inseminatie vergeleken met intra-uteriene inseminatie met gecryopreserveerd donorsperma in de natuurlijke cyclus betreffende effectiviteit en kosten.

In **hoofdstuk 1** verschaffen wij de algemene introductie van dit proefschrift en beschrijven wij de doelen van dit proefschrift.

In **hoofdstuk 2** beschrijven we de resultaten van een studie waarin we onderzochten of er een discrepantie is tussen het aantal inzetbare spermadonoren en het aantal vrouwen dat wil starten met een behandeling met donorsperma in Nederland. Acht spermabanken in Nederland werden gevraagd een enquête in te vullen over het aantal inzetbare spermadonoren en het aantal vrouwen dat zich aanmeldde voor een behandeling met donorsperma in 2010. Vijf spermabanken hebben de vragenlijst ingevuld. In 2010 waren er 162 inzetbare spermadonoren voor 644 vrouwen. Drie spermabanken maakten ook gebruik van sperma van buitenlandse spermabanken. Er waren 193 (30%) doorgaande zwangerschappen binnen zes inseminatie cycli. De gemiddelde wachtlijst was 15 maanden (gemiddeld 0-30 maanden). Een berekening liet zien dat met een maximum van 25 kinderen per spermadonor, 52 spermadonoren ($(2 \times 644) / 25$) genoeg zouden zijn voor alle 644 vrouwen die wilden starten in 2010. De resultaten van deze berekening moeten voorzichtig worden geïnterpreteerd, aangezien er een aantal beperkingen zijn. Allereerst is er een maximum aantal kinderen van 25 per spermadonor, vanzelfsprekend kan een spermadonor ervoor kiezen om voor minder kinderen inzetbaar te zijn. Van de 162 spermadonoren was het onbekend hoeveel kinderen er al waren geboren ten tijde van de enquête, dit illustreert dat niet alle spermadonoren inzetbaar waren voor 25 kinderen op dat moment. Ten tweede hebben we de resultaten van de screening op cytomegalie virus niet meegenomen, terwijl dit wel de inzetbaarheid van een spermadonor beperkt. Tenslotte was het onduidelijk hoeveel nieuwe spermadonoren zich ieder jaar aanmelden. De resultaten van onze studie laten zien dat het onduidelijk is of er een discrepantie is tussen vraag en aanbod. Aangezien er lange wachtlijsten bestaan en er gebruik werd gemaakt van sperma van spermabanken uit het buitenland is dit indirect bewijs dat er mogelijk een tekort aan spermadonoren bestaat.

In **hoofdstuk 3** onderzochten we of semen parameters een determinant zijn in het succesvol zijn van intra-cervicale inseminatie met gecryopreserveerd donorsperma. We voerden een retrospectieve cohortstudie uit en includeerden alle vrouwen die startten met intra-cervicale inseminatie tussen april 1999 en december 2015. Spermadonoren in dit cohort doneerden van 1984 tot 2015. We analyseerden de sperma parameters na ontdooien en berekenden de kansen op een doorgaande zwangerschap door restricted cubic splines regressie analyses.

Gebaseerd op de spline functies definieerden we nieuwe afkapwaarden. We includeerden 1186 vrouwen die 7103 cycli intra-cervicale inseminatie met gecryopreserveerd donorsperma ondergingen van 129 spermadonoren. Onze resultaten lieten zien dat een totale motiliteit en de total motile count (TMC) na ontdooien waren geassocieerd met kansen op doorgaande zwangerschap. De hoogste doorgaande zwangerschapskansen werden bereikt door te insemineren bij intra-cervicale inseminatie met een totale motiliteit van $\geq 20\%$ en een total motile count (TMC) van $\geq 8 \times 10^6$ na ontdooien.

In **hoofdstuk 4** presenteren we een update van een systematische review waarin werd getracht de effectiviteit van intra-cervicale inseminatie te vergelijken met intra-uteriene inseminatie met gecryopreserveerd donorsperma. We zochten gerandomiseerde gecontroleerde studies die intra-cervicale inseminatie vergeleken met intra-uteriene inseminatie in de natuurlijke cyclus en met ovariële stimulatie en gerandomiseerde studies die verschillende co-interventies vergeleken in intra-cervicale inseminatie en intra-uteriene inseminatie. Twee review-auteurs screenden 456 titels en 19 volledige artikelen die waren geïdentificeerd door de uitgevoerde zoekstrategieën. We includeerden zes gerandomiseerde gecontroleerde studies en maakten vier vergelijkingen (708 vrouwen werden geanalyseerd).

De eerste vergelijking onderzocht intra-cervicale inseminatie en intra-uteriene inseminatie in de natuurlijke cyclus. Er was onvoldoende bewijskracht of er een duidelijk verschil in levendgeborenen was tussen intra-uteriene inseminatie en intra-cervicale inseminatie in de natuurlijke cyclus (odds ratio (OR) 3.24, 95% confidence interval (CI) 0.12 tot 87.13; 1 RCT, 26 vrouwen; zeer lage bewijskracht). Intra-uteriene inseminatie resulteerde in een hoger aantal klinische zwangerschappen (OR 6.18, 95% CI 1.91 tot 20.03; 2 RCTs, 76 vrouwen; $I^2 = 48\%$; zeer lage bewijskracht). Er waren geen meerlingen.

De tweede vergelijking onderzocht intra-cervicale inseminatie en intra-uteriene inseminatie in cycli met gonadotrofines. Er was onvoldoende bewijskracht om een duidelijk verschil aan te tonen in het aantal levend geboren tussens intra-uteriene inseminatie en intra-cervicale inseminatie in cycli met stimulatie middels gonadotrofines (OR 2.55, 95% CI 0.72 tot 8.96; 1 RCT, 43 vrouwen; zeer lage bewijskracht). Intra-uteriene inseminatie zou geassocieerd kunnen zijn met een hoger aantal tweelingzwangerschappen vergeleken met intra-cervicale inseminatie (OR 2.77, 95% CI 1.00 tot 7.69; 2 RCTs, 131 vrouwen; $I^2 = 0\%$; zeer lage bewijskracht).

De derde vergelijking onderzocht de timing van intra-cervicale inseminatie. We vonden onvoldoende bewijs om aan te tonen dat er een duidelijk verschil was in klinische zwangerschappen tussen intra-cervicale inseminatie getimed met urinaire luteïniserend

hormoontesten versus een stijging in de basale temperatuurcurve met cervicale mucus score (OR 1.31, 95% CI 0.42 tot 4.11; 1 RCT, 56 vrouwen; zeer lage bewijskracht).

De vierde vergelijking onderzocht de timing van intra-uteriene inseminatie. Er was een hoger aantal klinische zwangerschappen wanneer de intra-uteriene inseminatie werd uitgevoerd één dag nadat het luteïniserend hormoon positief was getest in het bloed vergeleken met een intra-uteriene inseminatie twee dagen nadat het luteïniserend hormoon positief was getest in het bloed (OR 2.00, 95% CI 1.14 tot 3.53; 1 RCT, 351 vrouwen; lage bewijskracht). Over het algemeen was het wetenschappelijk bewijs van zeer lage kwaliteit, vooral door het risico op bias door slecht rapporteren van de studiemethoden en serieuze onnauwkeurigheid. Bewijskracht is van zeer lage kwaliteit. Gezien dit gebrek aan wetenschappelijk bewijs concluderen wij dat er geen voorkeur is voor intra-cervicale inseminatie of intra-uteriene inseminatie in de natuurlijke cyclus of in gestimuleerde cycli in de behandeling met donorsperma.

In **hoofdstuk 5** beschrijven we de resultaten van een retrospectieve cohortstudie bij alle acht spermabanken in Nederland naar de effectiviteit van intra-cervicale inseminatie versus intra-uteriene inseminatie in de natuurlijke cyclus met gecryopreserveerd donorsperma. We includeerden alle vrouwen die inseminaties ondergingen met gecryopreserveerd donorsperma in de natuurlijke cyclus tussen januari 2009 en december 2010. We vergeleken de tijd tot aan zwangerschap binnen de eerste zes behandelcycli intra-cervicale inseminatie en intra-uteriene inseminatie. We includeerden 1843 vrouwen; 680 vrouwen ondergingen 2345 cycli intra-cervicale inseminatie en 1163 vrouwen ondergingen 4269 cycli intra-uteriene inseminatie. De cumulatieve doorgaande zwangerschapscijfers waren binnen zes cycli 40.5% na intra-uteriene inseminatie en 37.9% na intra-cervicale inseminatie. Dit correspondeert met een hazard ratio van 1.02 (95% betrouwbaarheidsinterval (CI) 0.84-1.23) na gecorrigeerd te hebben voor leeftijd van de vrouw en indicatie. Een toenemende vrouwelijke leeftijd was geassocieerd met lagere doorgaande zwangerschapscijfers, na intra-uteriene inseminatie en intra-cervicale inseminatie met een hazard rate voor doorgaande zwangerschap van 0.94 per jaar (95% CI 0.93-0.97). Wij concluderen dat er onvoldoende voordelig effect wordt waargenomen van intra-cervicale inseminatie vergeleken met intra-uteriene inseminatie in de natuurlijke cyclus met gecryopreserveerd donorsperma.

In **hoofdstuk 6** vergeleken we de effectiviteit van intra-cervicale inseminatie met intra-uteriene inseminatie in de natuurlijke cyclus met gecryopreserveerd donorsperma. We verrichtten een open-label gerandomiseerd non-inferioriteitsonderzoek in vijf centra in Nederland en één centrum in België. We randomiseerden vrouwen die in aanmerking

kwamen voor een behandeling met donorsperma tussen zes cycli intra-cervicale inseminatie en zes cycli intra-uteriene inseminatie. Onze primaire uitkomstmaat was conceptie binnen acht maanden na randomisatie leidend tot de geboorte van een levendgeborene. Secundaire uitkomsten waren doorgaande zwangerschap, meerlingzwangerschap, klinische zwangerschap, miskraam en de tijd tot conceptie leidend tot een levendgeborene. Tussen juni 2014 en februari 2019 werden 421 vrouwen gerandomiseerd waarbij er 211 werden toegewezen aan intra-cervicale inseminatie en 210 vrouwen werden toegewezen aan intra-uteriene inseminatie. Van de vrouwen die waren toegewezen aan intra-cervicale inseminatie kregen er 51 vrouwen (24%) een levendgeborene en van de vrouwen die waren toegewezen aan intra-uteriene inseminatie kregen 81 vrouwen (39%) een levendgeborene (RR 0.63, 95% CI: 0.47 tot 0.84). Dit komt overeen met een absoluut risicoverschil van min 15%; 95% CI: -24% tot -6.9%, dit suggereert inferioriteit van intra-cervicale inseminatie. Intra-cervicale inseminatie resulteerde ook in een lager aantal levendgeborenen in de tijd (HR 0.58, 95% CI: 0.41-0.82). Onze per protocol analyse laat zien dat 48 vrouwen (38%) toegewezen aan intra-cervicale inseminatie een levendgeborene hadden en dat 79 vrouwen (56%) toegewezen aan intra-uteriene inseminatie een levendgeborene hadden (RR 0.68, 95% CI: 0.52-0.88; RD-min 18%, 95% CI: -30% tot -6%). De resultaten van onze studie laten zien dat intra-uteriene inseminatie in de natuurlijke cyclus de eerstelijnsbehandeling moet zijn in de behandeling met gecryopreserveerd donorsperma.

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PhD Portfolio

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List of publications

- 2012 M. Visser, P.A.L. Kop, M. van Wely, F. van der Veen, G.J.E. Gerrits, M.C.B. van Zwieten. Counselling on disclosure of gamete donation to donor offspring: a search of facts. *Facts an vision in Gynaecology* 2012 4(3)
- 2014 P.A.L. Kop, P.M.W. Janssens, M.H. Mochtar. Kunstmatige inseminatie met donorsperma in Nederland: toekomstbestendig? *NTVG* 2014 ; 158
- 2015 P.A.L. Kop, B. Arends, M.H.J. Curfs, P.M.W. Janssens, E. Rijnders, H. Ruis, A.H.J. Simons, S. Repping, F. van der Veen, M.H. Mochtar. Intrauterine insemination or intra-cervical insemination with cryo-preserved donor sperm in the natural cycle: a nationwide cohort study. *Human reproduction* 2015; 603-7
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- 2020 P.A.L. Kop, M. van Wely, A.A. de Melker, F. van der Veen, M.H. Mochtar. Donor sperm treatment: the role of semen parameters in intracervical insemination, a retrospective cohort study. *Human Fertility*, February 2022, pages 1-7 2022 P.A.L. Kop, M. van Wely, A. Nap, A.T. Soufan, A.A. de Melker, B.W.J. Mol, R.E. Bernardus, M. De Brucker, P.M.W. Janssens, J.J.P.M. Pieters, S. Repping, F. van der Veen, M.H. Mochtar. Intracervical insemination versus intrauterine insemination with cryopreserved donor sperm in the natural cycle: a randomized controlled trial. *Human Reproduction*, April 2022;deac 071

Contribution to textbook

- 2012 Site of insemination in artificial insemination with donor sperm. Evidence based book on IUI. Publisher Prof. Ombelet and Mr. Peden.
- 2012 Geschiedenis van Kunstmatige Inseminatie met Donorsperma: CANON gynaecologie en obstetrie.

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General courses

2012	Practical biostatistics (1.1 ECTS)
2013	Good Clinical Practice (0.9 ECTS)
2013	Systematic Reviews (0.9 ECTS)
2014	Basic Course Legislation and Organization (BROK) (1.1 ECTS)

Seminars

2009-2014	weekly department lunch meetings (5ECTS)
2009-2014	weekly department seminars (5 ECTS)
2009-2014	weekly department journal clubs (5ECTS)
2013	The Donor conceived perspective. University College, London

Oral presentations

2010	KID-zorg in Nederland. DSRM-meeting, Amsterdam
2012	Intrauterine insemination or intra-cervical insemination with cryo-preserved donor sperm: a nationwide cohort study. DSRM-meeting, Amersfoort
2014	KID in Nederland, IVF-werkgroep, Utrecht
2015	Parenting decision making after failed TESE, Gynaecongres, Amersfoort
2019	Intracervical insemination compared to intrauterine insemination in donor sperm treatment: a randomized controlled trial. Gynaecongres, Arnhem
2020	Intracervical insemination compared to intrauterine insemination in donor sperm treatment: a randomized controlled trial, ASRM, virtual congress
2020	Intracervical insemination compared to intrauterine insemination in donor sperm treatment: a randomised controlled trial. NVML congress, Amersfoort

Poster presentations

- 2012 Intrauterine insemination or intra-cervical insemination with cryo-preserved donor sperm: a nationwide cohort study.
ESHRE, 28th Annual meeting, Istanbul
- 2019 Intracervical insemination compared to intrauterine insemination in donor sperm treatment: a randomised controlled trial,
ESHRE, 35th Annual meeting, Vienna, Austria.
- 2020 Intracervical insemination compared to intrauterine insemination in donor sperm treatment: a randomised controlled trial.
ESHRE, 36th Annual meeting, Virtual meeting

Attending conferences

- 2015 47e Gynaecongres, Amersfoort
- 2015 48e Gynaecongres, Arnhem
- 2016 49e Gynaecongres, Eindhoven
- 2016 50e Gynaecongres, Amersfoort
- 2017 52e Gynaecongres, Amersfoort
- 2018 53e Gynaecongres, Utrecht
- 2018 54e Gynaecongres, Amersfoort
- 2019 55e Gynaecongres, Utrecht
- 2019 56e Gynaecongres, Amersfoort
- 2019 ESHRE, 35th Annual Meeting, Vienna, Austria.
- 2020 57e Gynaecongres, virtueel
- 2020 ESHRE, 36th Annual Meeting, Virtual congress
- 2020 ASRM, Virtual congress

Teaching and student coaching

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Lieve Marije, beiden nageplaatst voor de studie geneeskunde in Amsterdam. Jij was al volop geïntegreerd in Groningen en ik in Leiden. We hadden allebei geen zin in dat Amsterdam, maar wilden wel graag geneeskunde studeren. Samen met Fre was het een geweldige tijd op de Borssenburg! Ik geniet altijd van onze etentjes en waardeer onze vriendschap enorm en ben super trots dat je vandaag naast me wil staan!

Mijn Zeeuwse tantes en ooms. Dat een genetische link niet het belangrijkste is, wist ik

eigenlijk al voordat ik met dit proefschrift begon. Heel veel dank voor jullie betrokkenheid.

Mijn lieve genetische familie, lieve tantes en ooms, neven en nichten van familie Kop en van Hooijdonk, wat is het heerlijk om te weten dat er een familie is die je onvoorwaardelijk steunt. Door alle Coronatoestanden hebben we elkaar veel te weinig gezien. Laten we dat snel veranderen.

Lieve tante Loes, woorden schieten te kort voor de enorme steun die je voor me bent geweest.

Lieve Wietske, dank dat je altijd al mijn stukken in het Nederlands hebt gecorrigeerd in dit proefschrift. En dat je ook je eigen dankwoord nog moest corrigeren is natuurlijk het toppunt, maar daar een fout in laten staan, zou me slapeloze nachten bezorgen. Wat heb je een heerlijk gezin! Ik ben trots dat ik deel uit mag maken van jullie familie.

Lieve Hans en Lies, jullie zijn de meest betrokken en zorgzaamste schoonouders die ik mij kan wensen. Dank voor alle keren dat jullie er voor mij, Jaap en de meisjes zijn. Ik ben ongelooflijk blij met jullie.

Lieve Ome Toine en tante Mion, mijn stand-in ouders en inmiddels ook opa en oma. Niets is jullie teveel, jullie zijn er altijd voor Jeroen, Veerle en mij en inmiddels ook voor onze aanhang. Ik kijk uit naar weer een gezellig vaaravontuur!

Lieve Jeroen en Veerle, jullie staan altijd voor me klaar. Ik heb heel veel respect voor alles wat jullie doen en hoe jullie het doen. Ik kijk uit naar weer een ontspannend broer en zus avondje met jullie en jullie warme gezinnen.

Mijn ouders, lieve pap, de liefde voor het vak heb ik van jou met de paplepel binnen gekregen. Als jong meisje zat ik in de kinderstoel te wachten tot je klaar was met een sectio bij een hond of kat. Later ging ik ook mee voor het grotere werk, koeien en schapen. Zelf hadden we vroeger wat koeien die je insemineerde. Je was dan ook heel verbaasd over mijn onderzoeksonderwerp: 'natuurlijk doe je dat intra-uterien, dat doen we bij koeien en paarden ook, is bewezen veel beter.' Inderdaad, je had gelijk. De wetenschap dat je altijd voor me klaarstaat maakt me ontzettend blij.

Lieve mama, als een rots in de branding voor haar kinderen. Samen met papa runde je de praktijk en voedde je ook ons op. Ik vond het toen allemaal heel normaal, maar nu vraag ik me wel eens af hoe je dat allemaal deed. Je bent mijn rots in de branding en de liefste en beste Oma Tonnie van de wereld.

Lieve Jaap, Sofie en Cato, mijn promotie is eindelijk af. Alle clichés zijn waar, jullie zijn waar het om draait in mijn leven. Ik hou van jullie! Nu hebben we eindelijk meer tijd om samen te genieten!

Curriculum Vitae

Femke Kop werd op 3 september 1982 geboren in Terneuzen. Na het behalen van haar VWO diploma op het Zeldenrust-Steelant college in Terneuzen, ging zij geneeskunde studeren aan de Universiteit van Amsterdam.

Tijdens haar coschap gynaecologie in het “ Onze Lieve Vrouwe Gasthuis” in Amsterdam en een tropencoschap in Turiani in Tanzania, besloot zij gynaecoloog te willen worden.

In 2008 kreeg zij in aansluiting op haar laatste coschap een baan aangeboden als ANIOS gynaecologie in het Zaans Medisch Centrum in Zaandam. Begin 2009 ging zij als ANIOS in het Amsterdam Medisch Centrum (AMC) werken.

In 2010 startte zij vervolgens als IVF-arts bij het Centrum Voor Voortplantingsgeneeskunde van het AMC. Vanaf 2010 startte zij naast haar werkzaamheden als IVF-arts als fertiliteitsarts bij Bergman/ KID Centrum Amsterdam en deed hier de medische intakes voor vrouwen die wilden starten met een behandeling met donorsperma. Tijdens haar werk binnen de KID-zorg ontstonden er vragen naar de meest effectieve behandelstrategie met donorsperma. Onder leiding van Prof. Dr. F. van der Veen, Prof. Dr. S. Repping, dr. M.H. Mochtar en dr. M. van Wely werd evaluatie onderzoek opgezet naar de behandelingen met donorsperma.

Dit resulteerde in haar promotie onderzoek naar de uitdagingen in de behandelingen met donorsperma.

In juni 2014 startte zij haar opleiding tot gynaecoloog binnen het cluster AMC in het Flevoziekenhuis. In 2016 vervolgde zij haar opleiding in het Amsterdam UMC, locatie AMC. In 2019 keerde zij terug naar het Flevoziekenhuis voor een differentiatie benigne gynaecologie. In 2020 deed zij een differentiatie voortplantingsgeneeskunde en endometriose in het Amsterdam UMC, locatie VUMC. In 2021 heeft zij haar opleiding tot gynaecoloog afgerond in het Flevoziekenhuis. Momenteel werkt Femke als chef de kliniek in het Flevoziekenhuis in Almere.

Femke woont in Naarden met Jaap Brouwer en hun dochters Sofie en Cato.

