PhD THESIS

Individualized prognosis for live birth and selected safety aspects of infants in women entering fertility treatment programs in Denmark 2002 – 2012

SARA SOFIA MALCHAU LAUESGAARD

FACULTY OF HEALTH AND MEDICAL SCIENCES

DEPARTMENT OF OBSTETRICS AND GYNECOLOGY,

COPENHAGEN UNIVERSITY HOSPITAL, HVIDOVRE

ACADEMIC SUPERVISORS

ANJA PINBORG

ANDERS NYBOE ANDERSEN

ANNE LOFT

ANNA-KARINA AARIS HENNINGSEN

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BY

SARA SOFIA MALCHAU LAUESGAARD

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SARA SOFIA MALCHAU LAUESGAARD, M.D.

Faculty of Health and Medical Sciences, University of Copenhagen
Department of Obstetrics and Gynecology, Copenhagen University Hospital, Hvidovre, DK

TITLE

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ACADEMIC SUPERVISIORS

Professor Anja Pinborg
Department of Obstetrics and Gynecology, Copenhagen University Hospital Hvidovre

Professor Anders Nyboe Andersen
Fertility Clinic, Copenhagen University Hospital, Rigshospitalet

Anne Loft, M.D., Senior Consultant
Fertility Clinic Copenhagen University Hospital, Rigshospitalet

Anna-Karina Aaris Henningsen, M.D.
Department of Obstetrics and Gynecology, Copenhagen University Hospital, Rigshospitalet

ASSESSMENT COMMITTEE

Professor Nicholas Macklon (chairman)
Department of Clinical Medicine, University of Copenhagen, DK

Professor Cecilia Ramlau-Hansen
Department of Public Health, Department of Epidemiology, University of Aarhus, DK

Dr. Sesh Kemal Sunkara
Queens Hospital, Barking Haveridge, Redbridge University Hospitals, UK

SUBMITTED

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1 Preface

This thesis is based on studies performed at the Department of Obstetrics and Gynecology, Hvidovre Hospital and the Fertility Clinic, Rigshospitalet in September 2013 – May 2017, during which time I was employed as a research fellow. In September 2014 – August 2015 I was on maternity leave. During my research fellow ship I have been responsible for data management and analyses and for writing and revising the manuscripts. Preparing the data for analysis has been the most time consuming part of the project.

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always being helpful. Further, I would like to express my gratitude to the Danish fertility clinics, reporting their treatment activity.

I would like to thank my research colleagues at Hvidovre Hospital and Rigshospitalet for good discussions and good times: Thank you Tanja, Sacha, Mette, Anne-Lærke, Fie, Hanne, Katrine, Thomas, Nina, Veronica, Mikkel, Robin, Mette, Lea, Astrid, Helene, Katrine and my sister Emma. I would like to express my warmest gratitude to my parents Inger and Henrik and siblings Emma and Erik, I want you to know how much I value your loving support, advice and encouragement. I would like to thank my mother- and father- in-law, Anne Beth and Jørgen, for unconditional and invaluable help. Finally, I would like to thank my beloved husband Jacob for giving me time to write, for enduring when I was absent or absent-minded and for being patient overall. And thank you Otto, Sigurd and Hedvig, for being gorgeous and funny and perfect.

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2 Funding

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3 Abbreviations

Assisted reproductive technology	ART
Clomiphene citrate	CC
Cumulative live birth rates	CLBR
Denmark	DK
Follicle stimulating hormone	FSH
Frozen embryo transfer	FER
Human Fertilization and Embryology Authority	HFEA
Human choriogonadotropin	hCG
Interquartile range	IQR
Intracytoplasmic sperm injection	ICSI
Intra uterine insemination	IUI
Intra uterine insemination with donor semen	IUI-D
Intra uterine insemination with homologous semen	IUI-H
In vitro fertilization	IVF
Large for gestational age	LGA
Low birth weight	LBW
Natural conception	NC
Ovarian hyperstimulation syndrome	OHSS
Oocyte donation	OD
Preterm birth	PTB
Small for gestational age	SGA
Spontaneous conception	SC

4 List of papers

Three manuscripts are included in this thesis. In the text, they are referred to as paper I, paper II and paper III:

I. Malchau SS, Henningsen AA, Loft A, Rasmussen S, Forman J, Nyboe Andersen A, Pinborg A. **The long-term prognosis for live birth in couples initiating fertility treatments.** Hum Reprod. 2017 May 4:1-11.

II. Malchau SS, Henningsen AA, Forman J, Loft A, Nyboe Andersen A, Pinborg A. **Cumulative live birth rate prognosis based on the number of aspirated oocytes in previous ART cycles.** Submitted to Human Reproduction June 2017.

III. Malchau SS, Loft A, Henningsen AK, Nyboe Andersen A, Pinborg A. **Perinatal outcomes in 6,338 singletons born after intrauterine insemination in Denmark, 2007 to 2012: the influence of ovarian stimulation.** Fertil Steril. 2014 Oct;102(4):1110-1116.

5 Introduction

In Denmark, 18% of women aged 25-44 years at some point experience infertility (1). For subfertile couples fertility treatment is an available option and most costs are covered by the national health care system. However, going through fertility treatment may be trying. The couples may experience side-effects and there are no guarantees of a successful outcome (2,3). Providing information on success rates is an important tool in adjusting expectations and advising the women and couples through the trajectory of treatments. There are several steps in each treatment cycle that can fail or succeed; and since most couples undergo more than one treatment, there is a large variety of ways to report success rates. A recent study assessed how fertility clinics in the UK report treatment outcome, and found that 53 clinics reported 51 different types of outcome measures (4). Success can e.g. be measured as pregnancy rates, ongoing pregnancy rates, or live birthrates, and the treatment unit can be per started treatment cycle, per embryo transfer, or per complete treatment cycle (all embryo transfers resulting from one ovarian stimulation). For subfertile couples starting fertility treatments, the single most important objective is having a child, and a prognosis should be easy to understand and reflect the overall chances to achieve this. With this objective in mind, it is also important to report the occurrence of natural conception in couples who have initiated fertility treatment. Many couples do not achieve live birth in their first treatment attempt, and for couples considering further treatment, their prognosis should be adjusted with regard to their previous treatment history, in order to provide the most accurate estimate of their chances of having a child.

In Denmark, approximately 35.000 fertility treatments are performed every year (5). The compulsory Danish ART register has existed since 1994, and also includes intrauterine inseminations (IUI) since 2006. When cross-linked to the Medical birth registry it is possible to study the long term probability for live birth, regardless of whether the conception was assisted or natural. The registries make it possible to follow the individual woman and couple through a complete fertility treatment trajectory, with information on different treatment types, conception and birth. The registries also make it possible to assess risks related to treatment, such as ovarian hyperstimulation syndrome (OHSS) and safety in the children born after fertility treatment.

6 Background

6.1 Reporting success rates in fertility treatments

Traditionally, success rates after fertility treatment have been reported as pregnancy rates or live birthrates per treatment cycle. However in recent years, longitudinal studies with cumulated birthrates are increasingly replacing the per cycle based estimates (6–9). In longitudinal studies, live birthrates have been reported cumulated over complete treatment cycles, where one complete treatment cycle is defined as one ART-treatment with ovarian stimulation and fresh transfer and all subsequent frozen-thawed transfers resulting from that specific ovarian stimulation. Birthrates can also be cumulated over several complete treatment cycles, or over time (10,11). Cumulative live birthrates provide a more accurate prognosis for chances of livebirth, when starting treatments, compared to per-cycle based estimates. Still, a complete prognosis require records of the entire treatment course, including information of possible shifts between ART and IUI, shifts between fertility clinics as well as births due to natural conception during treatment pauses.

A large UK study from January 2016 report cumulative live birthrates after a number of complete ART-cycles, where one cycle refers to all fresh and frozen-thawed transfers, generated from one oocyte pick-up (11). Although reflecting the efficiency of ART, it may not be the optimal measure when advising couples, since the time-frame of one complete cycle can vary considerably. Furthermore, the incidence of natural conception was not described.

Couples may shift between ART and intrauterine insemination, but few studies include IUI. Only a few small studies have investigated the long-term prognosis for live birth after both ART and IUI, when initiating fertility treatment (10,12–14). A Danish questionnaire study, based on reports from 1338 subfertile couples, showed that 69.5 % had a child within 5 years of treatment. Of these couples, 54.6 % gave birth after ART, 18.2 % after natural conception and only 9.9 % after IUI (12). However the majority of these couples had intrauterine inseminations prior to ART, before inclusion in the study. This may have affected the birthrates reported after IUI. According to reports from the Danish Fertility Society, the estimated live birth rate per IUI cycle in Denmark is 12 % (5).

6.2 Predictors of treatment outcome

Several studies have shown that the number of aspirated oocytes is a strong predictor for chances of livebirth after ART (15–19). An increasing number of oocytes leads to increased birthrates in the fresh cycle, where the oocytes were aspirated, but also yield more embryos for later frozen-thawed transfers, and thereby increased cumulated live birthrates in the first complete cycle (16). A large UK-study further showed that women with 12 or more aspirated oocytes in the first cycle had increased odds for live birth within six complete cycles, compared to women with less than 5 aspirated oocytes in the first cycle (20). This may indicate that the response to ovarian stimulation in the first cycle is likely to be reproduced in later cycles. Previous studies have shown, that women defined as poor responders, according to the Bologna criteria, have reduced cumulated live birthrates in repeated cycles, compared to normal responders (21–25). A Danish single-center study showed an effect on number of aspirated oocytes after dose adjustments in 385 women returning for a second treatment cycle. Women with an increased starting dose in the 2nd cycle compared to the 1st had more aspirated oocytes in the 2nd cycle compared with the first cycle (9.8 vs 8.3 oocytes, p=0.002) (26). Similar results were described in a UK-study, where 244 patients were stratified in two age groups: over and under 33 years. In both groups, the number of aspirated oocytes significantly improved in the 2nd cycle (27).

6.3 Fertility treatments in Denmark

Denmark is one of the countries in the world, that performs most fertility treatments per capita, and is only exceeded by Israel, Belgium and Lebanon worldwide (28). The treatments include intrauterine insemination (IUI) and assisted reproductive technology (ART). In Denmark, the use of intrauterine insemination exceeds the use of ART. IUI combined with mild ovarian stimulation is predominantly the first line treatment to subfertile couples with mild to moderate male factor infertility, unexplained infertility, mild endometriosis and anovulatory infertility (5,29). Women with low AMH values and aged 38 years or more may go straight to ART. Couples are offered 3 cycles with intrauterine insemination (when anovulatory infertility is the cause of infertility the couples will be offered up to six cycles). If this does not result in a live birth, the couples proceed to ART (29,30). Intrauterine insemination with the use of donor semen is also used in single women and in women with a

female partner. Several European countries as well as the U.S have a more aggressive approach with ART as first line fertility treatment. The national Danish IUI strategy, as described above, results in a less expensive and milder fertility treatment program with only limited side effects. However, when IUI does not result in live-birth, this treatment strategy will of course sometimes have prolonged the treatment period.

In Denmark fertility treatments are reimbursed by the National health system, when the following criteria are fulfilled: the woman is under 40 years old and either single and childless or in a relationship with no common children. Women/couples are offered a maximum of three fresh ART treatments including potential additional frozen-thawed transfers. When IUI is indicated, a maximum of six cycles is recommended before proceeding to ART (30). Fertility treatment in women aged more than 45 years is prohibited by law (30).

6.4 Children born after intrauterine insemination

It is well established that children born IVF and ICSI have increased risk of adverse perinatal outcomes such as preterm birth (PTB) low birth weight (LBW) and being born small for gestational age (SGA) (31–35). For children born after frozen-thawed transfer, an increased risk of being large for gestational age has been reported (36). A few studies have reported perinatal outcome in children conceived after intrauterine insemination, and describe an increased risk of adverse perinatal outcomes compared with naturally conceived children (37–42). Two smaller studies have compared perinatal outcomes in children conceived after IUI with children conceived after IVF/ICSI and found similar adverse perinatal risks in the two groups (43,44).

Although numerous studies have shown that ART children have an increased risk of being born with adverse perinatal outcomes, the casual explanation behind this association is still not fully understood. The high prevalence of multiple gestations after ART together with the effect of vanishing twins is part of the explanation. However, with the increasing use of elective single embryo transfer in ART the risk of many adverse perinatal outcomes have decreased over the years (31,45). Still an increased risk of adverse perinatal outcomes in ART singletons persist, and can be explained by both the subfertility of the couples together with the ART techniques itself (46–48). To improve safety, it is important to clarify the contributions of the different factors, especially considering the rapid development in

fertility treatments. Since intrauterine insemination in Denmark can occur in both natural and stimulated cycles, a study on children born after IUI may help disentangle the effect of the parental factors versus the ovarian stimulation.

6.5 Justification and relevance of this thesis

Obtaining an individualized prognosis improves the physician's ability to early identify women with risk of poor treatment outcome. This is valuable, when advising couples entering fertility treatment. Assessing birthrates after natural conception in a sub-fertile population provides couples with a more accurate overall prognosis for having a child, and can help identify treatment failures. Assessing perinatal outcome in children born after intrauterine insemination helps towards a better understanding of the increased risks seen in children conceived with fertility treatments.

To our knowledge this is the first national cohort study to explore the long-term prognosis for Danish couples in fertility treatment. The results of this study will be valuable, when counseling infertile couples both entering fertility treatment as well as during the treatment course.

7 Aim of thesis

7.1 Purpose and Objectives

Paper I

The purpose was to obtain a comprehensive long-term prognosis, to use when counselling couples initiating fertility treatment, based on individual patient characteristics.

The objective was to estimate cumulative live birthrates at 2, 3 and 5 years after the first treatment with assisted reproductive technology (ART) and intrauterine insemination (IUI) with homologous gametes in couples with no previous births due to fertility treatment. The birthrates were based on observed livebirths in the cohort and further stratified by mode of conception: treatment-related (IUI/ART) and natural conceptions. Furthermore, we aimed to assess if maternal age, smoking, BMI and cause of infertility had an impact on the estimated birthrates.

Paper II

The purpose of this study was to provide women and couples undergoing ART treatment with a revised prognosis according to the treatment response in their previous ART cycles.

The objective was to assess the association between the number of oocytes, aspirated in the first cycle, and the cumulated chances of livebirth in the 2nd and 3rd fresh ART-cycles including subsequent frozen-thawed transfers, in women receiving ART treatments with homologous eggs and homologous or donated semen. Secondly to assess changes in number of aspirated oocytes between the first and second stimulated ART-cycle. Last we wished to estimate cumulative live birthrates per initiated complete treatment cycle, after both treatment-related and natural conception based on observed livebirths in the cohort.

Paper III

The purpose was to describe perinatal outcome in singletons born after intrauterine insemination, and to gain a better understanding of factors influencing perinatal outcome in children conceived after fertility treatments.

The objective was to estimate incidence of adverse perinatal outcome in singletons born after intrauterine insemination and to compare perinatal outcome in children conceived after IVF, ICSI or after natural conception. Further we aimed to assess predictors of poor perinatal outcome in children conceived after intrauterine insemination. We also evaluated if outcome was affected by ovarian stimulation or cause of infertility.

7.2 Hypothesis

Paper I

Only 20% of couples initiating treatment with IUI give birth after IUI. The majority of these couples will need ART to obtain live birth. Birthrates after natural conception are higher in women initiating treatment with IUI, than in women initiating treatment with ART, related to a less severe degree of subfertility in these couples. Advanced female age, smoking and BMI > 30 are associated with a poorer prognosis. Effect of cause of infertility on birthrates is dependent on type of treatment used, IUI or ART.

Paper II

The live birthrate in the first complete ART cycle is higher than in later cycles. Number of aspirated oocytes in the first cycle is a predictor for outcome in later cycles. The more oocytes retrieved the higher risk of ovarian hyperstimulation syndrome (OHSS).

Paper III

Singletons born after intrauterine insemination have increased risk of adverse perinatal outcomes compared with naturally conceived singletons, but comparable outcomes compared with children born after IVF and ICSI.

8 Study unit, target- and study populations

Paper I

The study unit was women initiating fertility treatment with homologous gametes and who were residents in Denmark. The target population was couples undergoing fertility treatment with homologous gametes. The study population was women registered in the Danish ART-registry, who initiated fertility treatment with homologous gametes.

Paper II

The study unit was women undergoing ART treatment with homologous eggs and homologous or donated semen and who were residents in Denmark. The target populations were women and couples returning for repeated ART treatment with homologous eggs and homologous or donated semen. The study population was women registered in the Danish ART-registry, who received fertility treatment with homologous eggs and homologous or donated semen.

Paper III

The study unit was singletons conceived after intrauterine insemination with homologous or donated semen and who were born in Denmark. The target population was singletons born after intrauterine insemination. The study population was singletons, born in Denmark of women registered in the Danish ART-registry as undergoing intrauterine insemination at the time of conception.

9 Materials and methods

9.1 Study design and sources of data

All three studies are cohort studies. The study populations were obtained from the Danish ART registry, which include all ART treatments since 1994 and also all intrauterine inseminations since 2006. During 2006 and 2007, the reporting to the ART registry was restructured from paper to electronic forms. The registry is mandatory and all private and public clinics must report their treatment activity. A personal identification number (social security number) makes it possible to identify all treatments in the same woman and thereby construct a complete treatment history. Women without a social-security number were excluded since the replacement social-security number not necessarily remains the same between treatments. Furthermore, these women are not Danish residents, and follow-up on childbirths therefore not possible.

Information on births/children in the cohort was retrieved from the medical birth registry, in which all live- and still-born children in Denmark are registered. The medical birth registry includes information on birthweight and multiple gestations and gestational age at time of delivery, which was used to cross-link the deliveries to a certain treatment cycle and thereby identify births due to spontaneous conceptions.

The National Patient registry contains information on hospital admissions and visits to outpatient clinics. From this registry we obtained diagnosis of OHSS, hypertensive disorders of pregnancy, placenta previa, caesarean section, induction of labor and admittance to neonatal intensive care unit.

Information on date of treatment, treatment type, type of gametes used (homologous or donated), number of aspirated oocytes, cause of infertility, and female age, is included in the ART-registry since 1994. In 2006 data on all intrauterine inseminations were added along with a number of additional variables such as BMI, smoking status, and partner characteristics.

Information on emigration outside Denmark, or cross-border fertility treatments, was not available.

9.2 Data collection

The database for papers I and II were constructed in August 2014. At this point, follow-up on deliveries was available until December 31st 2012. The database for paper III was constructed in September 2013. Special consultant Steen Rasmussen was responsible for creating the database and cross-linking deliveries to treatment-cycles, and identifying births due to natural conception.

9.3 Inclusion/exclusion criteria

Paper I

This study includes all Danish residents initiating IVF, ICSI, or IUI with homologous gametes from January 1st 2007 to December 31st 2010. Frozen-thawed cycles (FER/FET), subsequent to treatment with IVF and ICSI, were also included. Women, registered with a frozen embryo transfer as the first treatment, were excluded since it could not have been their first cycle. Couples, treated with donated gametes or testicular sperm aspiration, were excluded. Women treated with donor semen plus donated oocytes were excluded, since their fertility potential may be different than women receiving treatments with homologous gametes. During the study period, treatment with oocyte donation was confined due to regulations, limiting the supply of donated oocytes, and couples referred to oocyte donation (OD) treatment spent several years waiting for an oocyte. After the above described exclusions, the cohort consisted of 19,884 women. A flowchart of included and excluded women and treatment-cycles is presented in paper I.

Paper II

This study includes all women (Danish residents), who had their first ART cycle with homologous eggs between January 1st 2002 and December 31st 2011. Follow-up on deliveries in this cohort was available until Dec 31st 2012, and women were censored if follow-up was less than a year from the start-date of their latest treatment. Both single women and women in a heterosexual or same-gender relationship were included; hence treatments with partner or donated semen were included. Women with at least one OD-

treatment were excluded (N=631 women). The cohort included 30,486 women who had 94,025 treatment cycles.

Paper III

This study included singletons conceived after intrauterine insemination and were born in Denmark from 2007 to 2012, N=6,338. Twins and triplets, conceived after intrauterine insemination during the study period, were excluded (twins, n=1,517 and triplets, n=45). The control cohorts included all singletons born after IVF (n=4,135) and ICSI (n=3,635), during the study period. Children born after oocyte donation, FER, preimplantation genetic diagnosis or testicular sperm aspiration were excluded from the controls. A third control group consisted of all naturally conceived singletons from 2008 to 2011 (n=229,749). Naturally conceived singletons in 2007 were excluded to avoid misclassification. The conversion of the ART-registry to the electronic version during 2006 and 2007 was expected to result in a random loss of data, and children conceived after IUI during this process were not always registered properly and would therefore appear as naturally conceived children. Naturally conceived children born in 2012 were also excluded to avoid misclassification, since the registrations in the ART registry for 2012 were not complete at the time of data collection.

9.4 Outcome measures

Paper I

The main outcome measure was livebirth resulting from fertility treatment or natural conception within 2, 3 and 5 years from first treatment.

Paper II

The main outcome measure was treatment-related live birth rate per started complete ART cycle (one complete cycle defined as one ovarian stimulation and all frozen-thawed transfers resulting from that oocyte aspiration), and cumulated live birth rates after several complete cycles.

Paper III

The main outcome measures were preterm birth (PTB), low birth weight (LBW) and small for gestational age (SGA).

9.5 Statistical Analysis

Paper I

In non-normal distributed data, the Mann-Whitney U test was used to compare distributions between groups.

Cumulative live birthrates were calculated as the proportion of women having at least one livebirth within 2, 3 and 5 years from the first treatment, among women with complete 2, 3 and 5 years follow-up, respectively. The numerator was women with a livebirth and the denominator was all women who started treatment. Follow-up on births in the cohort was available until December 31st 2012, so a minimum of two years follow-up was available in the entire cohort. The women were followed until their first treatment-related or naturally conceived livebirth, or until they shifted to treatment with donated gametes. Only the first livebirth since start of treatment was reported, the birth-rates do not include later siblings.

Birthrates were stratified according to type of first treatment (IUI or ART), mode of conception (IUI, ART or natural conception) and female age at first treatment. Further data was stratified according to cause of infertility, smoking status, secondary infertility and BMI.

Predictors of live birth were assessed with multivariable logistic regression analysis with spline assessment including variables: female age (linear spline with break point at 35 and 40 years), smoking (yes/no), BMI (linear spline with breakpoint at BMI 20 and 30), secondary infertility (yes/no), cause of infertility (categorical) and course of treatments (categorical).

Paper II

Descriptive statistics were outlined as numbers and percentages. The change in number of aspirated oocytes between the first and the second ART-cycle was compared with analysis of variance.

Cumulated live birthrates were reported per complete ART-cycle. The numerator was women with a livebirth within the complete cycle (including frozen-thawed transfers) and

the denominator was all women starting the corresponding fresh cycle. Live birthrates were also reported accumulated over 1 – 4 complete cycles, as the proportion with livebirth of all women starting the first treatment, without censoring drop-outs. A drop-out is defined as having no live-birth and no continued treatment within follow-up. The women were excluded from further analysis after the first livebirth in the study period. If the first livebirth since start of treatment was unrelated to ART, and before December 31st 2011, it was reported irrespective of time interval since first treatment. Non-ART related livebirths in women treated after January 1st 2006, were stratified according to mode of conception – natural or after insemination treatment. Embryos used in frozen-thawed cycles were assumed to originate from the most recent fresh cycle. The national recommendation is that all frozen embryos should be used before initiating another fresh cycle (30).

Live birthrates in complete cycle 1 – 4 were stratified according to female age and number of aspirated oocytes (0-3, 4-9, 10-15, >15). In women with one or more aspirated oocyte in the first cycle, the odds-ratio for livebirth in the first complete cycle was assessed with multivariable regression analysis including: number of aspirated oocytes (linear spline with break points at 7 and 12 oocytes), female age (linear spline with break points at 35 and 40 years) and cause of infertility (categorical).

Multivariable logistic regression analysis further assessed the association between number of aspirated oocytes in the 1st cycle and livebirth within the 2nd and 3rd complete cycle. Predictors included were number of oocytes in the first cycle (linear spline), female age (linear spline) and cause of infertility. The sum of retrieved oocytes in the 1st and the 2nd cycle, and odds for live birth in the 3rd complete cycle were also assessed with multivariable logistic regression analysis, including the predictors: sum of retrieved oocytes (6 categories), cause of infertility and female age (linear spline).

Paper III

Differences of means were compared with analysis of variance with Bonferroni correction. Non-normal distributed data were described with medians and interquartile range (IQR) and analyzed with the Kruskal-Wallis test. Distributions between groups were analyzed with χ^2-test and Bonferroni correction.

The incidence of SGA and LGA was calculated according to the 10th percentile with Marsal's formula using birth weight, gender and standard intrauterine growth curves for Scandinavia (49). Preterm birth (PTB) was defined as gestational age < 37 weeks. Low birth weight (LBW) was defined as <2500 grams. Multivariable logistic regression analysis assessed odds for LBW, PTB, SGA and LGA including the categorical predictors maternal age, parity, gender, year of birth, smoking, maternal BMI, elective caesarian section and induction of labor.

Impact of ovarian stimulation was also assessed with multivariable logistic regression analysis. Natural cycle IUI was defined as no treatment with clomiphene citrate (CC) or follicle stimulating hormone (FSH), but in 44% of natural cycles, hCG was used to trigger ovulation.

9.5.1 Database handling

The software used to create the database was SAS statistical software version 9.3. Data management and statistical analysis were performed with SAS statistical software version 9.4 and IBM SPSS statistics 22.

9.5.2 Power calculation

Sample size calculation based on estimating a proportion

- Estimated birthrate: 0.5
- Population size: 15 000 (~15 000 IUI and 15 000 ART treatments initiated annually in DK)
- Confidence level: 0.95
- Allowable error: 0.01

→ Required sample size: 5856

9.6 Ethical approval

The project was approved by the Danish Data Protection Agency (Paper I & II: J.nr 2012-41-1330; Paper III: CVR no. 11-88-37-29). In Denmark register-based observational studies do not require approval from an ethics committee.

10 Summary of results

The following section holds a summary of results. Studies I to III include the full and detailed description of the results. Results not included in the papers are presented in the section supplementary results.

10.1 Paper I

Background characteristics & follow-up

The mean age of women starting fertility treatments with IUI was 32.4 years (standard deviation, SD 4.8), which was slightly lower than women starting treatments with ART, 33.1 years (SD 5.1). The distribution of cause of infertility was significantly different in the two groups. Anovulatory infertility and idiopathic infertility were more prevalent in couples starting with IUI, and male factor infertility, tubal factor infertility and endometriosis were more prevalent in couples starting treatments with ART. The groups had similar prevalence of BMI>30 and smoking, but more women starting fertility treatments with IUI had previously given birth.

An overview of women with complete 2- 3- and 5-years follow-up is presented in Table I.

Table I, Overview of women with available follow-up

	All women N total	First treatment with IUI N women	First treatment with ART N women
2 years follow-up	19 884	12 488	7396
3 years follow-up	14 445	8816	5629
5 years follow-up	5165	3028	2137

Treatment activity and course of treatments

In women with complete 5-years follow-up, treatment activity mainly took place within two years from the first treatment, 92% of all treatments occurred within this time-frame. The median number of total treatments was 3 (interquartile range, IQR 2–5) in couples starting treatment with IUI, which was significantly more than in couples starting treatments with ART, who had a median of 2 (IQR 1–3) total treatments.

The course of treatments is displayed in table II. For couples starting treatments with IUI the majority (59.3%) exclusively had IUI-treatments, but a total of 38.1% shifted to ART

treatments after 1–6 attempts with IUI. For couples starting treatments with ART, 76.4% had 1–3 ART treatments in total, and only 2.8% had IUI treatments.

Table II, Course of treatments by patient within 2 years from first treatment in couples receiving fertility treatments with homologous gametes in Denmark 2007–2010

Starting with IUI			Starting with ART		
All couples	12,488		All couples	7396	
Course of treatments	%	[95% CI]	Course of treatments	%	[95% CI]
1–3 IUI	49.2	[48.3–50.0]	1–3 ART	76.4	[75.5–77.4]
4–6 IUI	10.1	[9.5–10.6]	4–6 ART	17.9	[17.0–18.8]
1–3 IUI → ART	26.8	[26.0–27.6]	7 + ART	2.9	[2.6–3.3]
4–6 IUI → ART	11.3	[10.7–11.8]	ART → IUI	2.8	[2.4–3.2]
Other	2.7	[2.4–3.0]			

Live birthrates within 2, 3 and 5 years.

In all couples starting treatments, 57% [95%CI 56.3–57.7] had a treatment related or naturally conceived livebirth within 2 years from first treatment, increasing to 65% [64.2–65.8] within 3 years and 71% [69.5–71.9] within 5 years. In women starting treatments with IUI, birthrates stratified on female age and mode on conception are presented in Table III. A total of 75% [73.7–76.7] of these women had given birth within 5 years, 35% [33.2–36.6] conceived with IUI, 24% [22.2–25.2] conceived with ART and 17% [15.3–17.9] conceived naturally. Birthrates decreased with increasing female age. In women starting treatments with ART, stratified birthrates are displayed in Table IV. The total birthrate in this group was 65% [62.4–66.5] within 5 years; 53% [50.8–55.0] conceived with ART, 11% [9.8–12.4] conceived naturally and 0.6% [0.3–0.9] conceived with IUI. Female age was strongly associated with outcome.

Table III Age stratified live birthrates 2, 3 and 5 years after first treatment with IUI and homologous gametes in Denmark, 2007–2010

		1st treatment with IUI			
Female age	N, total	IUI-conception, CLBR [95% CI]	ART-conception, CLBR [95% CI]	Natural conception, CLBR [95% CI]	Total CLBR [95% CI]
2 years follow-up					
<35 years	8753	37.8 [36.8–38.8]	16.8 [16.0–17.6]	10.6 [10.0–11.2]	65.2 [64.2–66.2]
35–39 years	2897	29.7 [28.0–31.4]	12.7 [11.5–13.9]	8.4 [7.4–9.4]	50.7 [48.9–52.5]
>=40 years	833	12.4 [10.2–14.6]	5.6 [4.0–7.2]	7.6 [5.8 –9.4]	25.6 [22.6–28.6]
3 years follow-up					
<35 years	6229	37.8 [36.6–39.0]	23.6 [22.6 – 24.7]	13.9 [13.0–14.8]	75.4 [74.3–76.5]
35–39 years	1981	30.2 [28.2–32.2]	17.3 [15.6–19.0]	11.3 [9.9–12.7]	58.9 [56.7–61.1]
>=40 years	601	13.1 [10.4–15.8]	7.5 [5.4–9.6]	9.7 [7.3–12.1]	30.3 [26.6–33.4]
5 years follow-up					
<35 years	2152	37.9 [35.9–40.0]	26.9 [25.0–28.8]	18.0 [16.4–19.6]	82.8 [81.2–84.4]
35–39 years	651	32.4 [28.8–36.0]	18.9 [15.9–21.9]	13.8 [11.2–16.5]	65.1 [61.4–68.8]
>=40 years	222	13.5 [9.0–18.0]	6.8 [3.5–10.1]	10.8 [6.7–14.9]	31.1 [25.0–37.2]

Table IV Age stratified live birthrates 2, 3 and 5 years after first treatment with ART and homologous gametes in Denmark, 2007–2010

		1st treatment with ART			
Female age	N, total	ART-conception, CLBR [95% CI]	IUI-conception, CLBR [95% CI]	Natural conception, CLBR [95% CI]	Total CLBR [95% CI]
2 years follow-up					
<35 years	4857	53.3 [51.9 – 54.7]	0.7 [0.5–0.9]	6.3 [5.6–7.0]	60.3 [58.9–61.7]
35–39 years	1769	39.3 [37.0–41.6]	0.3 [0.1–0.6]	5.1 [4.1–6.1]	44.8 [42.5–47.1]
>=40 years	753	15.5 [12.9–18.1]	0 [0–0.005]	5.6 [4.0–7.2]	21.1 [18.2–24.0]
3 years follow-up					
<35 years	3707	59.0 [57.4–60.6]	0.7 [0.4–1.0]	9.3 [8.4–10.2]	69.1 [67.6–70.6]
35–39 years	1328	44.7 [42.0 – 47.4]	0.2 [0.0–0.4]	6.6 [5.3–7.9]	51.5 [48.8–54.2]
>=40 years	580	15.5 [12.6–18.5]	0 [0–0.006]	7.4 [5.3–9.5]	22.9 [19.5–26.3]
5 years follow-up					
<35 years	1401	62.2 [59.7–64.7]	0.9 [0.4–1.4]	12.5 [10.8–14.2]	75.6 [73.4–77.9]
35–39 years	505	45.7 [41.4–50.0]	0.2 [0.2–0.6]	8.3 [5.9–10.7]	54.3 [50.0–58.6]
>=40 years	229	11.8 [7.6–16.0]	0 [0–0.02]	9.2 [5.5–12.9]	21.0 [15.7 – 26.3]

Predictors of live birth

Anovulatory infertility and non-smoking were predictors of high live birthrates in both women starting treatments with IUI and women starting with ART. Tubal factor infertility predicted low live birthrates, but only in women starting treatments with IUI. Male factor infertility predicted high live birthrates in women starting treatments with ART.

BMI had a significant impact on treatment outcome. For women with a BMI under 20, and who started treatment with IUI, adjusted odds ratio (AOR) for live birth increased with 11% (P=0.04) for each increasing BMI unit. For women starting with IUI who had a BMI between 20 and 30, the AOR decreased with 4% for each increasing BMI unit ($p<0.0001$), and for BMI >30, the AOR decreased with 7% for each increasing BMI unit ($p<0.0001$). A similar association was found in women starting treatments with ART.

10.2 Paper II

Background characteristics and follow-up

The mean age in women starting the first ART treatment was 33.1 (SD 4.9). A total of 98% of women who started the first fresh cycle had an aspiration, and 85% had an embryo transfer. The majority, 47% had 4–9 aspirated oocytes, 25% had 10–15 oocytes, 19% had 0–3 oocytes and 10% had >15 oocytes. Data on number of aspirated oocytes were available for 70% and missing for 30%.

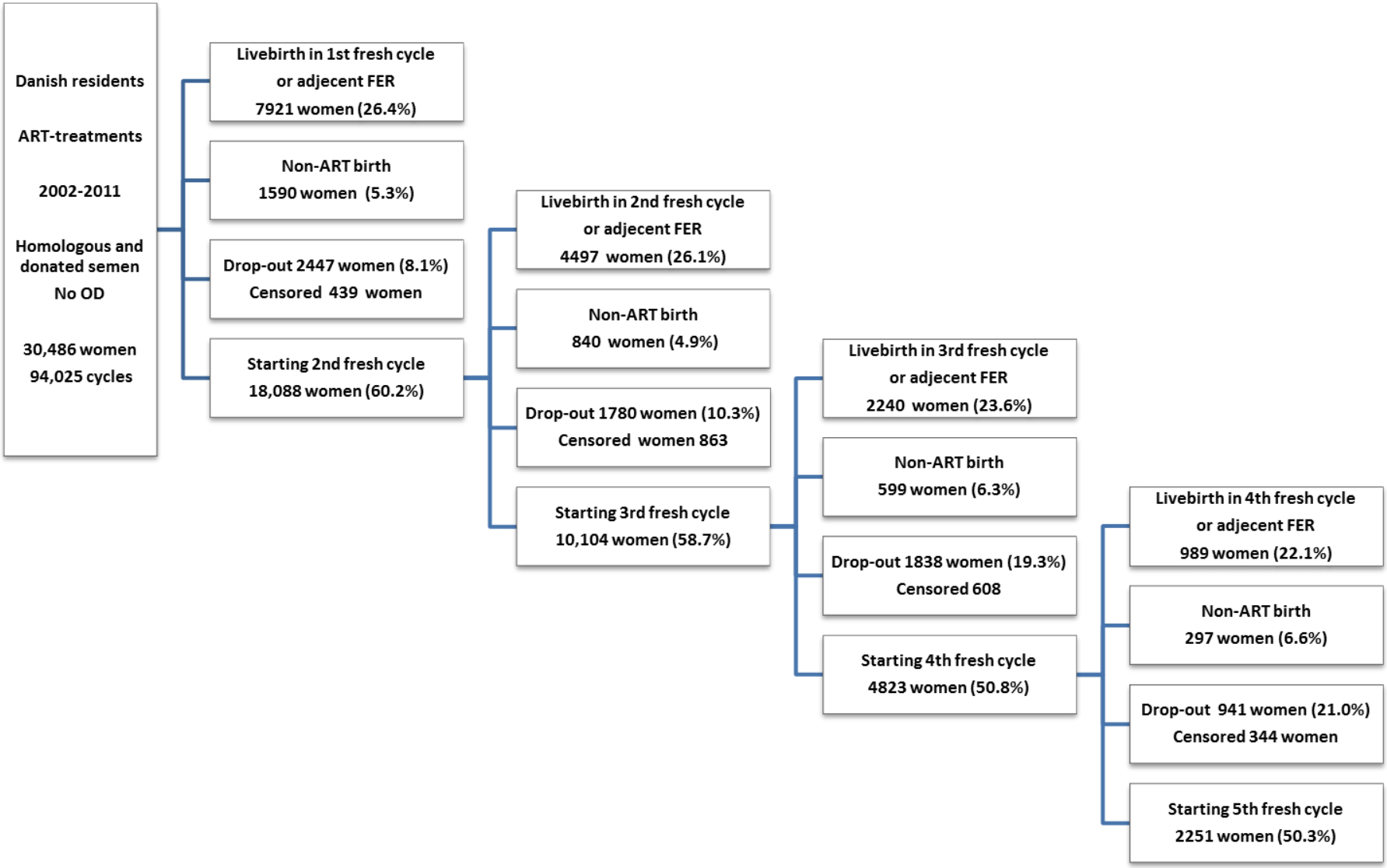
The median follow-up time was 72 months (IQR 45–99).

Course of treatments and treatment outcome

The treatment course and the cumulative live birthrates per complete ART cycle (fresh + frozen-thawed transfers) is displayed in figure 1. The birthrate decreased slightly in women having the third complete ART cycle, compared to women in the 1st and 2nd complete cycle.

Out of all women starting the 1st treatment 26.4% [25.9–26.9] had a treatment-related livebirth in the 1st complete ART cycle, 42.6% [42.0–43.1] had a livebirth within two complete cycles, 51.3% [50.7–51.9] had a livebirth within three cycles and 55.4% [54.8–56.0] had a livebirth within four cycles. For livebirths not related to ART treatment, it was possible to distinguish between children conceived with IUI-treatment and naturally conceived children in the years 2006 – 2011. Within 4 complete ART cycles, 8.2% of all women starting ART treatments had a livebirth due to natural conception. The median time interval between the first ART treatment and the naturally conceived livebirth was 20 months (IQR 13–31). Within four cumulated complete ART cycles, 1.6% had a livebirth conceived after insemination treatment, after a median time interval of 11 months (6 – 10) from 1st treatment. A total of 52% of livebirths after IUI were conceived with donor semen, and this may represent couples with male factor infertility, who started treatments with ICSI and moved on to insemination with donor semen.

Figure 1, Flow chart over included cases and their course of treatments, women followed until their first livebirth. Cases are censored if follow-up is less than one year from start-date of fresh or frozen-thawed treatment cycle; proportions are % of non-censored cases.



Number of retrieved oocytes and chances of live birth

For women with valid and missing data in number of aspirated oocytes, the mean female age, the CLBR and the incidence of OHSS was similar in the two groups.

As shown I Figure 2, the cumulative live birthrates in the first complete cycle increased with increasing number of oocytes until 15-16 aspirated oocytes, after which the CLBR plateaued; however, confidence intervals above 16 aspirated oocytes were too wide to determine a significant trend. In women with at least one aspirated oocyte in the first fresh cycle, the AOR for livebirth in the first complete cycle increased with 19.8% [16.8–22.8] for each added oocyte up to 8 aspirated oocytes. In women with 8 – 12 aspirated oocytes in the first fresh ART cycle, the AOR for livebirth increased with 4.3% [2.1–6.6] for each added oocyte. In women with over 12 aspirated oocytes, the AOR did not increase significantly by extra oocytes, 0.3% [-1.2 – 1.7].

For women who did not achieve livebirth in the first complete cycle, the number of retrieved oocytes in the first cycle was associated with CLBR in the 2nd and 3rd cycle. The AOR for livebirth in the 2nd and 3rd complete cycles increased with 3.7% [1.5–6.0] for each extra added oocyte in the first cycle, from 0 to 8 oocytes. For women with 8 – 12 aspirated oocytes in the 1st cycle the odds increased with 4.1% [1.6–6.6] per added oocyte and for women with >12 oocytes in the first cycle, the odds did not increase significantly with additional oocytes 1.0% [-0.8 – 2.7].

For women with no livebirth in the 1st and 2nd cycle, the sum of aspirated oocytes in the two first fresh cycles was associated to outcome in the 3rd cycle, as shown in Table V. Women with a sum of 0–6 aspirated oocytes and women with a sum of 7 –12 aspirated oocytes in the 1st and 2nd fresh cycles, had decreased AOR for livebirth, compared with women with 20 –30 aspirated oocytes. But women with 20 – 30 oocytes and women with >30 oocytes had similar outcome. Age stratified birthrates in the 3rd cycle, in women with a history of two fresh cycles with low ovarian response, were 21.5% for women under 35 and 5.6% in women aged 40 or more.

Figure 2, Cumulative live birthrates (CLBR) with 95% confidence intervals in the first fresh ART cycle with possible adjacent frozen-thawed transfers, by number of aspirated oocytes, Denmark 2002–2011

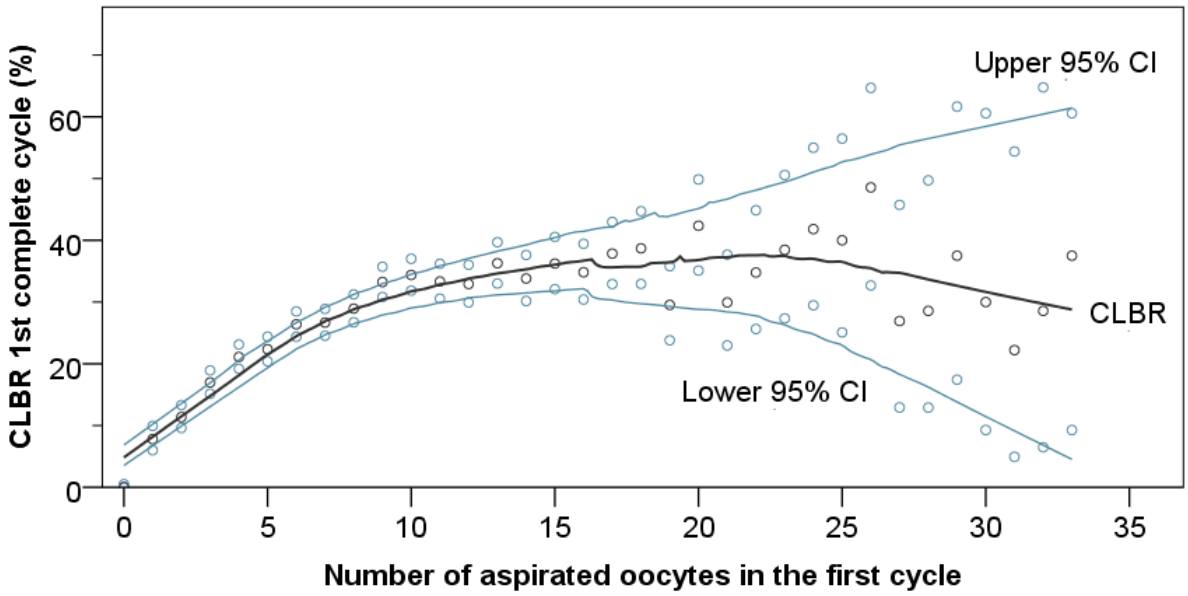


Table V, Adjusted[1] odds ratios for livebirth after ART-conception in the 3rd complete[2] ART-cycle, by number of aspirated oocytes in the 1st and 2nd cycle, in women with no livebirth in the 1st or 2nd complete cycle, Denmark 2002–2011

Sum of aspirated oocytes 1st & 2nd cycle	Livebirth 3rd cycle AOR [95% CI]
0 – 6	0.59 [0.46 – 0.75]
7 – 12	0.78 [0.66 – 0.93]
13 – 19	0.94 [0.80 – 1.11]
20 – 30	1.0 (ref)
>30	1.08 [0.82 – 1.41]

[1]Multivaraible logistic regression analysis further including female age (linear spline with break point at 35 and 40 years) and cause of infertility as predictors. [2]One complete cycle is a fresh ART cycle with possible subsequent frozen-thawed transfers.

Improving the number of aspirated oocytes in subsequent cycles

For women with a low number of aspirated oocytes in the first cycle, the incidence of remaining in the low response category with 0–3 aspirated oocytes depended on female age. In women aged <35 years, only 26.7% [24.3–29.2] stayed in this category in the 2nd cycle. In women aged 35–39 years 41.0% [38.4–45.6] repeated the initial low response and in women >40 years 60.5% [55.8–65.7] remained in the 0–3 category in the 2nd cycle.

OHSS

Within 4 cumulated complete cycles, 2.7 % [2.5–2.9] of women starting ART treatment were admitted with OHSS. Incidence increased with increasing number of oocytes. Further, incidence decreased with increasing cycle number. In the 1st cycle, incidence was 1.7% [1.6–1.9]. In the 2nd cycle, incidence was 1.3% [1.1–1.5]. However, compared to the 2nd cycle, the decrease in the 3rd and the 4th cycle was non-significant, 1.0% [0.8–1.2] and 0.9% [0.6–1.2], respectively.

10.3 Paper III

Background characteristics

There were 6338 singletons born after intrauterine insemination in the study period, of which 66.4% were conceived with insemination of homologous semen (IUI-H) and 29,7% were conceived with insemination of donated semen (IUI-D). The mean maternal age was 32.6 years (SD 4.3) in the IUI-H group and 34.4 years (SD 4.4) in the IUI-D group. Incidence of BMI >30 was 12.6% in the IUI-H group and 16.7% in the IUI-D group. Among all singletons born after insemination, 31% were conceived in a natural, unstimulated cycle, 24% were conceived after stimulation with clomiphene citrate (CC), 20% were conceived after stimulation with FSH and 24% were conceived in a cycle stimulated with a combination of CC and FSH. Among children born after IUI-H, 76% were conceived in a stimulated cycle. In children born after IUI-D, only 36% of children were conceived in a stimulated cycle.

Obstetric and perinatal outcome

Selected obstetric and perinatal outcomes are displayed in Table VI. The mean birthweight was lower in children born after IUI-H, compared with children born after IUI-D ($p<0.001$), and so was the mean gestational age ($p=0.007$). The mean birthweight and gestational age in children born after IUI-H was slightly higher than in children born after IVF, comparable to ICSI and slightly lower than in children born after natural conception. Both incidences of placenta previa and hypertensive disorders of pregnancy were lower compared with children born after IVF and ICSI, but comparable to naturally conceived children. The mean birthweight and the gestational age in children born after IUI-D was comparable to naturally conceived children and slightly higher compared with children born after IVF and ICSI. Incidence of placenta previa was comparable to naturally conceived children, and lower compared with children born after IVF and ICSI. Incidence of hypertensive disorders of pregnancy were higher compared to all three control groups. For children born after IUI-H and IUI-D, both groups had higher incidence of caesarian section and induction of labor than naturally conceived children, and comparable rates to children born after ART.

TableVI, Obstetric and perinatal outcome in singletons born after IUI- H and IUI-D, compared to IVF, ICSI and naturally conceived children (NC)

	IUI-H	**IUI-D**	**IVF**	**ICSI**	**NC**	**P-values**					
Singletons N, total	4208	1881	4135	3635	229.749	IUI-H vs. IVF	IUI-H vs. ICSI	IUI-H vs. NC	IUI-D vs. IVF	IUI-D vs. ICSI	IUI-D vs. NC
Mean birth weight ±SD, (grams)	3434±571	3505 ±590	3356 ±600	3420 ±574	3515 ±557	**<0.001**	1.0	**<0.001**	**<0.001**	**<0.001**	1.0
Mean gestational age ±SD, (days)	277.5 ±13.2	278.5 ±13.7	275.8 ±15.9	277.3 ±13.8	278.7 ±12.5	**<0.001**	1.0	**<0.001**	**<0.001**	**0.04**	1.0
Placenta Praevia, N (%)	45 (1.1)	9 (0.5)	97 (2.3)	71 (2.0)	923 (0.4)	**<0.001**	**0.004**	**<0.001**	**<0.001**	**<0.001**	0.60
Hypertensive disorders[a], N (%)	234 (5.6)	140 (7.4)	198 (4.8)	185 (5.1)	8577 (3.7)	0.11	0.36	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Caesarian section, N (%)	1165 (27.7)	489 (26.0)	1141 (27.6)	932 (25.6)	45799 (19.9)	0.925	0.16	**<0.001**	0.20	0.77	**<0.001**
Induction of labor, N (%)	559 (13.3)	287 (15.3)	557 (13.5)	484 (13.3)	26633 (11.6)	0.80	0.97	**0.004**	0.06	0.2	**<0.001**

[a]Gestational hypertension, preeclampsia, HELLP, and eklampsia

Table VII. Risks of adverse perinatal outcome in singletons born after IUI-H and IUI-D, compared to IVF, ICSI and natural conception (NC)

	AOR [95%CI]					
	IUI-H vs. IVF	IUI-H vs. ICSI	IUI-H vs. NC	IUI-D vs. IVF	IUI-D vs. ICSI	IUI-D vs. NC
LBW	**0.751 [0.618 – 0.914]**	0.933 [0.754 – 1.154]	**1.435 [1.235 – 1.667]**	**0.714 [0.554 – 0.920]**	0.886 [0.678 – 1.158]	**1.359 [1.089 – 1.696]**
PTB	**0.632 [0.512 – 0.779]**	0.948 [0.748 – 1.201]	**1.264 [1.071 – 1.490]**	**0.591 [0.447 – 0.781]**	0.885 [0.657 – 1.194]	1.177 [0.919 – 1.507]
SGA	1.118 [0.883 – 1.417]	1.245 [0.965 – 1.607]	**1.394 [1.180 – 1.648]**	1.077 [0.802 – 1.446]	1.199 [0.879 – 1.635]	**1.338 [1.048 – 1.707]**
LGA	1.096 [0.820 – 1.466]	0.917 [0.689 – 1.220]	0.966 [0.793 – 1.175]	1.314 [0.930 – 1.856]	1.098 [0.780 – 1.545]	1.157 [0.882 – 1.519]

Adjusted for year of birth, parity, maternal age, child sex, BMI, smoking, elective CS and induction of labor.

Adjusted odds ratios for risk of adverse perinatal outcome are shown in Table VII. In the adjusted analysis, in both children born after IUI-H and IUI-D, odds for being born with low birth weight was higher compared with naturally conceived children, lower compared with children born after IVF and similar to children born after ICSI. Adjusted odds for being small for gestational age was higher compared to naturally conceived children, and comparable to children born after ART.

Table VIII show adjusted odds ratios for risk of adverse perinatal outcomes in singletons born after IUI, by type of ovarian stimulation, cause of infertility and fertilization with homologous or donated semen. The AOR for preterm birth, being born with low birth weight or being born small for gestational age, were similar in children conceived with homologous or donated semen and were similar in different causes of infertility. However, the AOR for being born with low birth weight and small for gestational age were increased in children conceived in cycles stimulated with clomiphene citrate, compared to natural cycle-IUI. In cycles stimulated with FSH, odds for low birth weight and small for gestational age were similar compared to natural cycles. In cycles stimulated with a combination of CC and FSH, odds for being born with low birth weight were increased.

Table VIII. Adjusted odds ratios for risk of preterm birth (PTB), low birth weight (LBW) and small for gestational age (SGA) in singletons born after IUI.

	LBW	PTB	SGA
Clomiphene[a]	**1.459 [1.032 – 2.064]**	1.044 [0.710 – 1.537]	**1.617 [1.110 – 2.385]**
FSH[b]	0.872 [0.577 – 1.317]	0.814 [0.528 – 1.255]	1.233 [0.800 – 1.901]
Clomiphene + FSH	**1.637 [1.164 – 2.300]**	1.253 [0.867 – 1.813]	1.461 [0.983 – 2.172]
Natural cycle	1.0 (ref)	1.0 (ref)	1.0 (ref)
Idiopathic	1.142 [0.752 – 1.734]	1.124 [0.709 – 1.782]	0.961 [0.594 – 1.555]
Anovulation	1.024 [0.656 – 1.599]	1.070 [0.652 – 1.757]	0.796 [0.479 – 1.324]
Male factor	1.0 (ref)	1.0 (ref)	1.0 (ref)
IUI-H	1.014 [0.775–1.326]	0.966 [0.716 – 1.303]	0.992 [0.736 – 1.337]
IUI-D	1.0 (ref)	1.0 (ref)	1.0 (ref)

[a]Treatment with Clomiphene citrate, cases treated with Clomiphene citrate *and* follicle stimulating hormone (FSH) excluded. [b]Treatment with follicle stimulating hormone (FSH), cases treated with FSH *and* Clomiphene citrate excluded. [c]Time to pregnancy . [e]Adjusted for year of birth, parity, maternal age, child sex, BMI, smoking, elective CS and induction of labor.

10.4 Supplemental results

Since the registry went from paper to electronic reporting during 2006 – 2007 and further added insemination treatments in 2006, this may have resulted in administrative loss of data. This could lead to misclassification of children conceived with IUI; if registrations are missing they appear to be naturally conceived. Further, couples who are registered to start treatments with ART may in fact have started with IUI. In paper III the study period for naturally conceived children started in 2008 to avoid misclassified children. In paper I, the study period started in 2007. In the tables below, distribution of IUI and ART treatments with homologous gametes in 2005 – 2011 are reported. The proportion of IUI treatments seem to increase in 2008, compared with 2007, however possible missing IUI treatments is of limited extent.

Supplemental table SI, Distribution of total registered IUI and ART treatments with homologous gametes 2005-2010

Year	IUI	ART
2005	2.8%	97.2%
2006	44.5%	55.5%
2007	46.5%	53.5%
2008	51.5%	48.5%
2009	48.2%	51.8%
2010	54.0%	46%

Supplemental table SII Distribution of couples starting fertility treatments with IUI and ART, 2007-2010 (homologous gametes)

	Type of first treatment	
Year	IUI, N (%)	IVF N (%)
2007	3028 (58.6)	2137 (41.4)
2008	3024 (62.9)	1784 (37.1)
2009	2764 (61.8)	1708 (38.2)
2010	3672 (67.5)	1767 (32.5)
All years	12488 (62.8)	7396 (37.2)

11 Discussion

11.1 Main findings in comparison to previous studies

Live birth rates after a complete course of fertility treatments (Paper I)

In couples starting fertility treatments with own gametes, we found that 57% had a treatment-related birth within 5 years and that 14% delivered after natural conception. The chance of treatment success was strongly associated with female age. Live birthrates for women aged less than 35 years was 80%, compared to 26% in women aged 40 years or more. In couples, where IUI was the first choice of fertility treatment, total live birthrates were higher than in couples where ART was the first line treatment, which is most likely related to subfertility being less severe in these couples.

In couples starting fertility treatment with IUI, 34% conceived as a result of IUI within 2 years, with a marginal increase with further IUI treatment after 2 years, which is most likely related to that couples shift to ART treatments. The national treatment guidelines recommend shifting to ART treatment after 3 – 6 unsuccessful IUI attempts (30). IUI is an inexpensive alternative to ART, and has fewer side-effects, but is also less efficient per cycle (50–53). The long-term prognosis for live birth, over a complete treatment course, including both IUI and ART, have only been reported by a few smaller studies (10,12–14). A questionnaire study from Denmark, including 1338 couples, showed that 70% gave birth within 5 years (12). A total of 55% of the couples gave birth after ART, 10% after IUI and 18% after natural conception. The low birthrates after IUI, compared with the findings in our study, may be explained by that the majority of couples in the questionnaire study, had received IUI prior to inclusion. A prospective cohort study of 380 couples from Sweden, showed that 57% of couples had a livebirth within 2.5 years (14). In that cohort, 28% of couples conceived with ART, 19% conceived with ovulation induction or IUI, and 11% conceived naturally. The birthrate after IUI is lower than in our study, but 22% in the Swedish study did not have IUI or ovulation induction. In a Dutch prospective cohort study of 946 couples, 60% of couples became pregnant (32% with treatment and 28% naturally) and 51% had a live birth (10). Two smaller, single center studies from Korea and Germany report

cumulated pregnancy rates of 25–30% within 4 IUI cycles, in women aged less than 35 years (52,53). In a Dutch randomized controlled trial, birthrates within six fresh ART cycles were compared to birthrates within six cycles of IUI, in couples with idiopathic or male factor subfertility (50). Despite birthrates per cycle being higher in the ART group, the cumulated outcome was similar in the two groups, because drop-out rates were higher in the ART group. Another Dutch trial, from 2015, included 602 couples with idiopathic or mild male subfertility, randomized to have 3 complete ART cycles, six natural-cycle IVF or six IUI treatments with ovarian stimulation. Live birthrates were similar in the three groups (54).

In the present study, 34% of couples starting fertility treatment with IUI, delivered as a results of this treatment method. However 38% of the couples shifted to ART treatments within two years. In Denmark, national guidelines recommend couples with idiopathic, mild factor infertility, mild to minimal endometriosis and anovulatory infertility to start fertility treatment with IUI (29). Our results show that this may be a suitable treatment program, particularly in women under 35, where 45% of women with anovulatory infertility, 38% with idiopathic infertility and 32% with male factor infertility had an IUI-related livebirth within 2 years.

Naturally conceived children in couples receiving fertility treatment (Papers I and II)

We found that out of all women starting fertility treatment with homologous gametes, 14% had a live birth due to natural conception within 5 years. Birthrates after natural conception were higher in couples who started with IUI than in couples who started with ART (17% vs. 11% within 5 years, respectively). For women who received ART with own eggs (irrespective of semen source) 8.2% had a livebirth after natural conception within 4 complete cycles, with a median time interval of 20 months from the first cycle.

Even if our results indicate that for selected couples, IUI is a feasible first line treatment option to ART, the study does not address what can be achieved with expectant management. In Denmark, fertility treatment is offered to couples who have been trying to conceive for at least one year and after referral, there is a waiting time of 3 – 12 months or longer. Unfortunately, we did not have valid information on duration of infertility in the study period. A Danish prospective cohort study and a Danish randomized controlled trial report duration of infertility to be 3 and 4 years at baseline, respectively (12,55). In these

studies, the couples may have had IUI prior to inclusion. However, even with information on duration of infertility, effectiveness of expectant management cannot be assessed in a retrospective study, where the entire cohort had fertility treatment. Couples may have conceived while on the waiting list for fertility treatment, and active treatment may hinder natural conception. Therefore, the reported birthrates after natural conception in this study may not be a true reflection of what can be achieved with expectant management.

A randomized controlled trial from Scotland compared live birthrates in 580 couples with unexplained infertility, divided in three treatment groups: natural cycle IUI, expectant management and treatment with Clomiphene citrate (CC) alone (56). They reported live birthrates within 6 months from treatment start. In the natural cycle IUI group 23% had a live birth, as did 17% in the group with expectant management and 14% in the group who were treated with CC alone. There were no significant differences between the groups, indicating that expectant management may be as efficient as IUI or treatment with CC alone (56). A study from the Netherlands randomized 253 couples to 6 months of expectant management or treatment with IUI preceded by ovarian stimulation (57). There was no significant difference in ongoing pregnancy rates between the groups. However the pregnancy rate per IUI cycle was only 4% and furthermore, 20% of couples who started in the expectant group, had IUI treatments within 3 – 5 months from randomization.

Live birthrates after ART (Papers I and II)

We found that 53% of couples starting fertility treatments with ART and own gametes had a treatment related livebirth within 5 years. For women who received ART treatments with own eggs and partner or donor semen we found that 51% had a livebirth within 3 complete cycles. One complete cycle is defined as one oocyte pick-up and all resulting fresh and frozen-thawed embryo transfers. Our results are concurrent with previous studies from Sweden and the US, reporting conservative estimates after 3 – 6 ART cycles (7,58–60). A Swedish retrospective cohort study of 974 couples found that 56% had a livebirth within 3 complete cycles (58). A US study reported cumulative live birthrates of 45% within 3 consecutive ART cycles (59). The three cycles did not represent complete cycles, but were either fresh or frozen-thawed cycles. The women had a higher mean female age compared to our study. Another US-study reported cumulative live birthrates of 49% after 3 fresh or frozen-thawed cycles (7). A Study from UK reported conservative live birthrates of 44% after

8 complete cycles, but in this study 31% dropped out after the first complete cycle, which is higher than in our study. In our study the drop-out rate was 8% after the first complete cycle.

Number of aspirated oocytes and live birthrates (Paper II)

In couples having fertility treatment with own eggs and with homologous or donated semen, we found that the number of aspirated oocytes in the first fresh cycle, was a predictor for outcome in the 2nd and 3rd cycle. The adjusted odds for livebirth increased with increasing number of oocytes up to 12 aspirated oocytes in the first cycle. In women who had a low response to ovarian stimulation, in the first two fresh cycles, we found decreased odds for livebirth in the 3rd cycle.

The number of aspirated oocytes has previously been associated with live birthrates per fresh ART cycle (15,17–19). In a cohort study from the UK in 2011, Sunkara et al showed a strong association between number of oocytes and live birthrates per fresh ART-cycle (15). Birthrates increased with increasing oocytes up to 15 oocytes, then leveled and decreased if more than 20 oocytes were retreived. Similarly, a cohort study from the US showed that birthrates increased with increasing oocytes up to 15 oocytes (19). In these studies only fresh cycles were assessed, which excluded cycles where all embryos were frozen due to risk of OHSS. The number of oocytes has also been associated to cumulated live birthrates in a complete ART cycle (including frozen-thawed transfers) (16,61). A cohort study of 1099 women from Belgium assessed the association per complete ART cycle (16). In this study, women with more than 15 oocytes had higher cumulated live birthrates than women with less than 15 oocytes. In our study, we included live births from frozen-thawed transfers, and when assessing the first complete ART-cycle, the cumulated birthrate seem to plateau around 15-20 oocytes. However we did not have enough power to detect a potential increase or decline in birthrates beyond this point. Further, in recent years success rates in frozen-thawed cycles have improved, which may affect the number of oocytes needed to increase cumulated birthrates (5,28,62). Since frozen-thawed transfers are increasingly successful, one hypothesis could be that fewer oocytes are needed to optimize cumulated live birthrates. But it is also conceivable that the improvement gives even more value to each added aspirated oocyte.

Our results show that the number of aspirated oocytes in the first cycle is associated with cumulated live birthrates in the 2nd and 3rd complete cycles. A UK-study reports a similar finding with an association between number of retrieved oocytes in the first cycle, and outcome after 6 complete ART cycles (20). One explanation to this may be that ovarian response to stimulation is probable to be reproduced in later cycles. However, studies have shown that it is possible to regulate ovarian response with dose adjustments (26,27). A retrospective study from Denmark showed an improved number of aspirated oocytes after increasing the FSH start-dose, in 385 patients having their 2nd fresh ART cycle (26). A prospective cohort study from Sweden explored predictors for treatment outcome and found that high- medium and low responders could be identified by dividing the number of aspirated oocytes with total FSH dose (the ovarian sensitivity index) (63). The same research group also showed that a combination of Anti-Müllerian Hormone (AMH), age and antral follicle count (AFC) could predict ovarian response (64). Information on AMH and AFC is not included in the Danish ART registry, but our results show that female age alone is a useful predictor of chances of improved ovarian response in the 2nd cycle. We found that in women with 0 – 3 oocytes in the first fresh cycle, 73% of women under 35 had an improved ovarian response in the 2nd cycle, which was true for only 40% of women aged 40 years and more. One explanation to the high proportion of women under 35 improving their treatment response in the 2nd cycle may be that they were expected to be high responders in the 1st cycle, and therefore a low GnRH-dose was administered to prevent OHSS. This might have resulted in the women not reaching their threshold for follicular growth.

Women with a history of two treatment cycles with less than 0 – 3 aspirated oocytes per cycle (despite maximal stimulation) are defined as poor responders according to the Bologna criteria (24). We could not identify all groups of Bologna poor responders, since we did not have information on AMH or AFC. We found that women who had a sum of 0 – 6 oocytes in the 1st and 2nd fresh cycles, had reduced odds for livebirth compared with women who had a sum of 15 – 30 oocytes in the first two fresh cycles.

A Belgian study assessed cumulated live birthrates in 485 poor responders and found similar birthrates in women aged over and under 40 years (25). A Chinese study reports cumulative live birthrates of 18.6% in the first complete ART cycle (23). Two smaller Chinese studies report a marked decline in live birthrates in poor responders having their 3rd

treatment cycle, however sample size is very limited (21,22). Both studies report higher live birthrates in women less than 40 years, compared with women over 40 years.

In summary, concurrent with previous studies, we found that the cumulated live birthrate in the first complete cycle increased with increasing oocytes until 15 aspirated oocytes. Hereafter, it plateaued. Among women with a low treatment response in the first cycle, we found that female age predicted the chances of improving the treatment response in the 2nd cycle. Women with a history of two fresh cycles with less than 3 aspirated oocytes, but aged less than 35 years, still had acceptable birthrates of 21% in the 3rd cycle. In women aged over 40, with a history of two cycles with a low response, only 5.6% had a livebirth in the 3rd cycle.

OHSS and number of aspirated oocytes (paper II)

In our study, we obtained information on OHSS from the National Patient registry. This implies that the women with a diagnosis of OHSS were either admitted due to OHSS or seen in a gynecological outpatient clinic. It is possible that women with mild OHSS were followed in a fertility clinic without being admitted, and therefore the rates in this study are likely to be underestimated. Still, they may give an estimation of incidence of hospital admissions due to OHSS. We found an incidence of 1.7% in the first fresh cycle. Similar rates of hospital admission due to OHSS have been described in a Danish randomized controlled trial. The study reported that 1.7% of women in the Gonadotropin releasing hormone (GnRH) - antagonist protocol were admitted due to OHSS and so were 3.6% of women in the GnRH-agonist protocol (55). A Belgian cohort study reports incidence of hospital admissions due to OHSS of 2.1% (65). The Belgian study also showed, that the number of follicles sized over 11 mm was a better predictor of early-onset OHSS, than estradiol levels on the day of triggering ovulation. The study further reports that the risk of OHSS increased when more than 13 oocytes were collected.

We found that the incidence of OHSS increased with an increasing number of oocytes. As for the cumulated incidence of OHSS, we found that 2.7% of women were admitted due to OHSS within four complete cycles. Further, incidence of OHSS was lower in the 2nd, 3rd and 4th cycle than in the first cycle. The reduced incidence of OHSS in repeated cycles may be due to adjustments in ovarian stimulation, regulating ovarian response and increasing safety. However, the decreasing incidence of OHSS is probably also due to a selection of women

having repeated treatments. The number of oocytes is positively correlated with both live birthrates and OHSS; hence women returning for treatment may be less likely to have a high response to ovarian stimulation.

Perinatal outcome in singletons conceived after IUI (paper III)

We found slightly increased obstetric and perinatal risks in singletons conceived after IUI compared with naturally conceived singletons. Compared to children conceived after IVF and ICSI, outcome in children conceived after IUI was more favorable or comparable.

In singletons conceived after IUI, we found increased adjusted odds for preterm birth, being born with low birth weight or small for gestational age compared with naturally conceived singletons. Similar findings were reported in a large Belgian cohort study, in singletons born after IUI compared with naturally conceived singletons (37). In the Belgian study, children born after IUI have increased risk of preterm birth, very preterm birth (<32 weeks), low birth weight, very low birth weight (<1500 grams), respiratory distress, intracranial hemorrhage and admittance to neonatal intensive care unit. The study did not explore IUI-H vs IUI-D, and odds-ratios were not adjusted for maternal BMI and smoking. A Finnish study assessed outcome in children born after ovarian stimulation with or without IUI, and also found increased risks of low birthweight, very low birthweight, preterm birth and very preterm birth compared with naturally conceived children (38). In contrast, an Australian study of 1600 children, found no increased incidence of preterm birth or low birth weight in children conceived with IUI-D, compared with naturally conceived children (66). 23% of the children were conceived after a stimulated cycle. However, the study did find increased adjusted odds of preeclampsia, induced labor and caesarian section.

In our study, we found similar adjusted odds ratios for preterm birth, low birth weight and being born small for gestational age, in singletons conceived after IUI-H and IUI-D compared with singletons born after ICSI. Compared with singletons conceived after IVF, AOR for preterm birth and low birthweight were decreased in singletons born after IUI-H and IUI-D. Two smaller studies, from Belgium and Finland, compared children born after IUI with children born after IVF, and found similar outcomes in the groups (43,44). A Swedish/Australian study compared children born after IUI and children born after ART to naturally conceived children, and found that both groups had increased incidence of preterm birth compared with naturally conceived children, but the increased risk was highest in the

ART group (41). The favorable outcome, in children conceived after IUI, compared with children conceived with IVF, may be explained by characteristics in couples conceiving after IUI; they may have a shorter duration of infertility and a less severe degree of subfertility, than couples who conceive after ART. Further, IUI is a less invasive treatment, with fertilization occurring in vivo and with a milder or no ovarian stimulation. However, outcome was similar compared to children born after ICSI, despite the in vitro fertilization and ovarian stimulation performed in ICSI. Lower incidence of preterm birth and low birth weight in children conceived after ICSI, compared with IVF have been described previously (31). The reason for this is likely to be that mothers conceiving with ICSI are less reproductively ill, as the primary indication for ICSI is male-factor infertility and that female subfertility has a larger impact on perinatal outcome than male-factor infertility. In our study sub-analysis of children conceived after IUI showed similar AOR for preterm birth, low birthweight and small for gestational age, irrespective of the cause of infertility (male factor, anovulation or idiopathic infertility).

We found increased incidence of several obstetrical complications in singletons conceived with IUI. The incidence of placenta previa was increased in the IUI-H group compared with naturally conceived children, but was lower than in pregnancies conceived with ART. In pregnancies conceived with IUI-D, incidence of placenta previa was similar to naturally conceived children. A Japanese study associate placenta previa with tubal disease and endometriosis. These conditions are more prevalent among women undergoing ART treatments, and more prevalent in couples having IUI-H versus IUI-D (67).

In summary, concurrent with previous studies, we found that singletons conceived after IUI had increased risks compared with naturally conceived singletons, similar outcome as singletons conceived after ICSI, and more favorable outcome than children conceived after IVF. The different risk-profiles for being born preterm, with low birth weight or small for gestational age in the four groups are probably related to both different degrees of subfertility, causes of infertility and different treatment regimes. The impact of ovarian stimulation and source of semen will be further elaborated in the following section.

Ovarian stimulation, source of semen and perinatal outcome (paper III)

Couples receiving IUI with homologous semen differ in many aspects from women/couples receiving IUI with donated semen. IUI-H is predominantly performed in couples with a reproductive disease, with longer duration of infertility and more frequently in a stimulated cycle. In contrast, the IUI-D group includes women without a reproductive disease (male factor infertility, same-sex couples and single women), thus it may be hypothesized that perinatal outcome in the IUI-D group is more favorable. However, as found in the current study, as well as in previous studies, IUI-D is associated with an increased incidence of hypertensive disorders of pregnancy (68–70). This association may be explained by immunological mechanisms, since the woman has no previous exposure to the specific semen prior to fertilization. Although they differed with regard to parental characteristics, we found no difference in singletons conceived with IUI-D vs. IUI-H with regard to AOR of preterm birth, low birth weight or being born small for gestational age.

Our results indicate an association between treatment with clomiphene citrate and AOR for being born with low birth weight and small for gestational age. We found increased odds for these adverse perinatal outcomes in singletons conceived after IUI cycles stimulated with CC, compared with singletons born after IUI in a non-stimulated cycle. The association was also present in singletons conceived after IUI cycles stimulated with a combination of CC and FSH, compared with IUI in a natural cycle. Ovarian stimulation and its impact on perinatal outcome have been explored in a Swedish study of children born after ovarian stimulation (excluding IVF and ICSI) (40). This study reports increased risk of preterm birth and low birthweight in children born after ovarian stimulation compared with naturally conceived children. The increased risks remained after adjustment for duration of subfertility. Type of medication used was not specified. A Japanese study assessed impact of ovarian stimulation in IVF, comparing CC, FSH and CC+FSH to natural cycle IVF (71). Concurrent with our findings, the Japanese study found increased AOR for low birth weight in cycles stimulated with CC and CC+FSH. Another Japanese study found increased risk of preterm birth, low birth weight and small for gestational age in both children conceived after ovarian stimulation (not specifying type of medication) and after natural-cycle IUI, compared with naturally conceived children (72). A German study assessed dose-effect of gonadotropins, and found no effect of gonadotropins on birth weight in singletons conceived with ART, after stratification on duration of ovarian stimulation, dose and number of aspirated oocytes (73).

Although we did find an association between treatment with CC and incidence of low birth weight, causality cannot be established in a retrospective cohort study. However, in the following section, possible explanatory factors will be discussed. Clomiphene citrate displaces endogenous estrogen from receptors in the hypothalamus and pituitary gland (74,75). This triggers a negative feedback, leading to increased GnRH levels and thereby increased levels of endogenous FSH, thus stimulating follicular growth and ovulation. However, the anti-estrogenic effect of CC may be associated with thinning of the endometrium and affect cervical mucus (74,76–78). In a small study, decreased endometrial thickness, endometrial growth rate and increased serum estradiol levels have been reported in women treated with CC (77). Further, histological changes have also been described in relation to treatment with CC: decreased glandular density and increased number of vacuolated endometrial cells (79). It is not known if these described effects of CC on the endometrium have any impact on implantation, placental development and early fetal growth, but hypothetically this could represent a possible explanation to our findings.

In summary, we found similar incidence of preterm birth, low birth weight and small for gestational age in children conceived with IUI-H vs IUI-D. While IUI-H may have a higher degree of subfertility in the parents, and predominantly is performed in a stimulated cycle, pregnancy achieved with IUI-D is complicated with an increased incidence of hypertensive disorders of pregnancy. These different risk factors equalizes outcome in the two groups. As for IUI performed in a stimulated vs. natural cycle, we found that singletons conceived in CC-stimulated cycles may have slightly increased risks of low birth weight and small for gestational age. Previous literature on perinatal outcome after treatment with CC is sparse, but our findings is concurrent with one previous study in children conceived with ART (72). Further, other studies report of adverse effect on the endometrium associated with CC treatment (74,76,77).

11.2 Methodological considerations

Validity and bias

According to Danish legislation, the ART registry is mandatory for both public and private fertility clinics in Denmark, but it is not 100% complete. There may be random losses of data related to administration of the registry, especially in 2006 and 2007, when the registry converted from paper to electronic reporting. There may also be underreporting of cycles not resulting in pregnancy, however since the registry is mandatory; this kind of underreporting is of limited extent. Missing treatments may lead to an overestimation of the success-rates per treatment cycle, but it does not affect the cumulative live birthrates within 5 years from first treatment. Missing treatment cycles may however lead to misclassification of treatment related births to naturally conceived births. Missing information in the medical birth registry is unlikely.

With regard to correctly identifying the women's first treatment, registration of ART treatments started in 1994, so it is unlikely that women who started ART treatments prior to that, had a 9 years long treatment pause, before returning for further treatment in the study period. Thus, we have most likely correctly identified 1st ART treatments. IUI treatments were included in 2006, and couples who had IUI-treatment, but not ART, prior to 2006, and had no treatments at all in 2006 and then started ART treatments in the study period, may be misclassified as starting treatments with ART.

With the national registries we have detailed information on the women and couples receiving treatment, such as cause of infertility, parity, BMI, smoking , type of semen used for fertilization, obstetrical information etc. This information enables us to stratify or adjust results for confounders known to affect outcome measures. However, we were unable to stratify or adjust for duration of infertility. In previous studies, duration of infertility have been used as a proxy for degree of subfertility in the couples, and an association between increasing duration of infertility and incidence low birth weight have been reported (31). This suggests that risk of adverse perinatal outcome in children born after fertility treatments is related not only to the treatments but also to subfertility in the parents.

The proportion of couples with combined male/female factor infertility is lower than expected in our results. This may be due to missing information in one partner.

Information on number of aspirated oocytes was available for 70% of the fresh cycles. Sub-analysis showed that female age, incidence of OHSS and birthrates were similar in women with valid and missing data, suggesting that loss of information is random. Further, the number of aspirated oocytes is manly used as an explanatory variable, not an outcome variable, hence the main concern with the missing data is reduced power.

Limitations

Results are influenced by treatment guidelines from the Danish health authorities, reimbursement rules and legislation. Fertility treatments are reimbursed in couples with no common children and in childless single women, if the woman receiving treatment is below 40 years old. A total of three complete ART cycles (including frozen-thawed transfers) are reimbursed. However, not all medication costs are covered. Any additional ART treatment is at the women's own expense. IUI treatments are reimbursed and the recommendation is to offer a maximum of 3 – 6 cycles (30). Our results show that 97% of couples starting fertility treatments with homologous gametes had 1 – 6 IUI treatments while 1.4% had 7 IUI cycles and 1.5% had 8 or more IUI treatments.

Reimbursement of fertility treatments lead to inclusion of women from all socio-economic levels, and exclude financial reasons for discontinuing treatment. Low drop-out rates lead to higher cumulative birthrates, compared to countries without coverage of treatments. The cohort may be younger compared to other countries, but results are stratified or adjusted for female age. Women aged more than 40 years and women with more than 3 complete ART cycles, may have higher socio-economic level compared to the total cohort of women receiving treatments in Denmark. Socio-economic level may be related to degree of subfertility.

Results are further influence by treatment strategies recommended by the Danish health authorities; IUI is the first-line treatment to women with anovulatory, mild male factor infertility, mild endometriosis and idiopathic infertility (in addition to single women and women with a female partner). This affects distribution of cause of infertility in women starting IUI and ART treatments, compared to countries with other recommendations, but results have been stratified or adjusted for cause of infertility.

Longitudinal analysis

In longitudinal studies, estimates are influenced by decisions regarding censoring of drop-outs and the time axis. A cohort study from the UK showed that cumulated live birthrates, after 8 complete cycles, was 44% with a conservative estimate and 82% with an optimistic estimate (11).

A conservative estimate is the proportion women with a livebirth, within a specified time-frame or number of treatments, out of all women starting treatments, drop-outs included (11,59). Optimistic estimates are obtained with survival analysis using the number of treatments as the time-axis and censoring women who discontinue treatments (11,58,59). Optimistic estimates are based on the assumption of independent censoring, that couples who discontinue treatments would have had the same chance of treatment success (had they continued) as couples who do continue (6,8,9). However studies have shown that women who drop-out of treatment are more likely to have a poor prognosis than couples who continue (8,59,80). Further, natural conceptions in treatment pauses may be a reason for drop-out, a reason that is not independent of the outcome in question. Hence, occurrence of naturally conceived pregnancies is critical information when assessing treatment outcome in longitudinal studies.

11.3 Wider implications of the findings and future perspectives

This project included a national cohort of couples starting fertility treatments with homologous eggs and assessed a complete fertility treatment history with follow-up on both treatment-related and naturally conceived births, thus providing a robust prognosis for having a child when starting fertility treatments. Reporting natural conceptions is an important strength when assessing success rates, since it contributes substantially to the birthrates and may be a reason for dropping out of treatment. For couples having repeated ART-treatments, we used information from previous cycles to provide an adjusted prognosis that better reflect the current chances for achieving livebirth. We further described safety in the children conceived with IUI treatments, and found that they represent an intermediate risk group, compared to children conceived with IVF treatment and naturally conceived children. We found a slightly increased adjusted odds-ratio for low birth weight and small for gestational age associated to ovarian stimulation with clomiphene citrate, but not to treatment with FSH alone. The association between ovarian stimulation with CC and risk of being born small for gestational age, in infants conceived with IUI, contribute to the understanding of the adverse perinatal outcome seen in children conceived with fertility treatments, and may in the future result in improved safety. However, the findings regarding CC need to be reproduced and further explored in future studies.

Based on national data collected over four years this thesis assesses a treatment strategy offered to couples with idiopathic, mild male factor and anovulatory infertility, showing that the less-invasive and inexpensive IUI treatments may be a feasible first-line treatment alternative to ART in these couples. However, what can be achieved with expectant management is not addressed in this study, since we were not able to identify couples who had a natural conception while on the waiting list for treatments, and we did not have information on duration of infertility. Although information on duration of infertility exists in the IVF registry, it was poorly registered during our study period. Hopefully, it will be possible to explore expectant management, as a treatment strategy in subfertile couples, in future large register-based studies. We reported outcome after IUI-treatments regardless of insemination was performed in a natural or stimulated cycle, and in future studies, it would be interesting to stratify birthrates according to type of ovarian stimulation and type of medications used.

As for the association between the chances of treatment success and the number of aspirated oocytes, we found this to be a useful marker also to predict success rates in future cycles. We further described chances of an improved treatment response in different age groups. We found live birthrates more than halved in women aged >40 years in comparison with women <35 years. Oocyte donation is an efficient treatment, but in Denmark, oocyte donation has been limited due to a lack of donors. In 2016, a new more liberal reimbursement legislation for oocyte donors has been introduced. It is worthwhile considering whether this treatment should be offered in women >40 years of age at an earlier stage in the treatment process than it is now.

Concurrent with previous studies, we found that the number of oocytes needed to optimize live birthrates was 15–16. We could not detect increasing birthrates beyond this point, although power declined around 15–20 aspirated oocytes. However, in the regression analysis, adjusted for female age, there was no increasing effect on birthrates over 12 aspirated oocytes and with narrow confidence intervals. The declining number of observations in large number of oocytes most likely is a result of physicians avoiding OHSS. We further need to consider if the question, increasing treatment success with more than 15 oocytes, may be irrelevant considering the increasing risk of OHSS with a high ovarian response. However, in recent years, GnRH agonist trigger, blastocyst transfer and vitrification has opened the possibility of freeze-all treatment strategies with low risk of OHSS, opening a discussion on what the aimed number of aspirates oocytes should be. In principle we can stimulate more aggressively with agonist trigger and maintain a low risk of OHSS but still women will suffer from large ovaries and dizziness and the risk of haemorhage and torsion. Further, these treatment strategies may increase risks in the offspring, considering increased stimulation dose and the risk of being born large for gestational age related to frozen-thawed cycles. Based on the results in the current thesis, cumulative pregnancy rates after 15 oocytes do not seem to increase, which supports a stimulation strategy resulting in less than 15 oocytes.

12 Conclusion

Overall, 71% of couples starting fertility treatment with own gametes had a livebirth within 5 years from starting treatment, 57% had a treatment-related livebirth and 14% delivered after natural conception. Intrauterine insemination may be a beneficial first-line treatment alternative to ART in couples with anovulatory-, idiopathic and mild male factor infertility. The number of aspirated oocytes in the first ART cycle predicts treatment success in later cycles.

The results from this thesis may provide couples and women in fertility treatment with a comprehensible, individualized and age-stratified prognosis adjusted according to current treatment progression and previous treatment history.

Singletons conceived with intrauterine insemination had an intermediate risk-profile for adverse perinatal outcome compared with children conceived with ART and naturally conceived children. IUI treatment in cycles where the woman is stimulated with clomiphene citrate may be associated with an increased risk for the child to be born with low birth weight or small for gestational age.

13 Summary

Success rates in fertility treatments have traditionally been reported per treatment cycle, and no large studies have described the chances of live birth over a complete course of fertility treatments, including chances of natural conception. Fertility treatments in Denmark are highly accessible and costs are covered by the Danish health care system. IUI is first line treatment to couples with idiopathic, mild male factor infertility, and to couples with anovulatory infertility, combined with ovulation induction. All treatments performed in public and private clinics are registered in the mandatory ART registry. Through a personal identification number, treatment cycles can be cross-linked with the Medical Birth registry. The registries provide an opportunity to follow the couples through a trajectory of treatments, between different types of fertility treatments, between clinics and with complete follow-up on both treatment related and treatment independent births. Further, children born after fertility treatments can be identified and studied.

In this thesis we aimed to: I) Estimate the long-term prognosis for live birth in all couples starting fertility treatments II) Provide women with a revised prognosis based on previous treatment history and response to treatment and III) Study perinatal outcome in singletons conceived after insemination treatment and assess predictors of poor perinatal outcome.

Paper I

In couples starting fertility treatment with homologous gametes 57% had a treatment-related livebirth within 5 years from first treatment. In 14% of the couples, the first birth after starting treatments was conceived naturally. Age stratified total birthrates (after treatment and natural conception) was 80% in women <35 years, 61% in women aged 35-39 years and 26% in women aged >40 years. Based on our results, we consider that IUI treatment is a beneficial and patient friendly first-line treatment in selected couples.

Paper II

The number of aspirated oocytes in the first cycle was associated to cumulative live birthrates in the first complete cycle, including frozen-thawed transfers. Birthrates increased

with increasing oocytes up to 15-16 oocytes. The number of aspirated oocytes in the first cycle also predicted outcome in later cycles, although the association was less strong. For women with a low response to treatment (0 – 3 aspirated oocytes) the chance of an improved treatment outcome in subsequent cycles was strongly associated with female age.

Paper III

We found that singletons conceived with intrauterine insemination represent an intermediate risk group with regard to incidence of low birth weight and being small for gestational age, compared to singletons conceived with ART and naturally conceived singletons. This is likely related to a milder degree of subfertility in the parents and also to IUI being a less invasive treatment with milder or no ovarian stimulation and with fertilization occurring in vivo. We found a slightly increased risk of being born with low birth weight and small for gestational age in singletons conceived after IUI in cycles stimulated with clomiphene citrate, compared to singletons conceived after IUI in natural cycles.

14 Resumé (summary in Danish)

Succes-rater ved fertilitetsbehandling er traditionelt blevet rapporteret per behandlingscyklus. Der findes ingen store studier som beskriver chancerne for at få et barn over et komplet behandlingsforløb, inklusive chancen for naturligt opstået graviditet blandt par der starter behandling. Sammenlignet med andre lande, er fertilitetsbehandling i Danmark let tilgængelig og udgifterne dækkes af Sygesikringen. Intrauterin insemination (IUI) er førstevalgsbehandling til par med idiopatisk, mild mandlig faktor infertilitet og anovulation (kombineret med ovulationsinduktion). Alle behandlinger som udføres i offentlige og private klinikker i Danmark, bliver registreret i IVF-registeret, som det er obligatorisk at indrapportere til. Behandlinger i IVF-registeret kan kobles til fødselsregisteret. Registrene giver mulighed for at følge par, som starter fertilitetsbehandling, gennem et helt behandlingsforløb med forskellige typer af fertilitetsbehandling, også selvom parrene skifter klinik. Det er også muligt at følge op både på behandlingsrelaterede fødsler og fødsler efter naturlig undfangelse. Desuden kan børn som er født efter fertilitetsbehandling identificeres og studeres.

Formålet med denne afhandling er at I) Vurdere langtids-prognosen for at få et barn for par der påbegynder fertilitetsbehandling II) Lave en revideret prognose baseret på tidligere behandlinger og behandlingsrespons III) Undersøge enkeltfødte børn efter intrauterin insemination og estimere prædiktorer for lav fødselsvægt.

Studie I

Blandt par som påbegyndte fertilitetsbehandling med egne gameter, havde 57 % en levende behandlingsrelateret fødsel. Blandt 14 % af parrene var den første fødsel efter behandlingsstart undfanget naturligt, uden behandling. Totalt 80 % af kvinderne under 35 år fødte efter behandling eller naturlig undfangelse, i løbet af 5 år. Tilsvarende tal for kvinder 35–39 år var 61 %, og blandt kvinder over 40 år, var der kun 26 % som fødte. Baseret på vores resultater, mener vi, at intrauterin insemination er en god og patient venlig behandlingsmulighed, for udvalgte par.

Studie II

Antallet udtagne æg i den første ART behandling var associeret til den kumulative fødselsrate i den første friske cyklus og efterfølgende frysebehandlinger. Fødselsraten steg med antallet af udtagne æg op til 15-16 æg. Antallet af æg i den første friske cyklus var associeret til fødselsrater i senere behandlinger, men associationen var mindre udtalt. For kvinder med et lavt behandlingsrespons (0 – 3 udtagne æg) var chancerne for at forbedre behandlingsresponset i senere cykli stærkt relateret til kvindens alder.

Studie III

Vi fandt at enkeltfødte børn efter IUI-behandling havde større forekomst af for tidlig fødsel og lavere fødselsvægt end naturligt undfangede børn, men lavere forekomst end børn undfanget med IVF. Dette kan muligvis forklares af en mindre alvorlig grad af infertilitet hos forældrene samt en mildere og mindre invasiv fertilitetsbehandling, sammenlignet med børn fødte efter IVF. Desuden fandt vi en mulig let øget risiko for lav fødselsvægt og intrauterin væksthæmning hos børn født efter IUI og stimulation af æggestokkene med clomifen, sammenlignet med børn født efter IUI i en naturlig cyklus.

15 References

1. Olsen J, Küppers-Chinnow M, Spinelli A. Seeking medical help for subfecundity: a study based upon surveys in five European countries**Supported in part by The European Union (The Medical and Health Research Programme). The activities of the Danish Epidemiology Science Centre are financed by a g. Fertil Steril. 1996;66(1):95–100.

2. Sylvest R, Furbringer JK, Schmidt L, Pinborg A. Infertile men's needs and assessment of fertility care. Ups J Med Sci. 2016;121(4):1–7.

3. Klitzman R. How much is a child worth? Providers' and patients' views and responses concerning ethical and policy challenges in paying for ART. PLoS One. 2017;12(2):e0171939.

4. Wilkinson J, Vail A, Roberts SA, Haagen E, Tuil W, Hendriks J, et al. Direct-to-consumer advertising of success rates for medically assisted reproduction: a review of national clinic websites. BMJ Open. 2017;7(1):e012218.

5. Danish Fertility Society Annual Reports 1997-2016 [Internet]. [cited 2017 May 17]. Available from: www.fertilitetsselskab.dk/images/2017/dfs1997-2016 oversigt_140317.pdf

6. Bland JM, Altman DG. Topic collections Statistics Notes Survival probabilities (the Kaplan-Meier method). Bmj. 1998;317(7172):1572–80.

7. Stern JE, Brown MB, Luke B, Wantman E, Lederman A, Missmer SA, et al. Calculating cumulative live-birth rates from linked cycles of assisted reproductive technology (ART): Data from the Massachusetts SART CORS. Fertil Steril. 2010;94(4):1334–40.

8. Daya S. Life table (survival) analysis to generate cumulative pregnancy rates in assisted reproduction: Are we overestimating our success rates? Hum Reprod. 2005;20(5):1135–43.

9. Maheshwari A, McLernon D, Bhattacharya S. Cumulative live birth rate: Time for a consensus? Hum Reprod. 2015;30(12):2703–7.

10. Donckers J, Evers JLH, Land JA. The long-term outcome of 946 consecutive couples visiting a fertility clinic in 2001-2003. Fertil Steril. 2011;96(1):160–4.

11. McLernon DJ, Maheshwari A, Lee AJ, Bhattacharya S. Cumulative live birth rates after one or more complete cycles of IVF: A population-based study of linked cycle data from 178 898 women. Hum Reprod. 2016;31(3):572–81.

12. Pinborg A, Hougaard CO, Nyboe Andersen A, Molbo D, Schmidt L. Prospective longitudinal cohort study on cumulative 5-year delivery and adoption rates among 1338 couples initiating infertility treatment. Hum Reprod. 2009;24(4):991–9.

13. Sundström I, Ildgruben A, Högberg U. Treatment-related and treatment-independent deliveries among infertile couples, a long-term follow-up. Acta Obstet Gynecol Scand. 1997;76(3):238–43.

14. Aanesen A, Westerbotn M. Prospective study of a Swedish infertile cohort 2005-08: Population characteristics, treatments and pregnancy rates. Fam Pract. 2014;31(3):290–7.

15. Sunkara SK, Rittenberg V, Raine-Fenning N, Bhattacharya S, Zamora J, Coomarasamy A. Association between the number of eggs and live birth in IVF treatment: An analysis of 400 135 treatment cycles. Hum Reprod. 2011;26(7):1768–74.

16. Drakopoulos P, Blockeel C, Stoop D, Camus M, De Vos M, Tournaye H, et al. Conventional ovarian stimulation and single embryo transfer for IVF/ICSI. How many oocytes do we need to maximize cumulative live birth rates after utilization of all fresh and frozen embryos? Hum Reprod. 2015;31(2):370–6.

17. Briggs R, Kovacs G, MacLachlan V, Motteram C, Gordon Baker HW. Can you ever collect too many oocytes? Hum Reprod. 2015;30(1):81–7.

18. Baker VL, Brown MB, Luke B, Conrad KP. Association of number of retrieved oocytes with live birth rate and birth weight: An analysis of 231,815 cycles of in vitro fertilization. Fertil Steril. 2015;103(4):931–938.e2.

19. Steward RG, Lan L, Shah AA, Yeh JS, Price TM, Goldfarb JM, et al. Oocyte number as a predictor for ovarian hyperstimulation syndrome and live birth: An analysis of 256,381 in vitro fertilization cycles. Fertil Steril. 2014;101(4):967–73.

20. McLernon DJ, Steyerberg EW, Te Velde ER, Lee AJ, Bhattacharya S. Predicting the chances of a live birth after one or more complete cycles of in vitro fertilisation: population based study of linked cycle data from 113 873 women. BMJ. 2016;355:i5735.

21. Yang Y, Sun X, Cui L, Sheng Y, Tang R, Wei D, et al. Younger poor ovarian response women achieved better pregnancy results in the first three IVF cycles. Reprod Biomed Online. 2016;32(5):532–7.

22. Ke H, Chen X, Liu YD, Ye DS, He YX, Chen SL. Cumulative live birth rate after three ovarian stimulation IVF cycles for poor ovarian responders according to the bologna criteria. J Huazhong Univ Sci Technol - Med Sci. 2013;33(3):418–22.

23. Chai J, Lee VCY, Yeung TWY, Li RWH, Ho PC, Ng EHY. Live birth and cumulative live birth rates in expected poor ovarian responders defined by the Bologna criteria following IVF/ICSI treatment. PLoS One. 2015;10(3):1–10.

24. Ferraretti AP, La Marca A, Fauser BCJM, Tarlatzis B, Nargund G, Gianaroli L. ESHRE consensus on the definition of 'poor response to ovarian stimulation for in vitro fertilization: The Bologna criteria. Hum Reprod. 2011;26(7):1616–24.

25. Polyzos NP, Nwoye M, Corona R, Blockeel C, Stoop D, Haentjens P, et al. Live birth rates in Bologna poor responders treated with ovarian stimulation for IVF/ICSI. Reprod Biomed Online. 2014;28(4):469–74.

26. Popovic-Todorovic B, Loft A, Ziebe S, Nyboe Andersen A. Impact of recombinant FSH dose adjustments on ovarian response in the second treatment cycle with IVF or ICSI in “standard” patients treated with 150 IU/day during the first cycle. Acta Obstet Gynecol Scand. 2004;83(9):842–9.

27. Lashen H, Ledger W, López Bernal a, Evans B, Barlow D. Superovulation with a high gonadotropin dose for in vitro fertilization: is it effective? J Assist Reprod Genet. 1998;15(7):438–43.

28. Dyer S, Chambers GM, De Mouzon J, Nygren KG, Zegers-Hochschild F, Mansour R, et al. International committee for monitoring assisted reproductive technologies world report: Assisted reproductive technology 2008, 2009 and 2010†. Hum Reprod. 2016;31(7):1588–609.

29. Boujida, V H; Lemmen, F; Pedersen, DE; Svenstrup L. Danish Fertility Society Clinical Guidlines IUI-H [Internet]. [cited 2017 May 17]. Available from: http://www.fertilitetsselskab.dk/images/2016/guideline/iui-h_290316.pdf

30. Vævsbekendtgørelsen - Vejledning om sundhedspersoners og vævscentres virksomhed og forpligtelser i forbindelse med assisteret reproduktion - retsinformation.dk [Internet]. [cited 2017 May 17]. Available from: https://www.retsinformation.dk/Forms/R0710.aspx?id=172755#ide681ffa8-817c-4e1e-96ff-5ce8c2fa0a2a

31. Pinborg A, Wennerholm UB, Romundstad LB, Loft A, Aittomaki K, Sö derström-Anttila V, et al. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analysis. Hum Reprod Update. 2013;19(2):87–104.

32. Klemetti R, Gissler M, Hemminki E. Comparison of perinatal health of children born from IVF in Finland in the early and late 1990s. Hum Reprod. 2002;17(8):2192–8.

33. Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal Outcomes in Singletons Following In Vitro Fertilization: A Meta-Analysis. Obstet Gynecol. 2004;103(3):551–63.

34. Helmerhorst FM. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. Bmj. 2004;328(7434):261–0.

35. W. O, G. M. Pregnant after assisted reproduction: A risk pregnancy is born! 18-years results from a population-based registry in Flanders, Belgium. Hum Reprod. 2015;30(4):i366–7.

36. Pinborg A, Henningsen AA, Loft A, Malchau SS, Forman J, Andersen AN. Large baby syndrome in singletons born after frozen embryo transfer (FET): Is it due to maternal factors or the cryotechnique? Hum Reprod. 2014;29(3):618–27.

37. Ombelet W, Martens G, De Sutter P, Gerris J, Bosmans E, Ruyssinck G, et al. Perinatal outcome of 12 021 singleton and 3108 twin births after non-IVF-assisted reproduction: A cohort study. Hum Reprod. 2006;21(4):1025–32.

38. Klemetti R, Sevón T, Gissler M, Hemminki E. Health of children born after ovulation induction. Fertil Steril. 2010;93(4):1157–68.

39. Gaudoin M, Dobbie R, Finlayson A, Chalmers J, Cameron IT, Fleming R. Ovulation induction/intrauterine insemination in infertile couples is associated with low-birth-weight infants. Am J Obstet Gynecol. 2003;188(3):611–6.

40. Källén B, Olausson PO, Nygren KG. Neonatal outcome in pregnancies from ovarian stimulation. Obstet Gynecol. 2002;100(3):414–9.

41. Wang JX, Norman RJ, Kristiansson P. The effect of various infertility treatments on the risk of preterm birth. Hum Reprod. 2002;17(4):945–9.

42. Poon WB, Lian W Bin. Perinatal outcomes of intrauterine insemination/clomiphene pregnancies represent an intermediate risk group compared with in vitro fertilisation/ intracytoplasmic sperm injection and naturally conceived pregnancies. J Paediatr Child Health. 2013;49(9):733–40.

43. Nuojua-Huttunen S, Gissler M, Martikainen H, Tuomivaara L. Obstetric and perinatal outcome of pregnancies after intrauterine insemination. Hum Reprod. 1999;14(8):2110–5.

44. De Sutter P, Veldeman L, Kok P, Szymczak N, Van der Elst J, Dhont M. Comparison of outcome of pregnancy after intra-uterine insemination (IUI) and IVF. Hum Reprod. 2005;20(6):1642–6.

45. Henningsen AA, Gissler M, Skjaerven R, Bergh C, Tiitinen A, Romundstad LB, et al. Trends in perinatal health after assisted reproduction: A Nordic study from the CoNARTaS group. Hum Reprod. 2015;30(3):710–6.

46. Kondapalli LA, Perales-Puchalt A. Low birth weight: Is it related to assisted reproductive technology or underlying infertility? Fertil Steril. 2013;99(2):303–10.

47. Messerlian C, MacLagan L, Basso O. Infertility and the risk of adverse pregnancy outcomes: A systematic review and meta-analysis. Hum Reprod. 2013;28(1):125–37.

48. Romundstad LB, Romundstad PR, Sunde A, von Düring V, Skjærven R, Gunnell D, et al. Effects of technology or maternal factors on perinatal outcome after assisted fertilisation: a population-based cohort study. Lancet. 2008;372(9640):737–43.

49. Marsál K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. Acta Paediatr. 1996;85(7):843–8.

50. Goverde AJ, McDonnell J, Vermeiden JPW, Schats R, Rutten FFH, Schoemaker J. Intrauterine insemination or in-vitro fertilisation in idiopathic subfertility and male subfertility: A randomised trial and cost-effectiveness analysis. Lancet.

2000;355(9197):13–8.

51. Steures P, van der Steeg JW, Hompes PG, van der Veen F, Mol BW. Intrauterine insemination in The Netherlands. Reprod Biomed Online. 2007;14(1):110–6.

52. Schorsch M, Gomez R, Hahn T, Hoelscher-Obermaier J, Seufert R, Skala C. Success rate of inseminations dependent on maternal age? An analysis of 4246 insemination cycles. Geburtshilfe Frauenheilkd. 2013;73(8):808–11.

53. Jeon YE, Jung JA, Kim HY, Seo SK, Cho S, Choi YS, et al. Predictive factors for pregnancy during the first four intrauterine insemination cycles using gonadotropin. Gynecol Endocrinol. 2013;29(9):834–8.

54. Bensdorp a J, Tjon-Kon-Fat RI, Bossuyt PMM, Koks C a M, Oosterhuis GJE, Hoek a, et al. Prevention of multiple pregnancies in couples with unexplained or mild male subfertility: randomised controlled trial of in vitro fertilisation with single embryo transfer or in vitro fertilisation in modified natural cycle compared with intrauterine inse. BMJ. 2015;350(jan09_2):g7771.

55. Toftager M, Bogstad J, Bryndorf T, Løssl K, Roskær J, Holland T, et al. Risk of severe ovarian hyperstimulation syndrome in GnRH antagonist versus GnRH agonist protocol: RCT including 1050 first IVF/ICSI cycles. Hum Reprod. 2016;31(6):1253–64.

56. Bhattacharya S, Harrild K, Mollison J, Wordsworth S, Tay C, Harrold a, et al. Clomifene citrate or unstimulated intrauterine insemination compared with expectant management for unexplained infertility: pragmatic randomised controlled trial. BMJ. 2008;337(August):a716.

57. Steures P, van der Steeg JW, Hompes PG, Habbema JDF, Eijkemans MJ, Broekmans FJ, et al. Intrauterine insemination with controlled ovarian hyperstimulation versus expectant management for couples with unexplained subfertility and an intermediate prognosis: a randomised clinical trial. Lancet. 2006;368(9531):216–21.

58. Olivius K, Friden B, Lundin K, Bergh C. Cumulative probability of live birth after three in vitro fertilization/intracytoplasmic sperm injection cycles. Fertil Steril. 2002;77(3):505–10.

59. Malizia B, Hacker M, Penzias A. Cumulative Live-Birth Rates after In Vitro Fertilization. N Engl J Med. 2009;360:236–43.

60. Luke B, Brown MB, Wantman E, Lederman A, Gibbons W, Schattman GL, et al. Cumulative Birth Rates with Linked Assisted Reproductive Technology Cycles. N Engl J Med. 2012;366(26):2483–91.

61. Bdolah Y, Zemet R, Aizenman E, Lossos F, Abram TB, Shufaro Y. Frozen-Thawed embryo transfer success rate is affected by age and ovarian response at oocyte aspiration regardless of blasto- mere survival rate. J Bras Reprod Assist. 2015;19(4):210–5.

62. Kupka MS, D'Hooghe T, Ferraretti AP, De Mouzon J, Erb K, Castilla JA, et al. Assisted reproductive technology in Europe, 2011: Results generated from European registers by ESHRE. Hum Reprod. 2015;31(2):233–48.

63. Vaegter KK, Lakic TG, Olovsson M, Berglund L, Brodin T, Holte J. Which factors are most predictive for live birth after in??vitro fertilization and intracytoplasmic sperm injection (IVF/ICSI) treatments? Analysis of 100 prospectively recorded variables in 8,400 IVF/ICSI single-embryo transfers. Fertil Steril. 2017;107(3):641–648.e2.

64. Brodin T, Hadziosmanovic N, Berglund L, Olovsson M, Holte J. Comparing four ovarian reserve markers - Associations with ovarian response and live births after assisted reproduction. Acta Obstet Gynecol Scand. 2015;94(10):1056–63.

65. Papanikolaou EG, Pozzobon C, Kolibianakis EM, Camus M, Tournaye H, Fatemi HM, et al. Incidence and prediction of ovarian hyperstimulation syndrome in women undergoing gonadotropin-releasing hormone antagonist in vitro fertilization cycles. Fertil Steril. 2006;85(1):112–20.

66. Hoy J, Venn A, Halliday J, Kovacs G, Waalvvyk K. Perinatal and obstetric outcomes of donor insemination using cryopreserved semen in Victoria, Australia. Hum Reprod. 1999;14(7):1760–4.

67. Takemura Y, Osuga Y, Fujimoto A, Oi N, Tsutsumi R, Koizumi M, et al. Increased risk of placenta previa is associated with endometriosis and tubal factor infertility in assisted reproductive technology pregnancy. Gynecol Endocrinol. 2013;29(2):113–5.

68. Dekker G a, Sibai BM. Etiology and pathogenesis of preeclampsia: current concepts. Am J Obstet Gynecol. 1998;179(5):1359–75.

69. Need JA, Bell B, Meffin E, Jones WR. Pre-eclampsia in pregnancies from donor inseminations. J Reprod Immunol. 1983;5(6):329–38.

70. Kho EM, McCowan LME, North RA, Roberts CT, Chan E, Black MA, et al. Duration of sexual relationship and its effect on preeclampsia and small for gestational age perinatal outcome. J Reprod Immunol. 2009;82(1):66–73.

71. Nakashima A, Araki R, Tani H, Ishihara O, Kuwahara A, Irahara M, et al. Implications of assisted reproductive technologies on term singleton birth weight: An analysis of 25,777 children in the national assisted reproduction registry of Japan. Fertil Steril. 2013;99(2):450–5.

72. Hayashi M, Nakai A, Satoh S, Matsuda Y. Adverse obstetric and perinatal outcomes of singleton pregnancies may be related to maternal factors associated with infertility rather than the type of assisted reproductive technology procedure used. Fertil Steril. 2012;98(4):922–8.

73. Griesinger G, Kolibianakis EM, Diedrich K, Ludwig M. Ovarian stimulation for IVF has no quantitative association with birthweight: A registry study. Hum Reprod. 2008;23(11):2549–54.

74. Homburg R. Clomiphene citrate - End of an era? A mini-review. Hum Reprod. 2005;20(8):2043–51.

75. Kousta E, White DM, Franks S. Modern use of clomiphene citrate in induction of ovulation. Hum Reprod Updat. 1997;3(4):359–65.

76. Badawy A, Shokeir T, Allam AF, Abdelhady H. Pregnancy outcome after ovulation induction with aromatase inhibitors or clomiphene citrate in unexplained infertility. Acta Obstet Gynecol Scand. 2009;88(2):187–91.

77. Haritha S, Rajagopalan G. Follicular growth, endometrial thickness, and serum estradiol levels in spontaneous and clomiphene citrate-induced cycles. Int J Gynecol Obstet. 2003;81(3):287–92.

78. Gonen Y, Casper RF. Sonographic determination of a possible adverse effect of domiphene citrate on endometrial growth. Hum Reprod. 1990;5(6):670–4.

79. Sereepapong W, Suwajanakorn S, Triratanachat S, Sampatanukul P, Pruksananonda K, Boonkasemsanti W, et al. Effects of clomiphene citrate on the endometrium of regularly cycling women. Fertil Steril. 2000;73(2):287–91.

80. Smeenk JMJ, Verhaak CM, Stolwijk AM, Kremer JAM, Braat DDM. Reasons for dropout in an in vitro fertilization/intracytoplasmic sperm injection program. Fertil Steril. 2004;81(2):262–8.

16 Appendix

Paper I

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human reproduction

ORIGINAL ARTICLE *Reproductive epidemiology*

The long-term prognosis for live birth in couples initiating fertility treatments

S. S. Malchau[1,*], A. A. Henningsen[2], A. Loft[2], S. Rasmussen[1], J. Forman[3], A. Nyboe Andersen[2], and A. Pinborg[1]

[1]Department of Obstetrics and Gynecology, Copenhagen University Hospital Hvidovre, Kettegård Allé 26, DK-2650 Hvidovre, Denmark [2]Fertility Clinic, Copenhagen University Hospital Rigshospitalet, Blegdamsvej 9, DK-2100 KBH Ø, Denmark [3]Department of Public Health, Section of Biostatistics, University of Copenhagen, Øster Farimagsgade 5, DK-1014 KBH K, Denmark

*Correspondence address. Sara Sofia Malchau, Abildgaardsvej 131, DK-2830 Virum, Denmark. Tel: +4540548954; E-mail: smalchau@hotmail.com

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STUDY QUESTION: What are the long-term chances of having a child for couples starting fertility treatments and how many conceive with ART, IUI and without treatment?

SUMMARY ANSWER: Total 5-year live birthrates were strongly influenced by female age and ranged from 80% in women under 35–26% in women ≥40 years, overall, 14% of couples conceived naturally and one-third of couples starting treatments with intrauterine insemination delivered from that treatment.

WHAT IS KNOWN ALREADY: Few studies report success rates in fertility treatments across a couple's complete fertility treatment history, across clinics, evaluating live births after insemination, ART and natural conceptions.

STUDY DESIGN, SIZE, DURATION: This register-based national cohort study from Denmark includes all women initiating fertility treatments in public and private clinics with homologous gametes in 2007–2010.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Women were identified in the Danish ART Registry and were cross-linked with the Medical Birth Registry to identify live births. Subfertile couples were followed 2 years ($N = 19\,884$), 3 years ($N = 14\,445$) and 5 years ($N = 5165$), or until their first live birth. Cumulative live birthrates were estimated 2, 3 and 5 years from the first treatment cycle, in all women, including drop-outs. Birthrates were stratified by type of first treatment (ART/IUI), mode of conception (ART/IUI/natural conception) and female age.

MAIN RESULTS AND THE ROLE OF CHANCE: Within 5 years, in women aged <35 years ($N = 3553$), 35–39 years ($N = 1156$) and ≥40 years ($N = 451$), a total of 64%, 49% and 16% had a live birth due to treatment, respectively. Additionally, in women aged < 35 years, 35–39 years and ≥40 years, 16%, 11% and 10% delivered after natural conception, yielding total 5-year birthrates of 80%, 60% and 26%. In women starting treatments with IUI ($N = 3028$), 35% delivered after IUI within 5 years, 24% delivered after shift to ART treatments and 17% delivered after natural conception. Within 5 years from starting treatments with ART ($N = 2137$), 53% delivered after ART, 11% delivered after natural conception and 0.6% delivered after IUI.

LIMITATIONS, REASONS FOR CAUTION: Birthrates are most likely higher compared to countries without national coverage of treatments and results are influenced by laws and regulations. Information on duration of infertility prior to treatment was not available. Future prospective intervention studies should focus on the role of expectant management.

WIDER IMPLICATIONS OF THE FINDINGS: Our results can provide couples with a comprehensible age-stratified prognosis at start of treatment.

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TRIAL REGISTRATION NUMBER: The study was approved by the Danish Data Protection Agency (J.nr. 2012-41-1330).

Key words: assisted reproductive technology / intrauterine insemination / cumulative live birthrates / natural conception / long-term prognosis / fertility treatments

Introduction

When assessing success rates after fertility treatments, longitudinal studies are increasingly replacing the traditional per treatment-based estimates (Bland and Altman, 1998; Daya, 2005; Mahesshwari *et al.*, 2015). However, very few studies have evaluated a complete course of treatments, across all treatment types, with follow-up irrespective of shift between clinics and on both treatment related and treatment-independent births (Pinborg *et al.*, 2009; Donckers *et al.*, 2011; Aanesen and Westerbotn, 2014).

In Denmark, fertility treatments are highly accessible and additionally reimbursed by the National Health System, in childless couples where the woman is below 40 years of age. Three fresh ART treatments are reimbursed, including adjacent frozen embryo transfers. Treatments with IUI are also reimbursed, a maximum of three to six cycles is recommended. Unlike most countries in the world, insemination treatments exceed the use of ART (http://www.fertilitetssekskab.dk). IUI is first-line treatment to couples with unexplained infertility, mild to moderate male factor infertility, and is also used in anovulatory infertility (combined with ovulation induction), according to guidelines from the Danish fertility society (http://www.fertilitetssekskab.dk). Fewer side-effects and lower costs in IUI treatments may justify this treatment strategy, but little is known of long-term results, since few studies assess long-term live birthrates including shifts between IUI- and ART treatments (Sundström *et al.*, 1997; Pinborg *et al.*, 2009; Donckers *et al.*, 2011; Aanesen and Westerbotn, 2014).

In Denmark, all public and private fertility clinics have mandatory reporting of treatment activity to the national ART Registry, and all births are registered in the Medical Birth registry. With the registries, the individual couple can be followed over time; between IUI- and ART cycles, between fertility clinics, to conception and birth, both natural conceptions and as a result of fertility treatment.

The purpose of this study was to obtain a comprehensive long-term prognosis, suitable for counseling couples entering treatment. Further, to assess the Danish treatment strategy, using IUI as first-line treatment to a selected group of infertile couples. The objective was to estimate cumulative live birthrates at 2, 3 and 5 years after the first treatment with ART or IUI, basing estimates on observed live births after treatment and natural conception, including only couples treated with homologous gametes and with no previous births due to fertility treatments but including couples with previous live birth after natural conception.

Methods

Study design and patients

This is a national cohort study based on the Danish ART registry and the Medical Birth registry. The Danish ART registry includes all ART treatment-cycles performed in public and private fertility clinics in Denmark since 1994; IUI-cycles were added in 2006. IUI is performed in unstimulated as well as stimulated cycles with either clomiphene or gonadotropins; however a clear majority are stimulated with clomiphene (Malchau *et al.*, 2014). Reporting to the ART registry is mandatory for both public and private clinics. A personal identification number enables identification of all treatment cycles received by the same woman, and thereby construction of a complete fertility treatment history.

The study included 19 884 couples initiating fertility treatment in the period January 1st 2007 to December 31st 2010. Follow-up on births were available until December 31st 2012, hence a minimum of 2-year follow-up from first treatment, was available. Inclusion criteria were Danish residents, first treatment within the study period, treatment with ART (fresh or frozen-thawed cycles) or IUI with homologous (own) gametes. Couples receiving treatments with donated gametes or testicular sperm aspiration were excluded. Due to strict regulations regarding egg donation during the study period, treatment with egg donation was very limited and included several years on waiting list before treatment. All included and excluded treatment cycles are shown in Fig. 1.

Subjects identified in the Danish ART Registry were cross-linked with the Medical Birth Registry to identify all live births of the same woman. The gestational age at birth, recorded in the medical birth registry, was used to link births to treatment cycles, or to identify births due to natural conception. Women were followed for two, three and 5 years from the first treatment, until the first live birth or until they shifted to treatment with donated gametes. Only the first live birth since start of treatment is reported; later siblings are not included in the birthrates. One treatment cycle is defined as any started fresh or frozen-thawed ART-cycle (including treatments that are canceled before transfer), or insemination treatment.

Statistical analyses

For non-normal distributed data, the Mann–Whitney *U* test was used to compare distributions between groups. Cumulative live birthrates were estimated as the proportion of women having achieved at least one live birth within follow-up, among all women with complete 2-, 3- and 5-year follow-up. Complete 2-, 3- and 5-year follow-up was available if start date of the first treatment cycle was at least 2, 3 and 5 years prior to Dec 31st 2012. Information on emigration outside Denmark was not available. All birthrates were calculated separately for subgroups according to type of first treatment (IUI/ART). Stratification was made according to mode of conception (IUI/ART/natural conception), female age at time of first treatment, cause of infertility, female body mass index (BMI), smoking status and secondary infertility (defined as previous natural conception-birth). Female BMI > 55 was considered an extreme value most likely due to errors in registration of height or weight, and were excluded in analyses regarding BMI. Missing data was not imputed. Cause of infertility was reported as female factor infertility, male factor, combined female/male factor and idiopathic infertility. Female factor infertility was further stratified in subgroups, such as anovulatory infertility, tubal factor and endometriosis. The subgroups included only women with one type of female factor infertility and did not include couples with combined male/female factor infertility.

Predictors of live birth were analyzed in multivariable logistic regression analysis with spline assessment including categorical and continuous variables; female age (linear spline with break point at 35 and 40 years), smoking (yes, no), BMI (linear spline with break point at BMI 20 and 30),

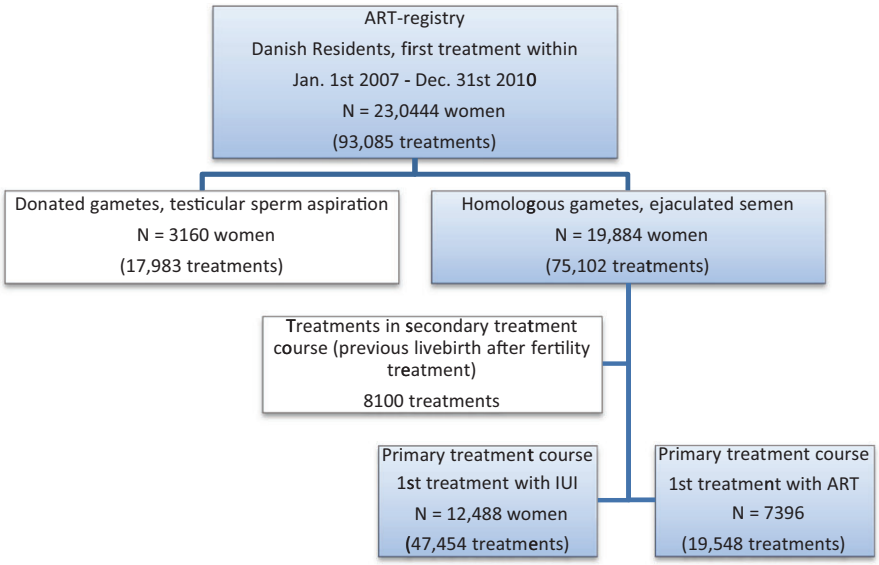

Figure 1 Overview of included and excluded treatment-cycles. ART, assisted reproductive technology; IUI, intrauterine insemination.

secondary infertility (yes, no), cause of infertility (categorical, including idiopathic, male factor, combined male/female factor, anovulation, tubal factor, endometriosis, other female factors, two female factors and ovarian factor) and course of treatments (categorical).

Statistical software SAS version 9.4 and IBM SPSS statistics version 19 were used for data management and analyses.

Role of funding source

The funders had no role in the study design, data collection and interpretation, or decision to submit the work for publication.

Ethical approval

The study was approved by the Danish Data Protection Agency (J.nr. 2012-41-1330). In Denmark, register-based studies do not require approval from ethics committees.

Results

Baseline characteristics at time of first treatment are shown in Table I. Women starting treatments with IUI were slightly younger than women starting with ART: 32.4 years versus 33.1 years. Cause of infertility was significantly different in the two groups. In couples starting with IUI, anovulatory infertility and idiopathic infertility were more prevalent. In couples starting with ART, male factor infertility, tubal factor infertility and endometriosis were more prevalent. Prevalence of obesity (BMI ≥ 30) and smoking were similar in the two groups, but secondary infertility was slightly more prevalent in women starting treatments with IUI.

Complete 2-year follow-up was available for 19 884 women: 12 488 women starting treatments with IUI and 7396 women starting treatments with ART (Table II). Complete 3-year follow-up was available for 8816 women starting treatments with IUI and 5629 women starting treatments with ART. Complete 5-year follow-up was available for 3028 women starting treatments with IUI and 2137 women starting treatments with ART.

Treatment activity at 2-year follow-up

Among women with complete 5-year follow-up, 92% of all IUI and ART treatments occurred within 2 years from first treatment. The total number of IUI and ART treatments per woman at 2-year follow-up is shown in Fig. 2. The median number of total treatments was 3 (interquartile range, IQR 1–4). For couples starting with IUI, the median number of total IUI and ART treatments was 3 (IQR 2–5). For couples starting with ART, the median number of total treatments was 2 (IQR 1–3), significantly lower compared to women starting treatments with IUI, $P < 0.0001$.

All outcomes after 2, 3 and 5 years are shown in Table II. In all women (starting treatments with either IUI or ART) the cumulative live birthrates 2, 3 and 5 years after first treatment were: 57.0%, 65.0% and 71.0%.

Course of treatments and live birthrates when starting treatments with IUI

The course of treatments is shown in Table III. The majority (49.2%) had 1–3 IUI treatments within the first 2 years. A total of 10.1% had 4–6 IUI treatments. A total of 38.1% of couples starting with insemination subsequently shifted over to ART (26.8% shifted after 1–3 IUI attempts and 11.3% after 4–6 IUI attempts). Details on distribution of IUI treatments are shown in Supplementary Table SI.

Birthrates, stratified on type of first treatment and type of conception, are shown in Table II and illustrated in Fig. 3. In couples starting treatments with IUI, the total live birthrates (after treatment and natural conception) within 2, 3 and 5 years were 59.0%, 68.6% and 75.2%. When stratifying on mode on conception, 34.2% delivered after IUI-conception within 2 years, and birthrates increased to 34.4%

Table I Background characteristics at time of first treatment cycle with IUI or ART with homologous gametes in Denmark 1997–2010.

	First treatment with IUI		First treatment with ART	
	Women, *N*	% [95% CI]	Women, *N*	% [95% CI]
All	12 488	100	7396	100
Overall cause of infertility				
Female	2418	19.4 [18.7–20.1]	1860	25.1 [24.2–26.2]
Male	2401	19.2 [18.5–19.9]	3158	42.7 [41.6–43.8]
Combined	481	3.9 [3.5–4.2]	527	7.1 [6.5–7.7]
Idiopathic	7188	57.6 [56.7–58.4]	1851	25.0 [24.0–26.0]
Female infertility, specified				
Anovulation[a]	1716	13.7 [13.1–14.4]	307	4.2 [3.7–4.6]
Tubal factor[a]	214	1.7 [1.5–2.0]	1025	13.9 [13.1–14.7]
Endometriosis[a]	87	0.7 [0.6–0.9]	241	3.3 [2.9–3.7]
BMI ≥ 30	1055	11.2 [10.7–11.8]	645	10.7 [10.0–11.4]
Smoking, yes	1563[b]	16.0 [15.3–16.7]	915[c]	14.8 [13.9–15.7]
Secondary infertility	3539	28.3 [27.5–29.1]	1575	21.3 [20.4–22.2]
	Mean ± SD	95% CI	Mean ± SD	95% CI
Female age (years)	32.4 ± 4.8	32.3–32.5	33.1 ± 5.1	32.9–33.2

[a]Subgroup of female factor infertility, not all subgroups are reported.
[b]Missing information smoking *N* = 2710 (21.7%).
[c]Missing information smoking *N* = 1229 (16.6%)

and 34.9% within 3 and 5 years, respectively. Shift to ART treatment resulted in birthrates after ART-conception of 15.1%, 21.1% and 23.7%, 2, 3 and 5 years from first treatment, respectively (Table II). After 5 years, 16.6% of women starting treatments with IUI had delivered after natural conception.

Live birthrates when starting treatments with ART

The course of treatments within the first 2 years of treatment, are shown in Table III. The vast majority (76.4%) had 1–3 ART treatments. Only 2.8% of couples starting with ART also had IUI within the first 2 years.

In women where ART was the initial treatment, the cumulative total live birthrates after 2, 3 and 5 years were 52.5%, 60.1% and 64.5% (Table II and Fig. 3). The majority of live births were after ART treatment, with birthrates of 46.1%, 51.1% and 52.9% within 2, 3 and 5 years. Within 5 years 11.2% had given birth after natural conception, but only 0.6% gave birth after IUI.

Age-stratified analyses

Age-stratified total birthrates (both treatment-related and treatment-independent) are shown in Fig. IV. Live birthrates stratified on age, type of first treatment and mode of conception, are shown in Table IV. For all women, starting treatments with either IUI or ART, the total birthrates at 5-year follow-up were: <35 years 80.0%, 35–39 years 60.5% and >40 years 26.2%. Birthrates for natural conceptions within 5 years in women aged <35, 35–39, >40 were: 15.8%, 11.4% and 10.0%. At 5-year follow-up, there was no difference in total number of treatments between the age-groups.

Predictors of live birth

Adjusted odds ratios for chances of live birth are shown in Supplementary Table SII. For all women, non-smoking and anovulatory infertility were predictors of high live birthrates. For women starting treatments with IUI, tubal factor infertility was a predictor of lower live birthrates. For women starting treatments with ART, male factor infertility was a predictor of high success rates.

Female age

In women starting treatments with IUI and aged less than 35, the adjusted odds-ratio (AOR) for live birth decreased with 1% for each year the women got older, not statistically significant. In women aged 35–39, AOR for live birth decreased significantly with 16% ($P < 0.0001$) for each year the woman was older. In women aged 40 and over, AOR for live birth decreased with 37% ($P < 0.0001$) for each year the woman was older.

In women starting treatments with ART and aged less than 35, AOR for live birth decreased with 4% ($P < 0.0001$) for each year the women was older. Adjusted odds for live birth in women aged 35–39 years decreased with 15% ($P < 0.0001$) each year. In women over 40 years old, chances decreased with 33% ($P < 0.0001$) each year.

BMI

In women starting treatments with IUI, who had a BMI under 20, adjusted odds for live birth increased with 11% ($P = 0.04$) for each increasing BMI unit. For women with BMI 20–29, AOR for live birth decreased with 4% ($p < 0.0001$) for each increasing BMI unit. For women with BMI ≥ 30, AOR for live birth decreased with 7% ($P < 0.0001$) for

Table II **All outcomes 2-, 3- and 5 years after first treatment with IUI or ART with homologous gametes, in women with no previous births due to fertility treatment, Denmark 2007–2010.**

	N	Live birth % [95% CI]				No live birth % [95% CI]				Total %
	Total couples	IUI-conception	ART-conception	Natural conception	Total live births	Continue treatment[a]	Shift to donor semen	Shift to donor egg	No live birth[b]	All outcomes[c]
2-year follow-up										
All	19 884	21.7 [21.3–22.3]	26.6 [26.0–27.2]	8.4 [8.0–8.8]	57.0 [56.3–57.7]	12.5 [12.0–13.0]	3.1 [2.9–3.3]	0.9 [0.8–1.0]	26.8 [26.2–27.5]	100
First treatment IUI	12 488	34.2 [33.4–35.0]	15.1 [14.5–15.7]	9.9 [9.4–10.4]	59.0 [58.1–59.9]	13.5 [12.9–14.1]	2.6 [2.3–2.8]	0.6 [0.4–0.7]	24.2 [23.5–25.0]	100
First treatment ART	7396	0.5 [0.3–0.7]	46.1 [45.0–47.2]	5.9 [5.4–6.4]	52.5 [51.5–53.6]	10.8 [10.1–11.6]	4.1 [3.7–4.6]	1.4 [1.2–1.7]	31.3 [30.2–32.3]	100
3-year follow-up										
All	14 445	21.2 [20.5–21.9]	32.8 [32.0–33.6]	11.2 [10.7–11.8]	65.0 [64.2–5.8]	4.9 [4.6–5.3]	3.6 [3.3–3.9]	1.1 [0.9–1.3]	25.3 [24.6–26.0]	100
First treatment IUI	8816	34.4 [33.4–35.4]	21.1 [20.3–22.0]	13.1 [12.4–13.8]	68.6 [67.6–69.6]	5.6 [5.2–6.2]	3.0 [2.6–3.4]	0.7 [0.6–0.9]	22.1 [21.3–23.0]	100
First treatment ART	5629	0.5 [0.3–0.7]	51.1 [49.8–52.4]	8.5 [7.8–9.2]	60.1 [58.8–61.4]	3.8 [3.4–4.4]	4.4 [3.9–4.9]	1.6 [1.3–2.0]	30.3 [29.1–31.5]	100
5-year follow-up										
All	5165	20.7 [19.6–21.8]	35.8 [34.5–37.1]	14.2 [13.3–15.2]	70.7 [69.5–71.9]	0.5 [0.3–0.7]	4.2 [3.7–4.8]	1.1 [0.9–1.4]	23.5 [22.4–24.7]	100
First treatment IUI	3028	34.9 [33.2–36.6]	23.7 [22.2–25.2]	16.6 [15.3–17.9]	75.2 [73.7–76.7]	0.7 [0.4–1.0]	4.2 [3.5–4.9]	0.8 [0.6–1.2]	19.2 [17.8–20.6]	100
First treatment ART	2137	0.6 [0.3–0.9]	52.9 [50.8–55.0]	11.0 [9.8–12.4]	64.5 [62.4–66.5]	0.2 [0.0–0.4]	4.1 [3.3–4.9]	1.5 [1.1–2.1]	29.7 [27.8–31.7]	100

The table reports the first live birth since start of treatment; later siblings are not reported.
[a]No live birth within follow-up, but record of further treatment beyond follow-up.
[b]No live birth or record of further treatment until 31st Dec 2012.
[c]Live birth + no live birth.

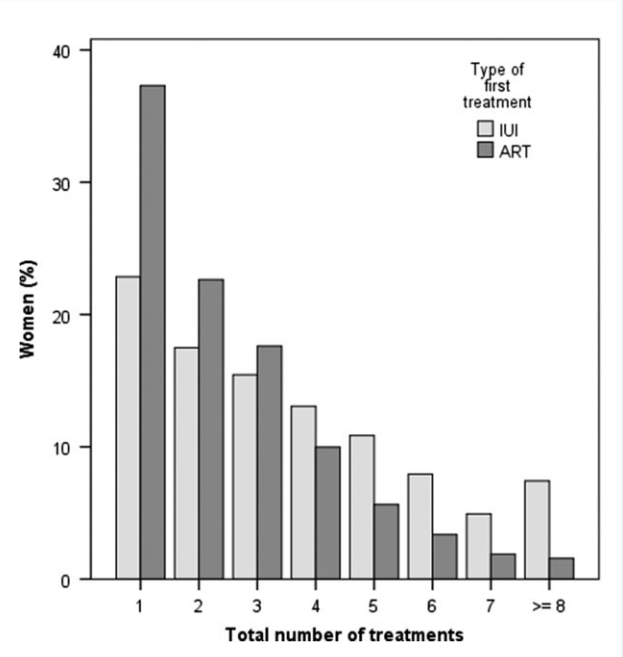

Figure 2 Total number of treatments with ART or IUI within 2 years from first treatment in women receiving fertility treatments with homologous gametes in Denmark 2007–2010, stratified on type of first treatment. Based on women with complete 2-year follow-up: 12 488 women starting treatments with IUI and 7396 women starting treatments with ART.

each increasing BMI unit. Similar results were found in women starting treatments with ART.

Live birthrates stratified on type of first treatment, female age, type of conception, cause of infertility and female characteristics are shown in Supplementary Tables SIII and SIV.

Multiples

Multiples rates stratified on type of conception are displayed in Supplementary Table SV. In couples who conceived with IUI, twin rates were 11.3% [10.4–12.3], which was lower compared to couples conceiving with ART. Among ART-conceived deliveries, twin rates were 14.0% [13.1–15.0]. Triplet and quadruplet rates were 0.2 and 0.0 in both groups.

Discussion

In couples receiving fertility treatments with their own gametes, 57% gave birth as a result of treatment within 5 years, and 14% conceived naturally. Female age was the most important factor to predict outcome, and the total live birthrates within 5 years declined from 80% in women under 35–26% in women aged 40 and over. Most treatments (92%) occurred within the first 2 years, and the median number of total treatments was then 3 (IQR 1–4). Couples starting treatments with IUI had higher birthrates after treatments and natural conceptions, probably related to less severe infertility than in couples allocated directly to ART treatment.

In couples starting treatments with IUI, 34% conceived with IUI within 2 years, with a minimal increase between years 2 and 5, which reflect the Danish treatment guidelines: to offer a maximum of 3–6 cycles of IUI before shifting to ART (http://www.fertilitetssekskab.dk). IUI is a well-tolerated and inexpensive alternative to ART, but it is also known to be less efficient per treatment cycle (Goverde *et al.*, 2000; Steures *et al.*, 2007; Jeon *et al.*, 2013; Schorsch *et al.*, 2013). Only a few smaller studies have investigated the long-term prognosis for live birth after ART and IUI (Sundström *et al.*, 1997; Pinborg *et al.*, 2009; Donckers *et al.*, 2011; Aanesen and Westerbotn, 2014). A Danish questionnaire study, based on reports from 1338 couples, showed that 70% gave birth within 5 years (Pinborg *et al.*, 2009). Of these couples, 55% gave birth after ART, 18% after natural conception and only 10% after IUI. However, the majority of couples had received fertility treatments with IUI prior to inclusion in the study, which may explain the low birthrate after IUI-conceptions. A Swedish prospective cohort study of 380 couples, reported total live birthrates of 57% within 2.5 years (Aanesen and Westerbotn, 2014). Of all couples, 19% delivered after IUI or ovulation induction, 30% delivered after ART and 12% delivered after natural conception. The birthrate after IUI is lower than in our study, but numbers cannot be directly compared, since 22% of couples in the Swedish study never had IUI treatments. A Dutch study, with a cohort of 946 couples, found that 51% had a live birth and that 28% had a naturally conceived pregnancy (Donckers *et al.*, 2011). Cumulated pregnancy rates of 25–30% after four IUI-cycles, in women under 35, are previously reported in two smaller, single center studies from Korea and Germany (Jeon *et al.*, 2013; Schorsch *et al.*, 2013). A randomized trial from the Netherlands compared birthrates after a maximum of six fresh cycles of IVF or six cycles of IUI in couples with idiopathic or male subfertility (Goverde *et al.*, 2000). Even though per-cycle birthrates were higher in the ART group, so were the drop-out rates, leading to similar cumulated outcome in the groups. A Dutch trial from 2015 compared live birthrates in 602 couples with idiopathic or mild male subfertility, randomized to start treatment with either three fresh IVF treatments (plus subsequent frozen-thawed cycles), six natural-cycle IVF treatments, or six insemination treatments with ovarian stimulation. Live birthrates were similar in the three groups, indicating that both types of IVF treatments are non-inferior to IUI with ovarian stimulation (Bensdorp *et al.*, 2015). A Scottish randomized controlled trial reports live birthrates of 17% after 6 months of expectant management, birthrates of 23% after 6 months of natural-cycle IUI and birthrates of 14% when treatment was clomiphene citrate alone. The differences were non-significant, indicating that expectant management may be as effective as natural-cycle IUI or clomiphene alone (Bhattacharya *et al.*, 2008). A Dutch study randomized 253 couples to 6 months of expectant management or treatment with IUI and ovarian stimulation. There was no significant difference in the ongoing pregnancy rates between the groups (23% in the intervention group versus 27% in the expectant group) (Steures *et al.*, 2006). In this study the ongoing pregnancy rate per started IUI cycle was only 4.1%. Further, 20% of couples in the expectant group started IUI treatments within 3–5 months from randomization.

Our results show, that in couples who start with insemination treatments 34% delivered after IUI-conception, and 38% changed to ART treatments within 2 years. The national guidelines recommend that couples with idiopathic, mild male factor as well as anovulatory infertility should initiate treatments with insemination. Our numbers indicate

Table III Course of treatments by patient within 2 years from first treatment in couples receiving fertility treatments with homologous gametes in Denmark 2007–2010, overall and stratified by cause of infertility.

First treatment with IUI	Total couples, *N*	1–3 IUI % [95% CI]	4–6 IUI % [95% CI]	1–3 IUI → ART % [95% CI]	4–6 IUI → ART % [95% CI]	Other % [95% CI]
Overall	12 488	49.2 [48.3–50.0]	10.1 [9.5–10.6]	26.8 [26.0–27.6]	11.3 [10.7–11.8]	2.7 [2.4–3.0]
Female factor infertility	2418	54.3 [52.3–56.3]	9.9 [8.7–11.1]	25.5 [23.8–27.2]	8.6 [7.6–9.8]	1.7 [1.2–2.3]
Anovulation[a]	1716	54.8 [52.5–57.2]	11.0 [9.6–12.6]	23.1 [21.2–25.2]	9.2 [7.9–10.6]	1.8 [1.3–2.5]
Tubal factor[a]	214	43.5 [36.9–50.2]	7.0 [4.2–11.0]	42.1 [35.6–48.7]	7.0 [4.2–11.0]	0.5 [0.1–2.2]
Endometriosis[a]	87	49.4 [39.1–59.8]	3.4 [1.0–8.9]	37.9 [28.3–48.4]	6.9 [2.9–13.7]	2.3 [0.5–7.2]
Male factor infertility	2401	46.6 [44.6–48.6]	8.6 [7.5–9.7]	32.0 [30.2–33.9]	11.0 [9.8–12.3]	1.8 [1.3–2.4]
Combined male female factor	481	49.5 [45.0–53.9]	11.9 [9.2–15.0]	26.4 [22.6–30.5]	10.0 [7.5–12.9]	2.3 [1.2–3.9]
Idiopathic infertility	7188	48.3 [47.1–49.4]	10.5 [9.8–11.2]	25.6 [24.6–26.6]	12.3 [11.6–13.1]	3.3 [2.9–3.8]

First treatment with ART	Total *N*	1–3 ART % [95% CI]	4–6 ART % [95% CI]	7 + ART % [95% CI]	ART → IUI % [95% CI]
Overall	7396	76.4 [75.5–77.4]	17.9 [17.0–18.8]	2.9 [2.6–3.3]	2.8 [2.4–3.2]
Female factor infertility	1860	76.3 [74.3–78.2]	18.0 [16.3–19.8]	2.9 [2.2–3.7]	2.8 [2.2–3.7]
Anovulation[a]	307	73.3 [68.1–78.0]	13.7 [10.2–17.9]	3.6 [1.9–6.1]	9.4 [6.6–13.1]
Tubal factor[a]	1025	76.4 [73.7–78.9]	19.6 [17.3–22.1]	2.7 [1.9–3.9]	1.3 [0.7–2.1]
Endometriosis[a]	241	76.8 [71.1–81.8]	19.1 [14.5–24.4]	2.5 [1.0–5.1]	1.7 [0.6–3.9]
Male factor infertility	3158	76.5 [75.0–77.9]	18.5 [17.2–19.9]	3.0 [2.4–3.6]	2.0 [1.6–2.6]
Combined male female factor	527	82.7 [79.3–85.8]	13.9 [11.1–17.0]	1.9 [1.0–3.3]	1.5 [0.7–2.8]
Idiopathic infertility	1851	74.7 [72.7–76.7]	17.8 [16.1–19.6]	3.1 [2.4–4.0]	4.3 [3.5–5.3]

One ART-cycle is defined as any started fresh or frozen-thawed cycle. [a]Subgroup of female factor infertility, not all subgroups are reported.

that this may be a feasible treatment program, especially in women under 35, were 45% in the anovulation group had a live birth due to IUI within 2 years, as did 38% in the idiopathic group, and 32% in the male factor group. Considering the benefits of the non-invasive IUI treatments, we believe that it qualifies as a first-line option to couples with these specific causes of infertility.

The role of expectant management is not addressed in this study. According to national guidelines, treatments are offered to couples who have been trying to conceive for at least 1 year. Two Danish studies, a prospective survey and a randomized controlled trial, report a mean duration of infertility of 3 and 4 years prior to inclusion. However in these studies the couples may have had previous insemination treatments (Pinborg *et al.*, 2009; Toftager *et al.*, 2016). The Danish ART registry does not contain individual data on how long the couples had tried to conceive before treatments, but even with that information, the question of expectant management cannot be addressed properly in a retrospective cohort analysis, where all couples had treatments. We do not know how many couples conceived while on the waiting list for treatments, and active treatment prevents natural conception; thus the potential for natural conception with expectant management in a subfertile cohort, is not reflected in our estimates. However, information on natural conceptions is vital to this report, to identify the true treatment-failures, and to give a complete prognosis. In women starting treatments with IUI, 10% delivered after natural conception within 2 years, increasing to 17% within 5 years.

A total of 53% of couples initiating treatments with ART, conceived with this treatment within 5 years. The vast majority had less than four treatment cycles. Few women starting with ART had both ART and IUI treatments. Comparing with previous studies from Sweden and the US, birthrates are similar to conservative estimates after 3–5 cycles (Olivius *et al.*, 2002; Malizia *et al.*, 2009; Stern *et al.*, 2010). A UK study presented lower conservative estimates of 44% after eight fresh cycles with possible adjacent frozen-thawed cycles. None of the studies included birthrates after natural conception. In our study, natural conceptions occurred in women of all ages and in all causes of infertility. Birthrates after natural conception increased from 6% within the first 2 years to 11% within 5 years. Even though natural conceptions occur, we believe that treatments should not be postponed in women over 35, considering the rapidly declining treatment-success with increasing age.

Even though mandatory by Danish legislation, the Danish ART Registry is not 100% complete. Although underreporting of cycles not resulting in a pregnancy may occur, missing treatments may also be random losses of information due to administration, such as the conversion from paper to electronic reporting in 2006, and later changing the software for the electronic forms. Further, since IUI treatments were included in 2006, couples who had IUI treatment but no ART prior to 2006, and no treatment activity at all in 2006, may be misclassified as starting treatments with ART if returning for ART treatment within the study period. Missing treatments may lead to an overestimation of the success-rate per treatment cycle, but not of the cumulated birthrate 2, 3 and 5 years after first treatment. Missing births in the medical birth registry are unlikely, thus information on live births is complete. Since most women have repeated treatments, it is unlikely that we have underestimated the number of women with no birth, but

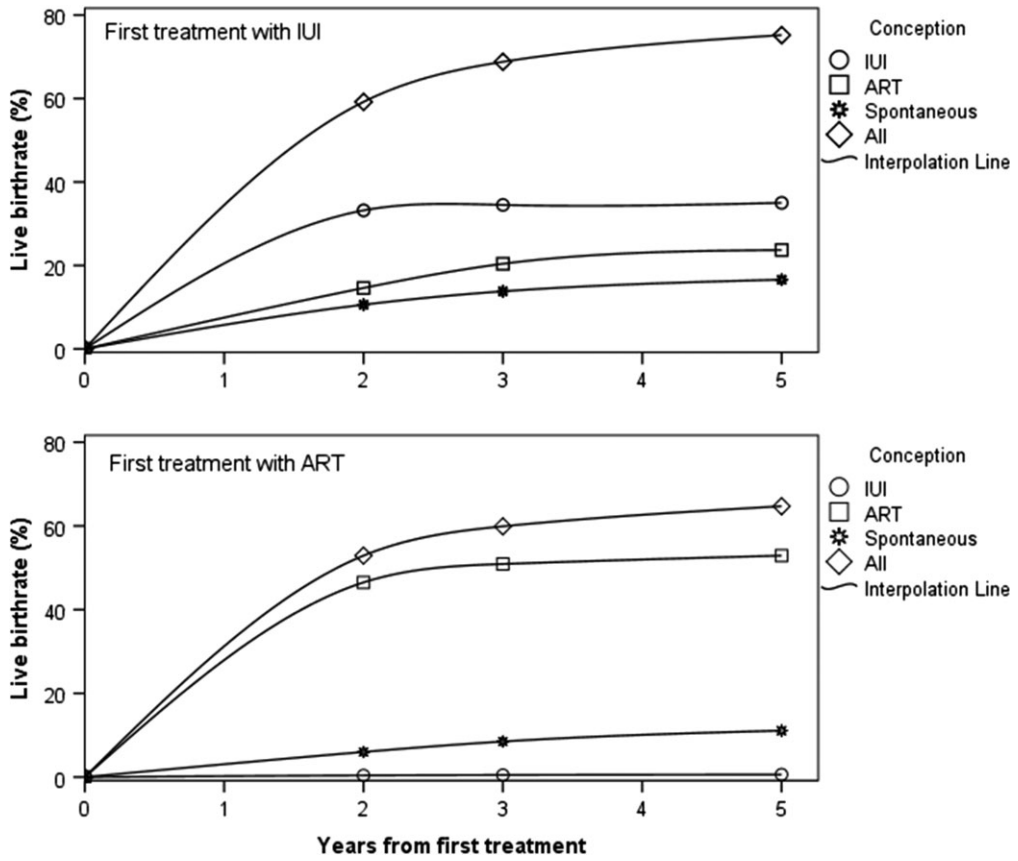

Figure 3 Live birthrates 2, 3 and 5 years after first treatment in women receiving fertility treatments with homologous gametes in Denmark 2007–2010, stratified on type of conception and type of first treatment. Based on women with complete 5-year follow-up: 3028 women starting treatments with IUI and 2137 women starting treatments with ART.

we may have allocated treatment-related births to natural conception births. Even though information on cause of infertility appear to be complete in the cohort, the fraction of couples with combined male/female factor infertility is lower than expected and this may be due to missing information in one partner.

Our results are influenced by national treatment guidelines, reimbursement rules and legislation.

The national treatment guidelines recommend IUI treatments with homologous semen as first-line treatment to couples with idiopathic, mild male factor infertility and anovulatory infertility. Couples with severe male factor infertility, tubal factor infertility or severe endometriosis are recommended to start treatments with ART (http://www.fertilitetssekskab.dk).

Inseminations and ART treatments are reimbursed in childless couples if the woman is below 40 years old. If the woman turns 40 during treatments, and still has frozen embryos, they may be transferred with reimbursement. Three fresh ART treatments are reimbursed, plus subsequent frozen embryo transfers. Further fresh cycles are at the couples own expense. It is recommended that all frozen embryos are used before proceeding to the next fresh cycle, but this is not regulated by legislation. The number of reimbursed ART treatments is unaffected by possible previous IUI treatments. The number of reimbursed insemination treatments is not strictly regulated, but a maximum of six inseminations is recommended. Our numbers indicate that this recommendation is followed in 97% of couples starting treatments with insemination, while 1.4% of couples have seven IUI-cycles, and 1.5% of couples have eight or more IUI-cycles in total. Legislation prohibits treatments to women above 45 years.

The national coverage of fertility treatments lead to inclusion of all socio-economic levels in our cohort, and exclude financial reasons for dropping out within the first three fresh ART cycles. Birthrates are most likely higher than in countries without reimbursement. The regulations concerning female age lead to a younger cohort, compared to other countries. The total number of treatments was similar across the age-groups, probably because the youngest women need fewer treatments to conceive, and that treatment activity in the highest age-group is limited by reimbursement rules. Because women over 40 finance their treatments personally, this may lead to selection-bias with regard to socio-economic level, which may be related to fertility potential. This may also be true for couples who receive more than three fresh ART cycles.

When assessing longitudinal outcome after fertility treatments, decisions regarding the time axis and censoring of drop-outs, have major influence on the estimates. This is demonstrated in a UK study

Table IV Age-stratified live birthrates by patient 2, 3 and 5 years after first treatment with homologous gametes in Denmark, 2007–2010.

Female age	first treatment with IUI					first treatment with ART				
	N, total	IUI-conception, live birthrate [95% CI]	ART-conception, live birthrate [95% CI]	Natural conception, live birthrate [95% CI]	Total live birthrate [95% CI]	*N*, total	ART-conception, live birthrate [95% CI]	IUI-conception, live birthrate [95% CI]	Natural conception, live birthrate [95% CI]	Total live birthrate [95% CI]
2-year follow-up										
<35 years	8753	37.8 [36.8–38.8]	16.8 [16.0–17.6]	10.6 [10.0–11.2]	65.2 [64.2–66.2]	4857	53.3 [51.9–54.7]	0.7 [0.5–0.9]	6.3 [5.6–7.0]	60.3 [58.9–61.7]
35–39 years	2897	29.7 [28.0–31.4]	12.7 [11.5–13.9]	8.4 [7.4–9.4]	50.7 [48.9–52.5]	1769	39.3 [37.0–41.6]	0.3 [0.1–0.6]	5.1 [4.1–6.1]	44.8 [42.5–47.1]
≥40 years	833	12.4 [10.2–14.6]	5.6 [4.0–7.2]	7.6 [5.8–9.4]	25.6 [22.6–28.6]	753	15.5 [12.9–18.1]	0 [0–0.005]	5.6 [4.0–7.2]	21.1 [18.2–24.0]
3-year follow-up										
<35 years	6229	37.8 [36.6–39.0]	23.6 [22.6–24.7]	13.9 [13.0–14.8]	75.4 [74.3–76.5]	3707	59.0 [57.4–60.6]	0.7 [0.4–1.0]	9.3 [8.4–10.2]	69.1 [67.6–70.6]
35–39 years	1981	30.2 [28.2–32.2]	17.3 [15.6–19.0]	11.3 [9.9–12.7]	58.9 [56.7–61.1]	1328	44.7 [42.0–47.4]	0.2 [0.0–0.4]	6.6 [5.3–7.9]	51.5 [48.8–54.2]
≥40 years	601	13.1 [10.4–15.8]	7.5 [5.4–9.6]	9.7 [7.3–12.1]	30.3 [26.6–33.4]	580	15.5 [12.6–18.5]	0 [0–0.006]	7.4 [5.3–9.5]	22.9 [19.5–26.3]
5-year follow-up										
<35 years	2152	37.9 [35.9–40.0]	26.9 [25.0–28.8]	18.0 [16.4–19.6]	82.8 [81.2–84.4]	1401	62.2 [59.7–64.7]	0.9 [0.4–1.4]	12.5 [10.8–14.2]	75.6 [73.4–77.9]
35–39 years	651	32.4 [28.8–36.0]	18.9 [15.9–21.9]	13.8 [11.2–16.5]	65.1 [61.4–68.8]	505	45.7 [41.4–50.0]	0.2 [0.2–0.6]	8.3 [5.9–10.7]	54.3 [50.0–58.6]
≥40 years	222	13.5 [9.0–18.0]	6.8 [3.5–10.1]	10.8 [6.7–14.9]	31.1 [25.0–37.2]	229	11.8 [7.6–16.0]	0 [0–0.02]	9.2 [5.5–12.9]	21.0 [15.7–26.3]

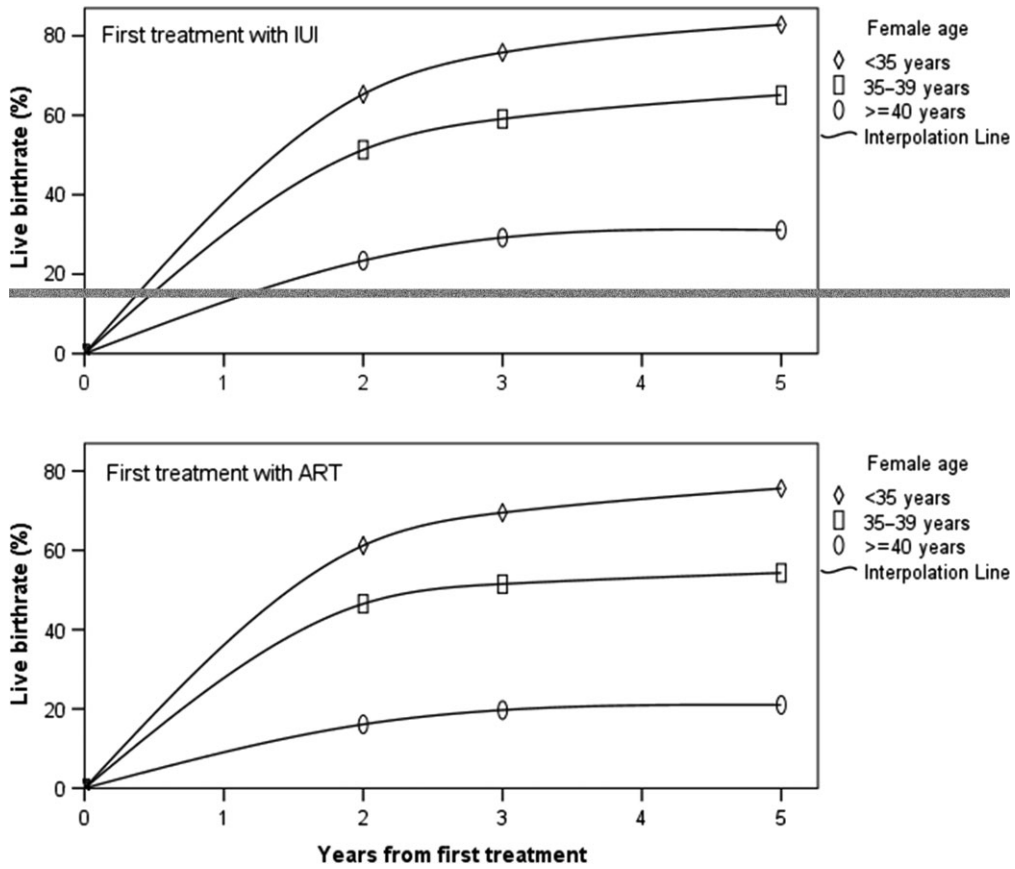

Figure 4 Total live birthrates after treatment and natural conception 2, 3 and 5 years after first treatment, in women receiving fertility treatments with homologous gametes in Denmark 2007–2010, stratified on female age and type of first treatment. Based on women with complete 5 years follow-up: 3028 women starting treatments with IUI and 2137 women starting treatments with ART.

from 2016 were birthrates after eight complete cycles were analyzed both in a conservative and optimistic way, yielding estimates of 44% and 82%, respectively (McLernon *et al.*, 2016). The conservative estimate is the proportion of women who deliver out of all couples entering treatment, including drop-outs (Malizia *et al.*, 2009; McLernon *et al.*, 2016). Optimistic estimates are assessed with survival analyses, such as Kaplan–Meier, were the time axis corresponds to the number of cycles and women are censored when they drop out of treatments (Olivius *et al.*, 2002; Malizia *et al.*, 2009; McLernon *et al.*, 2016). Such estimates are made on the assumption that censored couples would have reached the same birthrates as the couples who continue treatments, had they continued (Bland and Altman, 1998; Daya, 2005; Mahesshwari *et al.*, 2015). Previous studies have raised concern that the assumption, independent censoring, may not be met in an infertile population, where drop-outs/censored women have been shown to be older, have higher FSH levels on day three of menstrual cycle, lower peak estradiol levels, have fewer retrieved oocytes and fewer frozen embryos, and higher anxiety levels, thus are more likely to have a poor prognosis (Daya, 2005; Malizia *et al.*, 2009; Smeenk *et al.*, 2004). Further, natural conceptions in treatment pauses may be a reason for drop out. Therefore drop-outs may represent the couples with the best and the worst prognosis. Considering this, information on natural conceptions is crucial when assessing birthrates in longitudinal studies, and an important strength in this study.

Conclusion

Overall, 71% of the women had a live birth within 5 years from the first treatment, of which 57% conceived after treatment and 14% conceived naturally. Insemination treatments may be a feasible first-line option to couples with idiopathic and mild male factor as well as anovulatory infertility. Based on these results, we are able to provide couples with a comprehensible and individual, age-stratified long-term prognosis at start of treatment.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

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Authors' roles

S.S.M., A.A.H., A.L., J.F., A.N.A. and A.P. contributed substantially to the study design, analysis and interpretation of the data. S.R. cross-linked treatment cycles in the ART registry with the MBR-registry. S.R. and S.S.M. were responsible for data management. The first draft of the manuscript was written by S.S.M. All co-authors revised the manuscript. All co-authors approved the final manuscript.

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Conflict of interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: S.S.M. received an unconditional grant from Ferring Pharmaceuticals; A.A.H. has received personal fees from Ferring Pharmaceuticals not related to this work; A.N.A. reports grants and personal fees from Ferring Pharmaceuticals, personal fees from Merck Serono, grants and personal fees from MSD, outside the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

References

Aanesen A, Westerbotn M. Prospective study of a Swedish infertile cohort 2005–08: population characteristics, treatments and pregnancy rates. *Fam Pract* 2014;**31**:290–297.

Bland JM, Altman DG. Survival probabilities (the Kaplan–Meier method). *Br Med J* 1998;**317**:1572.

Bensdorp AJ, Tjon-Kon-Fat RI, Bossuyt PM, Koks CA, Oosterhuis GJ, Hoek A, Hompes PG, Broekmans FJ, Verhoeve JP, de Bruin JP *et al*. Prevention of multiple pregnancies in couples with unexplained or mild male subfertility: randomised controlled trial of in vitro fertilisation with single embryo transfer or in vitro fertilisation in modified natural cycle compared with intrauterine insemination with controlled ovarian hyperstimulation. *Br Med J* 2015;**350**:g7771.

Bhattacharya S, Harrild K, Mollison J, Wordsworth S, Tay C, Harrold A, McQueen D, Lyall H, Johston L, Burrage J *et al*. Clomifene citrate or unstimulated intrauterine insemination compared with expectant management for unexplained infertility: pragmatic randomised controlled trial. *Br Med J* 2008;**337**:a716.

Danish Fertility Society. http://www.fertilitetsselskab.dk (15 marts 2016, date last assessed).

Daya S. Life table (survival) analysis to generate cumulative pregnancy rates in assisted reproduction: are we overestimating our sucessrates? *Hum Reprod* 2005;**20**:1135–1143.

Donckers J, Evers JLH, Land JA. The long-term outcome of 946 consecutive couples visiting a fertility clinic in 2001–2003. *Fertil Steril* 2011;**96**:160–164.

Goverde AJ, McDonnell J, Vermeiden JPW, Schats R, Rutten FF, Schoemaker J. Intrauterine insemination or in-vitro fertilization in idiopathic subfertility and male subfertility; a randomized trial and cost-effectiveness analysis. *Lancet* 2000;**355**:13–18.

Jeon YE, Jung JA, Kim HY, Seo SK, Cho S, Choi YS, Lee BS. Predictive factors for pregnancy during the first four intrauterine insemination cycles using gonadotropin. *Gynecol Endocrinol* 2013;**29**:834–838.

Mahesshwari A, McLernon D, Bhattacharya S. Cumulative live birthrate: time for a consensus? *Hum Reprod* 2015;**30**:2703–2707.

Malchau SS, Loft A, Henningsen AKA, Nyboe Andersen A, Pinborg A. Perinatal outcomes in 6,338 singletons born after intrauterine insemination in Denmark, 2007 to 2012: the influence of ovarian stimulation. *Fertil Steril* 2014;**102**:1110–1116.

Malizia B, Hacker MR, Penzias A. Cumulative live birth rates after in vitro fertilization. *N Engl J Med* 2009;**360**:236–243.

McLernon D, Maheshwari A, Lee AJ, Bhattacharya S. Cumulative live birth rates after one or more complete cycles of IVF: a population-based study of linked cycle data from 178,898 women. *Hum Reprod* 2016;**31**:572–581.

Olivius K, Friden B, Lundin K, Bergh C. Cumulative probability of live birth after three in vitro fertilization/intracytoplasmic sperm injection cycles. *Fertil Steril* 2002;**77**:505–510.

Pinborg A, Hougaard CO, Nyboe Andersen A, Molbo D, Schmidt L. Prospective longitudinal cohort study on cumulative 5-year delivery and adoption rates among 1338 couples initiating an ART program (Copenhagen Multicentre Psychosocial Infertility Research Programme (COMPI)). *Hum Reprod* 2009;**24**:991–999.

Schorsch M, Gomez R, Hahn T, Hoelscher-Obermaier J, Seufert R, Skala C. Success rate of inseminations dependent on maternal age? An analysis of 4246 insemination cycles. *Geburtsh Frauenheilk* 2013;**73**:808–811.

Smeenk JM, Verhaak CM, Stolwijk AM, Kremer JA, Braat DD. Reasons for dropout in an in vitro fertilization/intracytoplasmic sperm injection program. *Fertil Steril* 2004;**81**:262–268.

Stern JE, Brown MB, Luke B, Wantman E, Lederman A, Missmer SA, Hornstein MD. Calculating cumulative live birth-rates from linked cycles of assisted reproductive technology (ART): data from the Massachusetts SART CORS. *Fertil Steril* 2010;**94**:1334–1340.

Steures P, van der Steeg JW, Hompes PG, Habbema JD, Eijkemans MJ, Broekmans FJ, Verhoeve HR, Bossuyt PM, van der Veen F, Mol BWJ. Intrauterine insemination with controlled ovarian hyperstimulation versus expectant management for couples with unexplained subfertility and an intermediate prognosis: a randomised clinical trial. *Lancet* 2006;**368**:216–221.

Steures P, van der Steeg JW, Hompes PG, van der Veen F, Mol BW. Intrauterine insemination in The Netherlands. *Reprod Biomed Online* 2007;**14**:110–116.

Sundström I, Ildgruben A, Högberg U. Treatment related and treatment independent deliveries among infertile couples, a long-term follow-up. *Acta Obstet Gynecol Scand* 1997;**76**:238–243.

Toftager M, Bogstad J, Bryndorf T, Løssl K, Roskær J, Holland T, Prætorius L, Zedeler A, Nilas L, Pinborg A. Risk of severe ovarian hyperstimulation syndrome in GnRH antagonist versus GnRH agonist protocol: RCT including 1050 first IVF/ICSI cycles. *Hum Reprod* 2016;**31**:1253–1264.

Supplemental data paper I

Supplementary Table I. Total number of received insemination treatments within 2 years from starting fertility treatments with homologous gametes, Denmark 2007-2010, stratified on type of first treatment.

N, total IUI treatments	First treatment with IUI		First treatment with ART	
	N, couples	%	N, couples	%
0	0	0.0	7191	97.2
1	3657	29.3	109	1.5
2	2723	21.8	53	0.7
3	3026	24.2	21	0.3
4	1518	12.2	11	0.1
5	786	6.3	5	0.1
6	417	3.3	5	0.1
7	172	1.4	1	0.0
8	87	0.7	0	0.0
9	39	0.3	0	0.0
10	25	0.2	0	0.0
11	19	0.2	0	0.0
12	12	0.1	0	0.0
13	4	0.0	0	0.0
14	0	0.0	0	0.0
15	1	0.0	0	0.0
16	1	0.0	0	0.0
17	1	0.0	0	0.0
Total	**12,488**	**100.0**	**7396**	**100.0**

Supplementary Table II. Predictors of live birth after treatment or natural conception within 2 years from first fertility treatment with homologous gametes in Denmark 2007–2010

	Livebirth within 2 years from 1st treatment with IUI		Livebirth within 2 years from 1st treatment with ART	
	AOR[1] [95%CI]	P-value	AOR[1] [95%CI]	P-value
Female age <35 years	0.99 [0.98 – 1.01]	0.47	0.96 [0.94 – 0.98]	<0.0001
Female age 35–39 years	0.84 [0.80 – 0.87]	<0.0001	0.85 [0.81 – 0.89]	<0.0001
Female age >=40 years	0.63 [0.55 – 0.72]	<0.0001	0.67 [0.59 – 0.76]	<0.0001
BMI<20	1.11 [1.01-1.23]	0.039	1.17 [1.03 – 1.33]	0.017
BMI 20 – 29	0.96 [0.94 – 0.97]	<0.0001	0.95 [0.94 – 0.97]	<0.0001
BMI >=30	0.93 [0.91 – 0.96]	<0.0001	0.94 [0.90 – 0.99]	0.016
Non-smokers	1.0 (ref)	0.009	1.0 (ref)	<0.0001
Smokers	0.85 [0.76 – 0.96]		0.62 [0.53 – 0.72]	
Primary infertility	1.0 (ref)	0.7	1.0 (ref)	0.6
Secondary infertility	1.02 [0.92 – 1.14]		0.97 [0.84 – 1.11]	
Cause of infertility				
Idiopathic	1.0 (ref)	0.001	1.0 (ref)	0.001
Male	0.98 [0.87 – 1.09]	0.7	1.24 [1.07 – 1.44]	0.005
Combined male/female factor	0.87 [0.70 – 1.08]	0.2	1.01 [0.80 – 1.28]	0.9
Anovulation	1.31 [1.15 – 1.50]	<0.0001	1.57 [1.18 – 2.11]	0.002
Ovarian	0.98 [0.70 – 1.36]	0.9	1.28 [0.85 – 1.92]	0.2
Endometriosis	0.89 [0.55 – 1.44]	0.6	1.29 [0.94 – 1.78]	0.1
Tubal factor	0.67 [0.49 – 0.92]	0.014	1.14 [0.95 – 1.37]	0.2
Other female factors	0.94 [0.66 – 1.35]	0.7	0.67 [0.41 – 1.08]	0.1
Two female factors	N/A		1.35 [0.39 – 4.69]	0.6

[1]Adjusted Odds Ratio, adjusted for female age (linear spline with break point at 35 and 40 years), BMI (linear spline with break point at BMI 20 and 30), course of treatments (categorical), smoking (yes/no), secondary infertility (yes/no) & cause of infertility (including idiopathic infertility, combined male/female factor infertility, male factor infertility, endometriosis, other female factors, ovarian factors, anovulation, two female factors & tubal factor infertility).

Supplementary Table III. Stratified live birthrates by patient 2 years after first treatment with intrauterine insemination with homologous gametes

	Female age <35					Female age >=35				
	N, total couples	IUI-conception livebirths [95% CI]	ART-conception livebirths [95% CI]	NC livebirths [95% CI]	Total livebirths [95% CI]	N, total couples	IUI-conception livebirths [95% CI]	ART-conception livebirths [95% CI]	NC livebirths [95% CI]	Total livebirths [95% CI]
Overall cause of infertility										
Female factor	2042	42.2 [40.1–44.4]	14.9 [13.4–16.5]	10.8 [9.5–12.3]	67.9 [65.8–69.9]	433	25.2 [21.1–29.5]	9.7 [7.1–12.9]	6.7 [4.5–9.5]	41.6 [36.9–46.4]
Male factor	1773	31.9 [29.8–34.2]	20.2 [18.4–22.2]	8.5 [7.2–9.9]	60.6 [58.3–62.9]	706	25.1 [21.9–28.4]	12.9 [10.5–15.6]	7.4 [5.5–9.5]	45.3 [41.6–49.1]
Combined	365	34.0 [29.1–39.1]	18.1 [14.3–22.4]	10.4 [7.5–14.0]	62.4 [57.3–62.9]	137	17.5 [11.6–24.9]	16.8 [10.9–24.1]	13.9 [8.6–20.8]	48.2 [39.6–56.9]
Idiopathic	4573	38.4 [36.9–39.8]	16.3 [15.2–17.4]	11.3 [10.4–12.2]	65.9 [64.5–67.3]	207	26.6 [24.8–28.4]	10.5 [9.3–11.8]	8.4 [7.4–9.6]	45.5 [43.5–47.5]
Specified cause of female infertility										
Anovulation*	1524	45.3 [42.8–47.9]	14.8 [13.1–16.7]	11.0 [9.5–12.7]	71.2 [68.8–73.5]	189	31.7 [25.2–38.9]	6.9 [3.7–11.5]	7.9 [4.5–12.8]	46.6 [39.3–53.9]
Tubal factor*	130	23.8 [16.8–32.1]	15.4 [9.7–22.8]	11.5 [6.6–18.3]	50.8 [41.9–59.6]	84	22.6 [14.2–33.0]	16.7 [9.4–26.4]	7.1 [2.7–14.9]	46.4 [35.5–57.6]
Endometriosis*	69	33.3 [22.4–45.7]	23.2 [13.9–34.9]	8.7 [3.3–18.0]	65.2 [52.8–76.3]	18	5.6 [0.1–27.3]	16.7 [3.6–41.4]	5.6 [0.1–27.3]	27.8 [9.7–53.5]
Female characteristics										
BMI <30	6163	37.8 [36.6–39.0]	18.2 [17.2–19.2]	10.5 [9.7–11.3]	66.5 [65.3–67.7]	2387	26.7 [24.9–28.5]	12.7 [11.4–14.0]	8.3 [7.2–9.4]	47.8 [45.8–49.8]
BMI >=30	743	37.7 [34.2–41.2]	10.1 [7.9–12.3]	9.4 [7.3–11.5]	57.2 [53.6–60.8]	313	23.3 [18.6–28.0]	7.3 [4.4–10.2]	9.6 [6.3–12.9]	40.3 [34.9–45.7]
Smoking, no	7632	38.2 [37.1–39.3]	17.1 [16.3–17.9]	10.5 [9.8–11.2]	65.2 [64.1–66.3]	3288	25.5 [24.0–27.0]	10.9 [9.8–12.0]	8.1 [7.2–9.0]	44.6 [42.9–46.3]
Smoking, yes	1121	35.0 [32.2–37.8]	15.3 [13.2–17.4]	10.9 [9.1–12.7]	61.1 [58.3–64.0]	442	27.6 [23.4–31.8]	12.2 [9.2–15.3]	9.5 [6.8–12.2]	49.3 [44.6–54.0]
Primary infertility	6752	36.5 [35.4–37.7]	18.6 [17.7–19.5]	9.8 [9.1–10.5]	64.9 [63.8–66.0]	2192	23.0 [21.2–24.8]	12.7 [11.3–14.1]	6.8 [5.8–7.9]	42.6 [40.5–44.7]
Secondary infertility	2001	42.1 [39.9–44.3]	10.9 [9.5–12.3]	13.1 [11.6–4.6]	66.2 [64.1–68.3]	1538	29.7 [27.4–32.0]	8.8 [7.4–10.2]	10.3 [8.8–11.8]	48.8 [46.3–51.3]

*Subgroup of female factor, not all subgroups are reported. NC: natural conceptions.

Supplementary Table IV. Stratified live birthrates by patient 2 years after first treatment with assisted reproductive technology with homologous gametes

	Female age <35					Female age >=35				
	N, total couples	ART-conception birthrate [95% CI]	IUI-conception birthrate [95% CI]	NC birthrate [95% CI]	Total birthrate [95% CI]	N, total couples	ART-conception birthrate [95% CI]	IUI-conception birthrate [95% CI]	NC birthrate [95% CI]	Total birthrate [95% CI]
Overall cause of infertility										
Female factor	1214	51.8 [49.0–54.7]	0.9 [0.5–1.6]	7.4 [6.0–9.0]	60.1 [57.3–62.9]	639	32.6 [28.9–36.3]	0.0 [0.0–0.6]	4.5 [3.1–6.5]	37.1 [33.3–41.0]
Male factor	2304	56.3 [54.2–58.3]	0.3 [0.1–0.6]	5.0 [4.2–6.0]	61.5 [59.5–63.5]	850	37.3 [34.0–40.6]	0.4 [0.1–1.0]	5.2 [3.8–6.9]	42.5 [39.1–45.9]
Combined	292	49.7 [43.8–55.5]	1.4 [0.4–3.5]	8.2 [5.3–12.0]	59.2 [53.4–64.9]	235	25.5 [20.1–31.6]	0.0 [0.0–1.6]	8.5 [5.3–12.8]	34.0 [28.0–40.5]
Idiopathic	1047	49.7 [46.6–52.7]	1.2 [0.7–2.1]	7.1 [5.6–8.8]	58.0 [54.9–61.0]	798	28.6 [25.5–31.8]	0.3 [0.0–0.9]	5.0 [3.6–6.8]	33.8 [30.6–37.2]
Specified cause of female infertility										
Anovulation*	257	55.6 [49.3–61.8]	3.5 [1.6–6.5]	7.8 [4.8–11.8]	66.9 [60.8–72.6]	50	38.0 [24.7–52.8]	0.0 [0.0–7.1]	2.0 [0.1–10.6]	40.0 [26.4–54.8]
Tubal factor*	614	50.3 [46.3–54.4]	0.2 [0.0–0.9]	7.5 [5.5–9.9]	58.0 [54.0–61.9]	409	35.5 [30.8–40.3]	0 [0.0–0.9]	4.4 [2.6–6.9]	39.9 [35.1–44.8]
Endometriosis*	197	51.3 [44.1–58.4]	0.5 [0.0–2.8]	9.6 [5.9–14.7]	61.4 [54.2–68.3]	41	39.0 [24.2–55.5]	0 [0.0–8.6]	4.9 [0.6–16.5]	43.9 [28.5–]
Female characteristics										
BMI <30	3752	54.6 [53.0–56.2]	0.8 [0.5–1.1]	6.4 [5.6–7.2]	61.8 [60.3–63.4]	1752	35.7 [33.5–37.9]	0.2 [0–0.4]	5.4 [4.3–6.5]	41.3 [39.0–43.6]
BMI >=30	412	46.1 [41.3–50.9]	0.5 [0–1.2]	4.6 [2.6–6.6]	51.2 [46.4–56.0]	230	26.5 [20.8–32.2]	0 [0–0.02]	4.8 [2.0–7.6]	31.3 [25.3–37.3]
Smoking, no	4250	54.6 [53.1–56.1]	0.7 [0.5–1.0]	6.4 [5.7–7.1]	61.8 [60.3–63.3]	2216	32.1 [30.2–34.0]	0.2 [0–0.4]	5.6 [4.6–6.6]	37.9 [35.9–39.9]
Smoking, yes	607	44.2 [40.3–48.2]	0.7 [0–1.4]	4.9 [3.2–6.6]	49.8 [45.8–53.8]	306	33.0 [27.7–38.3]	0 [0–0.01]	3.3 [1.3–5.3]	36.3 [30.9–41.7]
Primary infertility	4088	54.2 [52.6–55.7]	0.7 [0.4–1.0]	5.9 [5.2–6.6]	60.8 [59.3–62.3]	1720	31.4 [29.2 –33.6]	0.1 [0–0.3]	4.8 [3.8–5.8]	36.3 [34.0–38.6]
Secondary infertility	769	48.8 [45.3–52.3]	0.5 [0–1.0]	8.3 [6.4–10.3]	57.6 [54.1–61.1]	802	34.0 [30.7–37.3]	0.4 [0–0.8]	6.4 [4.7–8.1]	40.8 [37.4–44.2]

*Subgroup of female factor, not all subgroups are reported. IUI: intrauterine insemination, ART: assisted reproductive technology, NC: natural conception.

Supplementary Table V. Multiple rates in treatment-related deliveries, with at least one live born child, at 2 years follow-up in couples receiving fertility treatments with homologous gametes, Denmark 2007-2010, stratified by type of conception.

All couples	Deliveries, N total	Singleton	Twin	Triplet	Quadruplet
IUI-conception, N % [95%CI]	4307	3811 88.5% [87.5 – 89.4]	486 11.3% [10.4 – 12.3]	10 0.2% [0.1 – 0.4]	0 0.0% [0.0 – 0.01]
ART-conception, N % [95%CI]	5297	4545 85.8% [84.8–86.7]	743 14.0% [13.1–15.0]	8 0.2% [0.1–0.3]	1 0.0 % [0.0 –0.1]

IUI: intrauterine insemination, ART: assisted reproductive technology

Paper II

May 6th, 2017

Cumulative live birth rate prognosis based on the number of aspirated oocytes in previous ART cycles

Authors

S S Malchau, A A Henningsen, J Forman, A Loft, A Nyboe Andersen, A Pinborg

S S Malchau, M.D., Dept. of Obstetrics and Gynecology , Copenhagen University Hospital Hvidovre, Kettegård Allé 26, DK-2650 Hvidovre, Denmark.
A A Henningsen, M.D., Fertility Clinic, Copenhagen University Hospital Rigshospitalet, Blegdamsvej 9, DK-2100 KBH Ø, Denmark.
A Loft, Senior Consultant, Fertility Clinic, Copenhagen University Hospital Rigshospitalet, Blegdamsvej 9, DK-2100 KBH Ø, Denmark.
J L Forman, Associate Professor, University of Copenhagen, Dept. of Public Health, Section of Biostatistics, Øster Farimagsgade 5, DK-1014 KBH K, Denmark.
A Nyboe Andersen, Professor, Fertility Clinic, Copenhagen University Hospital Rigshospitalet, Blegdamsvej 9, DK-2100 KBH Ø, Denmark.
A Pinborg, Professor, Dept. of Obstetrics and Gynecology , Copenhagen University Hospital Hvidovre, Kettegård Allé 26, DK-2650 Hvidovre, Denmark.

Corresponding author: smalchau@hotmail.com
Sara Sofia Malchau, Abildgaardsvej 131, DK-2830 Virum, Denmark.
Phone: +4540548954

ABSTRACT

Study question

Is the number of aspirated oocytes in the first assisted reproductive technology (ART) cycle associated with the cumulative live birthrates (CLBR) in subsequent cycles?

Summary answer

The number of aspirated oocytes in the first cycle was associated with CLBR in subsequent cycles. Previous treatment response predicts outcome in future cycles.

What is known already?

Previous reports have shown a positive association between the number of retrieved oocytes and live birthrate per fresh treatment cycle. This has also been shown for the CLBR in one complete ART-cycle, including possible subsequent frozen-thawed transfers (FER). It has been shown that women with less than 5 oocytes in the first cycle have poorer outcome within six complete cycles than women with more than 12 oocytes, suggesting that the number of aspirated oocytes in the first cycle may be reproduced in later cycles. However, other studies have shown that an initial low treatment response may be improved with increased gonadotrophin start-dose.

Study design, size, and duration

The Danish National IVF-registry includes all ART treatments in public and private clinics since 1994. Treatment-cycles were cross-linked with the Medical Birth Registry, identifying treatment-related births and natural conception births. This national cohort study includes all women starting ART treatments with homologous eggs between 2002 and 2011, N=30,486. Subjects were followed for up to four fresh ART-cycles including subsequent FER-cycles (=four complete cycles), until the first livebirth, or until December 2011.

Participants/materials, setting, methods

The CLBR within 1-4 complete ART-cycles were calculated as the proportion of women with a livebirth, out of all women initiating ART-treatment, including drop-outs (no livebirth or no continued treatment within follow-up). In women with complete follow-up, multivariate logistic regression analysis assessed impact of retrieved oocytes on CLBR, adjusting results for female age and cause of infertility. Hospital admission due to ovarian hyperstimulation syndrome (OHSS) was reported.

Main results and the role of chance

After one, two and three complete ART-cycles, the CLBR after ART-conception were 26.4% [95%CI 25.9–26.9], 42.6% [42.0–43.1] and 51.3% [50.7–51.9], respectively. The CLBR after non-ART related conception were 5.3% [5.0–5.6], 8.3% [8.0–8.7] and 10.6% [10.3–11.0], after one, two, and three complete cycles. In women without a live birth in the first complete cycle, the number of aspirated oocytes predicted the outcome in the 2nd and 3rd cycle: For women with 0–7 oocytes in the first cycle, the adjusted odds ratio (AOR) for live birth in the 2nd and 3rd cycle increased with 3.7% [1.5–6.0] per increasing oocyte. For women with 8–12 aspirated oocytes, the AOR for livebirth increased with 4.1% [1.6–6.6] per increasing oocyte, and for women with >12 oocytes the AOR did not increase with additional oocytes. For women without livebirth in the 1st and 2nd cycle, the sum of aspirated oocytes predicted outcome in the 3rd complete cycle. Compared to women with a sum of 20-30 oocytes, women with 0-6 oocytes had decreased odds for live birth in the 3rd cycle, AOR 0.59 [0.46–0.75]. Incidence of hospital-admission due to OHSS was 1.7% in the 1st cycle, decreasing to 1.3% and 1.0% in the 2nd and 3rd cycles.

Limitations, reasons for caution

Although mandatory, there may be treatment-cycles not registered in the IVF-registry.

Wider implications of the findings

With these results we can counsel couples returning for fertility treatments, providing an age-stratified revised prognosis for chances of live birth and risk of OHSS, reflecting prior failed attempts and previous ovarian response.

Trial registration number

The study was approved by the Danish Data Protection Agency (J.nr. 2012-41-1330).

INTRODUCTION

Fertility treatment is demanding. The couples face side effects, financial burdens and emotional strain (1,2). Qualified information, on individualized success-rates and predictors of outcome, is an important tool in adjusting the couples' expectations and guiding them through the treatment journey. Providing an individual prognosis requires that success estimates are based on complete treatment courses and not only based on single treatment cycles. Thus the outcome in previous cycles can be used to adjust the prognosis during the course of treatments.

Previous studies have shown that the number of aspirated oocytes is a predictor for treatment outcome. Live birthrates per fresh treatment cycle increase with the number of aspirated oocytes, up to 15 oocytes (3–5). A UK study from 2016 showed that women with more than 12 aspirated oocytes in the first cycle have increased odds of a live birth within 6 complete cycles (6 fresh cycles with frozen-thawed transfers), compared to women with less than 5 aspirated oocytes in the first cycle (6). This may indicate that a low treatment response in the first cycle is probable to be repeated in later cycles. However, there are studies that describe a positive effect on number of aspirated oocytes with increased follicle stimulating hormone (FSH) start dose in the second cycle, in women with a low treatment response in the first cycle (7,8).

In Denmark, fertility treatments are reimbursed in women below 40 years of age, though the costs for medication are only partly covered. Couples with no common children and childless single women are offered three fresh ART treatments including potential subsequent frozen-thawed transfers. All treatments are registered in a mandatory registry, to monitor treatment activity and outcome. Thus the Danish ART registry comprises all couples and single women, who have received ART treatments, with information on the complete treatment history.

The purpose of the present study was to provide couples in ART treatment with a prognosis that is adjusted according to the treatment response in their previous ART cycles. The primary objective

was to assess the association between the number of aspirated oocytes in the first cycle and the cumulated chances of live birth in the 2nd and 3rd fresh ART-cycles and subsequent frozen-thawed transfers, in women receiving ART treatments with homologous eggs, irrespective of the source of sperm. The second objective was to assess changes in number of aspirated oocytes between the first and second stimulated ART-cycle. Additionally, we wanted to estimate cumulative live birthrates after ART treatment and natural conception.

MATERIAL AND METHODS

This national cohort study is based on the Danish ART registry, the Danish Medical Birth registry and the National Patient registry. Registration of all ART treatments has been mandatory for public and private fertility clinics since 1994 and insemination treatments are included since 2006. Fertility treatments performed in the same women were identified through a personal identification number, and follow-up on treatment outcome and information on livebirths after natural conception was achieved through cross-linking of fertility treatment-cycles with date of birth in the Medical Birth registry. The Medical Birth registry holds information on gestational age at birth, which was used to determine the time of conception. If the time of conception did not match an ART-cycle, the birth was defined as unrelated to ART-treatment. For women receiving treatment after January 1st 2006, it was possible to determine if the non-ART related livebirth was due to insemination-treatment or natural conception.

The study included all Danish residents who had their first ART-treatment from January 1st 2002 to December 31st 2011. Follow-up on the births were available until December 31st 2012. Women were censored if follow-up was less than one year from the start-date of their last treatment. Natural conceptions were reported if they were the first delivery after treatment-start and within the study period (prior to December 31st, 2011), but regardless of time interval since first treatment. Treatments with both homologous and donated semen were included, but women with one or more oocyte donation treatments were excluded (N=631 women). The women were either in a heterosexual- or same-sex relationship or single. The cohort consisted of 30,486 women and 94,025 cycles. Women were excluded from further analysis after the first treatment-related or treatment-independent livebirth, hence couples returning for further treatment after a livebirth only contributed to the CLBR with their first live birth. Likewise, women with a naturally conceived livebirth following a treatment-related birth, only contributed to birthrates with their first live birth.

One complete treatment cycle is defined as an ovarian stimulation including all fresh and frozen-thawed transfers derived from that ovarian stimulation. Women with no livebirth and no continued treatment within follow-up were considered drop-outs. For cycle number two or more, we assumed that the embryo used in a frozen-thawed transfer originated from the most recent ovarian stimulation. According to recommendation by the Danish health authorities, all frozen embryos should be used before proceeding to the next ovarian stimulation (9). Information on emigration was not available.

Information on hospital admission due to ovarian hyper stimulation syndrome (OHSS) was retrieved from the National Patient registry. Thus, diagnosis of OHSS was only reported for moderate or severe cases of OHSS.

Statistical analyses

Descriptive statistics were summarized as numbers and percentages. Changes in number of aspirated oocytes between the first and second cycle were compared with analysis of variance with Bonferroni correction. Cumulated live birthrates were reported with 95% confidence intervals per complete ART-cycle (including frozen-thawed transfers) and further accumulated over 1–4 complete cycles, as the proportion of women with a livebirth, out of all women starting their first fresh treatment, including drop-outs. These cumulated live birth rates are conservative estimates, based on observed livebirths without censoring of couples that discontinue treatments.

The livebirth rates in the 1st to 4th cycle were stratified according to female age and number of aspirated oocytes (0–3, 4–9, 10–15, >15). In women with at least one retrieved oocyte in the first cycle, odds of livebirth in the 1st complete cycle was assessed with multivariable logistic regression analysis including the predictors: number of retrieved oocytes (linear spline with break points at 7

and 12 oocytes), female age (linear spline with break points at 35 and 40 years) and cause of infertility (categorical).

The association between number of aspirated oocytes in the 1st cycle and treatment success within the 2nd and 3rd complete cycles was likewise assessed with multivariable logistic regression analysis, including number of retrieved oocytes in the 1st cycle (linear spline), female age (linear spline), and cause of infertility as predictors.

The association between livebirth in the 3rd complete cycle and the sum of retrieved oocytes in the 1st and the 2nd fresh ART-cycle was assessed with multivariable logistic regression analysis including the sum of retrieved oocytes in 1st and 2nd cycle (6 categories), cause of infertility, and female age (linear spline) as predictors.

Data management and statistical analyses were performed with Statistical software SAS version 9.4 and IBM SPSS statistics version 19.

Etical approval

In Denmark register-based studies do not require approval from an ethics committee. The project was approved by the Danish Data Protection Agency (J.nr 2012-41-1330).

RESULTS

The median follow-up time in the cohort was 72 months (interquartile range 45–99). Background and treatment related characteristics are shown in Table I. The mean age in women starting their first fresh ART treatment was 33.1±4.9 years, and two thirds of the women starting their first treatment (65%) was less than 35 years old at the time of their first treatment.

Data on number of retrieved oocytes was available for 69.7% and missing for 30.3% of all fresh cycles. Female age, live birthrates and incidence of OHSS were similar in women with valid and missing information on number of aspirated oocytes (supplemental Table SI). The most prevalent treatment response was 4–9 retrieved oocytes, almost half of the women with an aspiration in the first fresh cycle had this outcome, while 25% had 10–15 oocytes, 19% had 0–3 oocytes and 10% had >15 oocytes.

Course of treatments and treatment outcome is shown in Figure 1. In women starting the first complete ART cycle, 26.4% [95%CI 25.9–26.9] had a livebirth after the first fresh or a following frozen-thawed transfer. In women not achieving livebirth in the first ART cycle, and returning for repeated complete cycles, the live birthrate was 26.1% [25.5–26.8] in the 2nd complete cycle, decreased slightly to 23.6% [22.8–24.5] in the third complete cycle and was 22.1% [20.9–23.3] in the 4th cycle. After one, two, three and four complete cycles respectively, the observed cumulative live birthrates after ART-conception were 26.4% [25.9–26.9], 42.6% [42.0–43.1], 51.3% [50.7–51.9] and 55.4% [54.8–56.0], out of all women starting the first treatment.

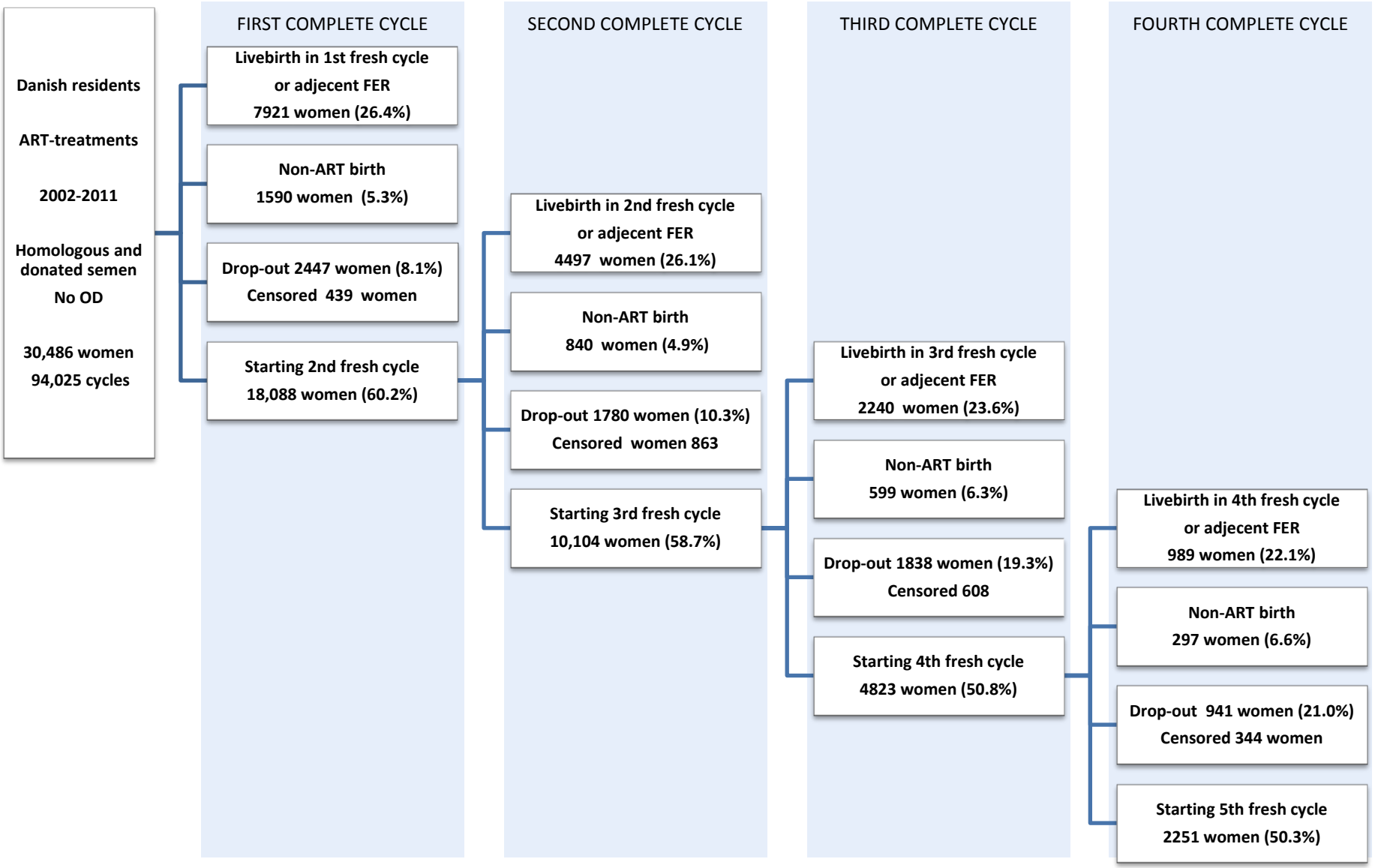
After one, two, three and four complete cycles, the cumulated live birthrates after non-ART conception (natural conception and intrauterine insemination, IUI) were 5.3% [5.0 – 5.6], 8.3% [8.0–8.7], 10.6% [10.3–11.0] and 11.8% [11.4–12.2], respectively. The median time interval between 1st treatment and non-ART birth was 20 months (interquartile range, IQR 13-31).

Table I, Background- and treatment characteristics in women starting 1st to 4th fresh ART cycle, 2002 – 2011

	1st fresh ART cycle N (%)	2nd fresh ART cycle N (%)	3rd fresh ART cycle N (%)	4th fresh ART cycle N (%)
Starting ovarian stimulation	30,486 (100)	17,500[1] (100)[1]	10,104[2] (100)	4569[3] (100)
Aspiration	29,741 (97.6)	17,274 (98.7)	9585 (98.8)	4536 (99.3)
Transfer	25,844 (84.8)	15,916 (90.6)	8935 (92.1)	4287 (93.8)
Donated semen[4]				
Yes	1137 (4.3)[5]	747 (5.0)[5]	457 (5.6)[5]	235 (6.5)[5]
No	22,990 (87.1)[5]	13,358 (89.6)[5]	7244 (89.4)[5]	3231 (88.9)[5]
No attempted fertilization	2277 (8.6)[5]	808 (5.4)[5]	406 (5.0)[5]	167 (4.6)[5]
Number of retrieved oocytes[6]				
0–3	3920 (18.7)[5]	1824 (15.2)[5]	1031 (15.7)[5]	498 (15.8)[5]
4–9	9791 (46.6)[5]	5841 (48.6)[5]	3191 (48.6)[5]	1533 (48.6)[5]
10–15	5279 (25.1)[5]	3321 (27.6)[5]	1812 (27.6)[5]	857 (27.2)[5]
>15	2026 (9.6)[5]	1032 (8.6)[5]	536 (8.2)[5]	266 (8.4)[5]
Ovarian Hyper stimulation syndrome	517 (1.7)	224 (1.3)	95 (1.0)	40 (0.9)
Female age at 1st treatment				
Age <35 years	19,828 (65.0)	11,061 (63.2)	5865 (60.5)	2771 (60.6)
Age 35–40 years	7976 (26.2)	4802 (27.4)	2830 (29.2)	1371 (30.0)
Age >=40 years	2643 (8.7)	1619 (9.3)	993 (10.2)	423 (9.3)
Mean age (years) ±SD	33.1 ±4.9	33.4 ±4.9	33.7 ±4.9	33.6 ±4.8
Overall cause of infertility				
Female	8562 (28.1)	4826 (27.6)	2606 (26.9)	1314 (28.8)
Male	10,827 (35.5)	6412 (36.6)	3484 (35.9)	1618 (35.4)
Combined	2079 (6.8)	1263 (7.2)	750 (7.7)	289 (6.3)
Ideopathic	9003 (29.5)	4990 (28.5)	2854 (29.4)	1345 (29.5)
Specified cause of infertility				
Anovulation[7]	2055 (6.7)	1082 (6.2)	527 (5.4)	247 (5.4)
Tubal factor[7]	4052 (13.3)	2324 (13.3)	1279 (13.2)	691 (15.1)

[1]588 women were censored in the first treatment of the second complete cycle. [2]411 women were censored in the first treatment of the third complete cycle. [3]254 women were censored in the first treatment of the fourth complete cycle. [4]Information was valid for 85.2% and missing for 14.8%. [5]Valid percent. [6]Information was valid for 69.7% and missing for 30.3%. [7]Subgroup of female factor, not all subgroups are reported.

Figure 1, Flow chart over included cases and their course of treatments, women followed until their first livebirth. Cases are censored if follow-up is less than one year from start-date of fresh or frozen-thawed treatment cycle, proportions are % of non-censored cases.



ART= assisted reproductive technology OD=oocyte donation FER= frozen embryo transfer

Information on IUI-conceptions was available for years 2006-2011. Data stratified on type of non-ART conception is displayed in supplementary Table SII. Within four cumulated complete ART cycles, 8.2% of all women starting ART had a livebirth after natural conception. The median time-interval between the first ART treatment and live birth after natural conception was 20 months (IQR 14-29). Within four cumulated cycles, 1.6% conceived after intrauterine insemination. More than half (51.8%) of the IUI-livebirths were conceived with donor semen and most likely represent couples starting treatments with ICSI and then moving on to IUI with donor semen. The median time interval between the 1st ART treatment and IUI conception was 11 months (IQR 6-10).

The cumulative proportion of women discontinuing treatment increased from 8.1% within one complete cycle to 14.5% within two cycles, 21.2% after three cycles and 24.8% after four cycles, however these results may be overestimated due to censoring of women at the end of follow-up.

Number of retrieved oocytes as a predictor of treatment outcome

Age-stratified live birthrates after the 1st, 2nd, 3rd and 4th complete cycle by number of aspirated oocytes in the corresponding cycle are shown in Figure 2 and Table II. Overall, the proportion of women with a livebirth increased with increasing number of aspirated oocytes. However, within the respective age-groups, the live birthrates were similar with 10–15 aspirated oocytes and >15 oocytes.

Cumulative live birthrates in the 1st complete ART cycle, by number of aspirated oocytes, are shown in figure 3. CLBR increased with increasing number of oocytes up to 16 oocytes, after which the birthrate seem to plateau, but confidence intervals were too wide to detect a possible decrease or increase beyond 16 oocytes. Adjusted odds ratios for live birth in the first complete cycle, in women with at least one aspirated oocyte, were assessed with multivariable logistic regression analysis with the number of aspirated oocytes as a continuous variable, to estimate the impact per added aspirated

oocyte. In women with 1–7 aspirated oocytes, odds for livebirth increased with 19.8% [16.8–22.8] for each added oocyte. In women with 8–12 aspirated oocytes, odds increased with 4.3% [2.1–6.6] for each added oocyte. In women with >12 aspirated oocytes, odds did not increase significantly by more oocytes, 0.3% [-1.2 – 1.7].

Figure 2, Live birthrates after the 1st, 2nd 3rd and 4th complete ART cycle (one fresh cycle including possible frozen-thawed transfers), by number of aspirated oocytes in the corresponding cycle, Denmark 2002-2011.

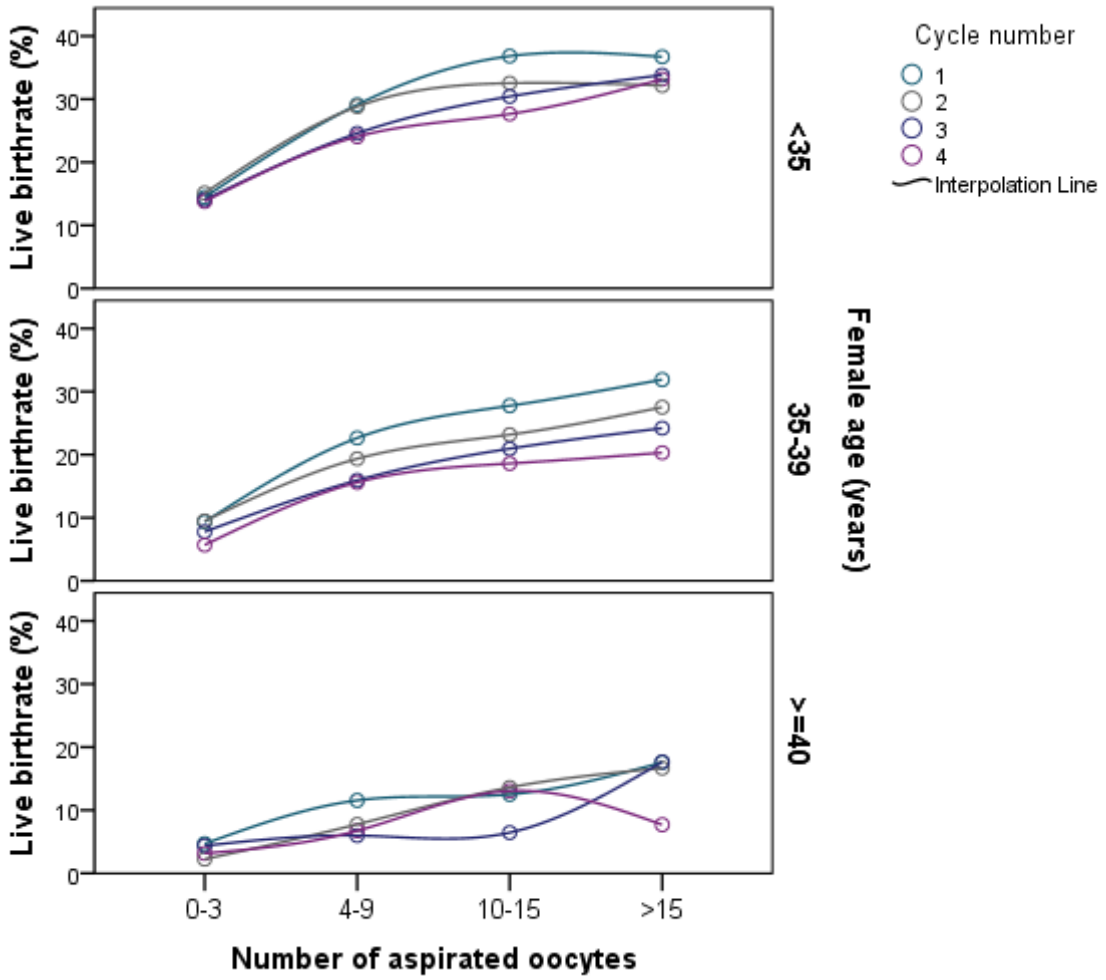
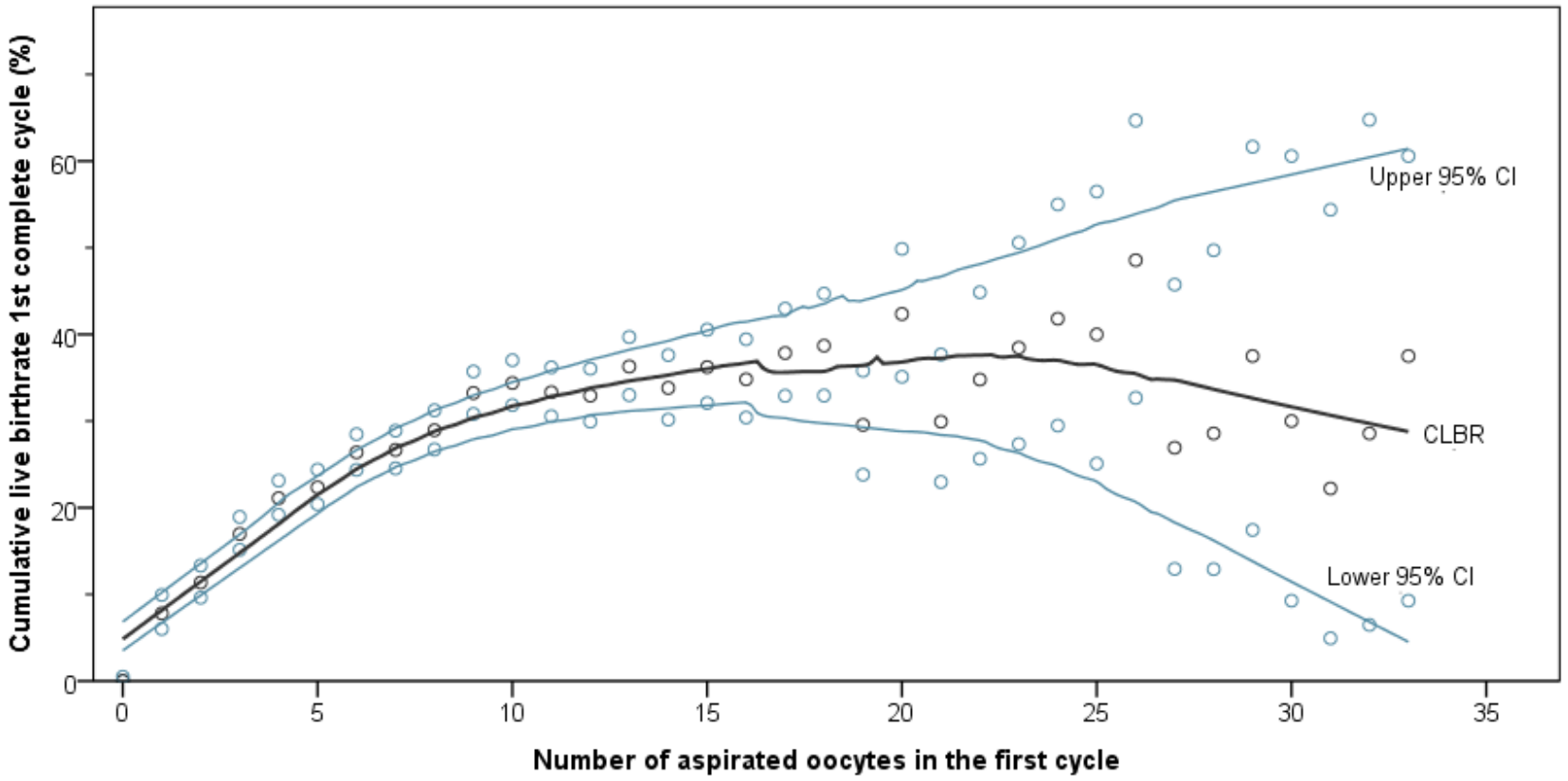


Table II, Live birthrates in 1st, 2nd, 3rd, and 4th complete[1] ART cycle by female age and number of aspirated oocytes, Denmark 2002-2011

Female age (years)	<35		35 – 40		>= 40	
No of retrieved oocytes	N, total	Livebirths %[95% CI]	N, total	Livebirths %[95% CI]	N, total	Livebirths %[95% CI]
First fresh cycle						
0-3	2039	14.7 [13.2–16.3]	1221	9.5 [8.0–11.2]	589	4.8 [3.3–6.7]
4-9	6170	29.7 [28.6–30.8]	2585	22.9 [21.4–24.6]	864	11.7 [9.7–14.0]
10-15	3728	37.7 [36.1–39.2]	1204	28.2 [25.8–30.8]	237	12.7 [8.9–17.3]
>15	1424	37.9 [35.4–40.5]	444	33.3 [29.1–37.8]	90	17.8 [11.0–26.6]
Second fresh cycle						
0-3	834	16.1 [13.7–18.7]	623	10.1 [7.9–12.7]	338	2.4 [1.1–4.4]
4-9	3528	31.0 [29.4–32.5]	1660	20.9 [19.0–22.9]	542	8.3 [6.2–10.9]
10-15	2280	34.5 [32.6–36.5]	787	24.9 [22.0–28.0]	183	14.2 [9.7–19.8]
>15	738	34.8 [31.5–38.3]	200	30.0 [24.0–36.6]	46	17.4 [8.6–30.2]
Third fresh cycle						
0-3	426	15.3 [12.1–18.9]	371	8.6 [6.1–11.8]	209	4.8 [2.5–8.3]
4-9	1838	26.5 [24.5–28.6]	964	17.4 [15.1–19.9]	304	6.9 [4.5–10.2]
10-15	1188	33.4 [30.8–36.1]	451	22.4 [18.7–26.4]	116	6.9 [3.3–12.6]
>15	371	37.5 [32.7–42.5]	115	26.1 [18.7–34.6]	31	19.4 [8.5–35.6]
Fourth fresh cycle						
0-3	215	14.9 [10.6–20.1]	191	6.3 [3.5–10.4]	82	3.7 [0.9–8.19]
4-9	851	26.3 [23.5–29.4]	486	17.1 [13.9–20.6]	160	7.5 [4.2–12.4]
10-15	576	31.3 [27.6–35.1]	193	20.7 [15.5–26.9]	60	15.0 [7.7–25.6]
>15	194	36.1 [29.6–43.0]	50	26.0 [15.4–39.2]	13	7.7 [0.8–30.7]

[1]One complete cycle defined as one fresh cycle and possible following frozen-thawed transfers

Figure 3, Cumulative live birthrates (CLBR) with 95% confidence intervals in the first fresh ART cycle with possible adjacent frozen-thawed transfers, by number of aspirated oocytes, Denmark 2002-2011



Adjusted odds for livebirth in the 2nd or 3rd complete cycles, by number of aspirated oocytes in the first cycle are shown in Table III. In women who did not achieve live birth in the first cycle, the number of aspirated oocytes in the first cycle predicted outcome in the 2nd and 3rd complete cycle. The odds for live birth in the 2nd and 3rd cycle increased with 3.7% [1.5–6.0] for each increasing oocyte from 0 up to 7 aspirated oocytes in the 1st cycle. For women with 8–12 aspirated oocytes in the 1st cycle, the odds increased with 4.1% [1.6–6.6] for each increasing oocyte, and for women with >12 oocytes the odds did not increase with additional oocytes, 1.0% [-0.8 – 2.7].

Table III, Adjusted[1] odds ratios for treatment related livebirth within 3 cumulated complete[2] cycles or in the 2nd or 3rd complete cycle, by number of aspirated oocytes in the first cycle, Denmark 2002-2011

Number of aspirated oocytes 1st cycle	Livebirth in 2nd or 3rd complete cycle Increase in odds of live birth per added aspirated oocyte in intervals, AOR[1] [95%CI]
0–7	1.037 [1.015–1.060]
8–12	1.041 [1.016–1.066]
>12	1.010 [0.992–1.027]

[1]AOR, adjusted odds ratio from multivariable logistic regression further including female age (spline with break points at 35 and 40 years) and cause of infertility (categories as in table 1) as predictors.
[2]One complete cycle defined as one fresh cycle and possible following frozen-thawed transfers

For women with no livebirth in the 1st and 2nd complete cycles, the sum of aspirated oocytes in the 1st and 2nd fresh cycle predicted outcome in the 3rd complete cycle, as seen in Table IV. Women with a sum of 0–6 aspirated oocytes, had decreased odds ratios for livebirth in the 3rd complete cycle, AOR was 0.59 [0.46–0.75] compared to women with a sum of 20–30 oocytes in the 1st and 2nd fresh cycle. For women with a sum of 7–12 oocytes in the two first fresh cycles, AOR was 0.78 [0.66–0.75] compared to women with a sum of 20–30 oocytes. There was no difference in outcome if the sum was over 30 oocytes, compared to women with the sum of 20–30 in the 1st and 2nd fresh cycle. The observed live birthrates in the 3rd complete cycle, in women with a sum of 20–30 aspirated oocytes in the two first fresh cycles, were 30.8% [27.9–33.8], 20.0% [16.1–24.3] and

9.5% [5.1–17.0] in women aged <35, 35–39 and >=40, respectively. For women with a sum of 0–6 oocytes in the first two cycles, the observed live birthrates in the 3rd complete cycle were 21.5% [17.3–26.4], 11.5% [8.2–16.0] and 5.6% [3.0–10.2] in women aged <35, 35–39 and >=40, respectively.

Table IV, Adjusted[1] odds ratios for livebirth after ART-conception in the 3rd complete[2] ART-cycle, by number of aspirated oocytes in the 1st and 2nd cycle, in women with no livebirth in the 1st or 2nd complete cycle, Denmark 2002-2011

Sum of aspirated oocytes 1st & 2nd cycle	Livebirth 3rd cycle AOR [95% CI]
0 – 6	0.59 [0.46 – 0.75]
7 – 12	0.78 [0.66 – 0.93]
13 – 19	0.94 [0.80 – 1.11]
20 – 30	1.0 (ref)
>30	1.08 [0.82 – 1.41]

[1]Multivaraible logistic regression analysis further including female age (linear spline with break point at 35 and 40 years) and cause of infertility as predictors. [2]One complete cycle is a fresh ART cycle with possible subsequent frozen-thawed transfers.

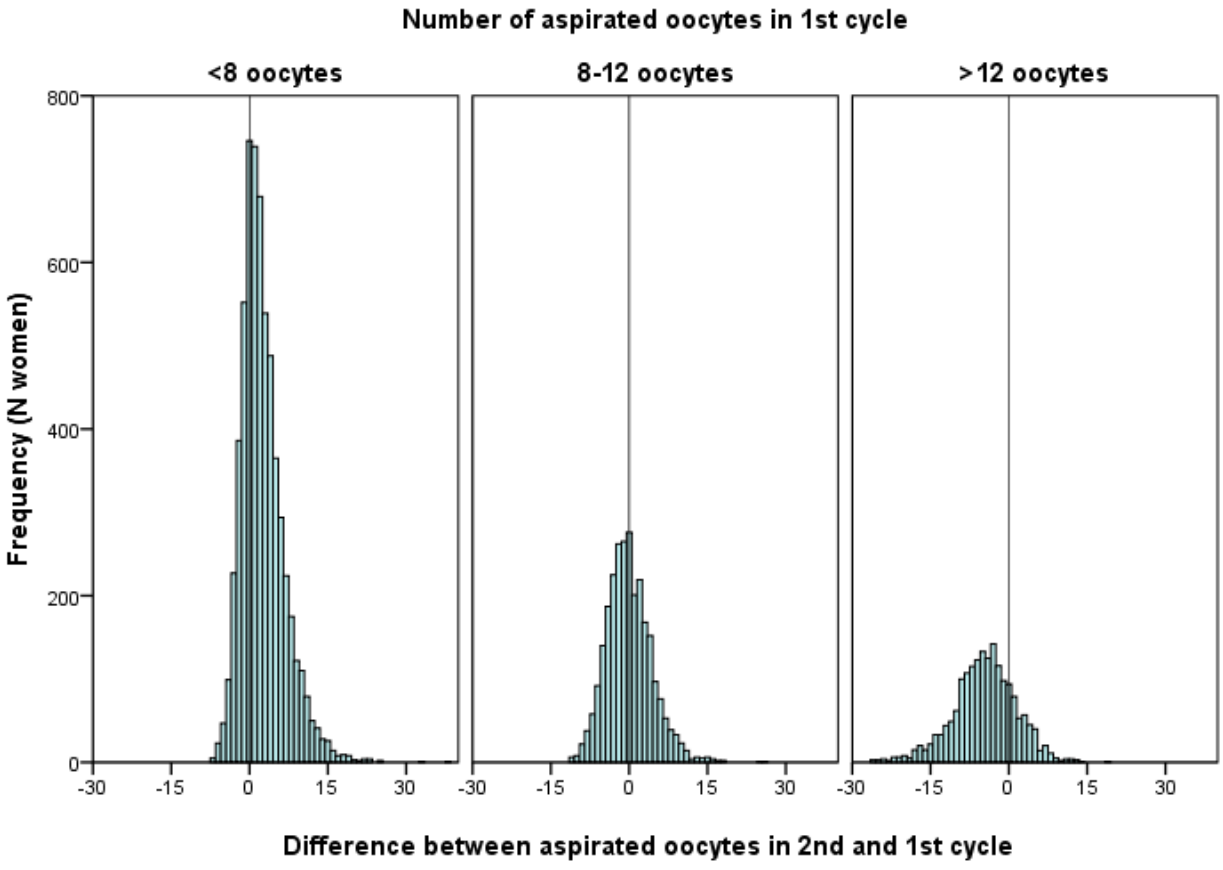
Dose adjustments in subsequent cycles

In women who returned for a second fresh cycle (who did not have a livebirth in the 1st cycle), we assessed the difference in the number of aspirated oocytes between the first and second cycle, as shown in Figure 4 and supplemental figures S1 and S2.

In women with <8 aspirated oocytes in the first cycle, the mean difference between the 1st and the 2nd cycle was +2.54 [95% CI 2.43–2.64] oocytes. In women with 8–12 aspirated oocytes in the first cycle, the mean difference was 0.00 [-0.16–0.17] oocytes between the 1st and 2nd cycle. In women with >12 oocytes, the mean difference was -4.67 [-4.95– (-4.39)] oocytes between the 1st and the 2nd cycle. The means differed significantly between the groups ($p<0.0001$).

For women who had 0–3 aspirated oocytes in the first cycle and aged <35 years, 26.7% [24.3–29.2] remained in this category in the 2nd cycle. For women aged 35–39 and >40 years, 41.0% [38.4–45.6] and 60.5% [55.8–65.7] remained in the 0–3 category in the 2nd cycle, respectively (supplemental figure S1).

Figure 4, Changes in number of aspirated oocytes in the second fresh ART cycle compared to the first fresh ART cycle, by initial treatment response, in women starting a minimum of two fresh ART cycles in Denmark 2002-2011



Incidence of OHSS

Overall, a total of 2.7% [2.5–2.9] of all women starting ART treatment had at least one episode with OHSS. Incidence of OHSS was 1.7% [1.6–1.9] in the first cycle, 1.3% [1.1–1.5] in the 2nd cycle, 1.0% [0.8–1.2] in the third cycle and 0.9% [0.6–1.2] in the 4th cycle (Table I). As expected the incidence of OHSS increased with higher number of aspirated oocytes (Supplemental Table SIII).

DISCUSSION

This national longitudinal cohort study assesses cumulative live birthrates within the first three complete ART-cycles. It shows that 51% of all couples starting treatments had a livebirth as a result of ART-treatments within the three cycles, 21% dropped out and 11% had a livebirth after non-ART conception. The number of aspirated oocytes in the first ART cycle was a predictor of outcome in the 2nd and 3rd cycle, the adjusted odds for live birth increased with an increasing number of oocytes up to 12 aspirated oocytes in the first cycle. Women with a poor treatment response in both the 1st and 2nd cycle had decreased odds for livebirth in the 3rd complete cycle. However, for women with 0–3 aspirated oocytes in the first cycle, the majority of women under 40 years of age had an improved treatment response in the 2nd cycle.

An association between number of aspirated oocytes and live birthrates has previously been demonstrated per fresh cycle (3,4,10,11) as well as per complete cycle (5,12). In a large cohort study, based on HFEA data, Sunkara et al demonstrated a strong association between increasing number of oocytes and live birthrates per fresh ART-cycle. Live birthrates increased up to 15 oocytes, then plateaued and further decreased when more than 20 oocytes were collected (3). Another large retrospective cohort study from the US, showed increasing live birthrates up to 15 oocytes (10). Common for these studies are that only fresh cycles were assessed and cycles with a freeze-all strategy (due to risk of OHSS) were excluded. In a Belgian cohort study of 1099 women that underwent a complete ART-cycle (including frozen-thawed transfers), women with more than 15 oocytes had higher live birthrates than women with 0-3, 4-9 or 10-15 oocytes (5). Our results indicate a plateau around 15-16 oocytes, even when possible frozen-thawed transfers are included in the birthrates. However, beyond 16 aspirated oocytes, we did not have enough power to detect a possible increase or decline. Moreover, results in frozen-thawed transfers have improved in recent years, which may alter the number of oocytes needed in order to optimize chances of livebirth (13–

15). Since a larger part of frozen-thawed transfers result in livebirth, one could speculate that fewer oocytes are needed to achieve livebirth, or one could speculate that this advancement gives even more value to each extra aspirated oocyte.

We found that the number of aspirated oocytes in the first cycle is associated with live birthrates in subsequent cycles. This may indicate that treatment response in the first cycle is likely to be reproduced, despite efforts to regulate a previous suboptimal response with dose-adjustments. However, previous studies have shown that it is possible to adjust ovarian response (7,8). A Danish retrospective study reported outcome in 385 patients returning for a 2nd ART cycle (after an unsuccessful 1st cycle) and showed improved number of aspirated oocytes when rFSH start-dose was increased in the 2nd ART cycle (8). Other studies have identified prognostic factors for the chances of an improved treatment response. A Swedish prospective cohort study found that the ovarian sensitivity index (number of retrieved oocytes/total FSH dose) is efficient in identifying high-, medium- and low responders, and is as a useful predictor for treatment outcome (16). Another Swedish study showed that a combination of Anti-Müllerian hormone, age and antral follicle count best predicted ovarian response (17). The IVF register do not include information on anti-Müllerian hormone and antral follicle count, however our results indicate that female age alone also predicts chances of an improved treatment response in the 2nd cycle. For women aged below 35 years, three in four had an improved treatment response in the 2nd cycle, but only 4 in 10 of women aged 40 and above.

Our results showed that in women who did not achieve livebirth in the first two complete cycles, and who had a low response to ovarian stimulation (0-3 oocytes) in both cycles, livebirth prognosis in the third complete cycle depended on maternal age. In women under 35, 1 in 5 achieved livebirth in the third complete cycle, despite two previous cycles with low ovarian response, which was true for only 5.6% of women aged 40 and older. According to the Bologna criteria, women with two

treatment cycles with less than 0-3 oocytes (despite maximal stimulation) are defined as poor responders (18). A Belgian study from 2014 compared 485 poor responders (defined by the Bologna criteria), aged over and under 40 years old, and found similar cumulated live birthrates in the two age groups within two cycles with ovarian stimulation (19). Similarly, in our study, confidence intervals were too wide to detect a significant difference between live birthrates in poor responders aged 35-39 and aged 40 or older, but this is most likely due to limited sample size, since there was a significant difference compared to women aged less than 35 years. Since antral follicle count and anti-Müllerian hormone is not included in the IVF registry, we cannot identify all groups of poor responders, according to the Bologna criteria. Cumulative live birthrates of 18.6% in poor responders as defined by the Bologna criteria were reported in a retrospective analysis of women undergoing their first complete ART cycle (20). A clear decline in treatment success for poor responders (defined by the Bologna criteria) returning for their third treatment cycle have previously been reported, in two smaller retrospective cohort studies from China. However, in both studies, less than 64 women had a third cycle (21,22). The studies both reported higher live birthrates in women aged less than 40 years, compared to women aged 40 and over.

A recent UK-study also found an association between number of aspirated oocytes in the 1st cycle and birthrates in later cycles (6). The study report birthrates within 3 complete cycles of ~25% for women aged 40, who had IVF treatment, 5 eggs collected, no embryos frozen, and transferal of a single cleavage stage embryo in the first cycle (6). The predicted estimates from the UK-study are roughly consistent with our observed live birthrates in women aged 40 and over, with 5 aspirated oocytes, were ~18% had a livebirth. The predicted estimates from the UK-study may be overly optimistic since they are based on the assumption that couples/women who discontinue treatment would have had the same chance of success, in a hypothetical future treatment, as couples who

continue treatments, but in fact drop-outs have been shown to have a worse prognosis than couples who continue treatments (23–25).

The information on OHSS in the present study was retrieved from the National Patient registry, which means that the women with a diagnosis of OHSS were either admitted or seen in a gynecological outpatient clinic. Most likely, some women with mild or even moderate OHSS may have been controlled in a fertility clinic, without being seen in a hospital outpatient clinic or admitted to hospital. Therefore, OHSS rates in the present study (incidence of 1.7% in the first cycle) are most likely underestimated. A Danish RCT, compared incidence of hospital admissions due to OHSS, in a GnRH antagonist vs. GnRH agonist treatment protocol (26). In the RCT 1.7% and 3.6% of the women were admitted due to OHSS in the 1st fresh cycle in the GnRH antagonist vs. agonist protocol respectively. A prospective cohort study from Belgium showed an incidence of hospital admissions due to OHSS of 2.1%. The study further showed that number of follicles over 11 mm was a better predictor of early OHSS than oestradiol levels on the day of ovulation triggering, and that incidence of OHSS increased when more than 13 oocytes were collected (27). Our results also showed increasing incidence of OHSS with increasing number of aspirated oocytes. In our study, 2.7% of women starting ART treatments had at least one episode of OHSS within four fresh complete cycles. We saw a decreasing incidence of OHSS with increasing cycle number. This may be explained by selection of the women continuing treatment: a decreasing oocyte number is associated with decreased live birthrates, and women returning for treatments may be less likely to have many oocytes and thereby a diminished risk of OHSS. However, it may also be due to dose adjustments regulating the ovarian stimulation in repeated treatments, optimizing the treatment response which leads to increase safety in subsequent cycles.

Strengths and weaknesses

This large national cohort study includes a complete fertility treatment history with follow-up on both treatment-related and treatment-independent births. Chances of successful treatment are described considering the number of previous treatment attempts and response to ovarian stimulation as well as female age. In Denmark, fertility treatments are reimbursed by the national health care system, which most likely contributes to the low drop-out rates within the first three cycles. Obviously, national rules and regulations concerning reimbursement and national treatment guidelines will affect our results. In Denmark treatments are only reimbursed if the woman is under 40 years old, and Danish law prohibits treatment to women older than 45 years of age (9). Our results are either stratified or adjusted for female age, which minimize the impact of this concern, but it is conceivable that women aged between 40 and 45 have a higher socioeconomic status than the younger women, which may affect their fertility potential.

The national treatment guidelines recommend that couples with anovulatory infertility, mild to moderate male factor infertility, idiopathic infertility and mild endometriosis, start fertility treatments with 3 cycles of insemination treatments (predominatly in combined with ovulation induction/ovarian stimulation). For couples with anovulatory infertility, up to 6 cycles are offered. A previous study from this research group has shown that these recommendations are followed in the vast majority of cases (28). This may affect the distribution of cause of infertility in this cohort as compared to other countries; however results have been adjusted for cause of infertility minimizing the risk of bias.

We do not have information on which fresh cycle produced the embryo transferred in the frozen-thawed cycle, and some of the association between aspirated oocytes in the 1st cycle and outcome in later cycles, may be due to frozen embryos originating from the 1st fresh cycle that were left over and not transferred until after oocyte pick-up in the 2nd or 3rd cycle. However, it is recommended by

the Danish health authorities that all frozen embryos are used before proceeding to the next ovarian stimulation (9).

Even though the Danish ART-registry is mandatory for all clinics, it is likely that there will be missing treatment cycles, and cycles not leading to pregnancy may be underreported. Loss of data for administrative causes may also occur, but is random. Missing data in number of aspirated oocytes is also a concern. However, our analyses showed that the group with missing data on number of aspirated oocytes did not differ significantly from the group with available data, with regard to age, live birthrates and incidence of OHSS, thus missing information is most likely random.

CONCLUSION

This study present cumulative live birthrates after a trajectory of complete ART-cycles, and the results can provide couples and women, returning for repeated fertility treatments, with an individualized prognosis that pinpoint their current treatment progression, their previous treatment response, as well as female age.

REFERENCES

1. Sylvest R, Furbringer JK, Schmidt L, Pinborg A. Infertile men's needs and assessment of fertility care. Ups J Med Sci. 2016;121(4):1–7.
2. Klitzman R. How much is a child worth? Providers' and patients' views and responses concerning ethical and policy challenges in paying for ART. PLoS One. 2017;12(2):e0171939.
3. Sunkara SK, Rittenberg V, Raine-Fenning N, Bhattacharya S, Zamora J, Coomarasamy A. Association between the number of eggs and live birth in IVF treatment: An analysis of 400 135 treatment cycles. Hum Reprod. 2011;26(7):1768–74.
4. Briggs R, Kovacs G, MacLachlan V, Motteram C, Gordon Baker HW. Can you ever collect too many oocytes? Hum Reprod. 2015;30(1):81–7.
5. Drakopoulos P, Blockeel C, Stoop D, Camus M, De Vos M, Tournaye H, et al. Conventional ovarian stimulation and single embryo transfer for IVF/ICSI. How many oocytes do we need to maximize cumulative live birth rates after utilization of all fresh and frozen embryos? Hum Reprod. 2015;31(2):370–6.
6. McLernon DJ, Steyerberg EW, Te Velde ER, Lee AJ, Bhattacharya S. Predicting the chances of a live birth after one or more complete cycles of in vitro fertilisation: population based study of linked cycle data from 113 873 women. BMJ. 2016;355:i5735.
7. Lashen H, Ledger W, López Bernal a, Evans B, Barlow D. Superovulation with a high gonadotropin dose for in vitro fertilization: is it effective? J Assist Reprod Genet. 1998;15(7):438–43.
8. Popovic-Todorovic B, Loft A, Ziebe S, Nyboe Andersen A. Impact of recombinant FSH dose adjustments on ovarian response in the second treatment cycle with IVF or ICSI in "standard" patients treated with 150 IU/day during the first cycle. Acta Obstet Gynecol

Scand. 2004;83(9):842–9.

9. Vævsbekendtgørelsen - Vejledning om sundhedspersoners og vævscentres virksomhed og forpligtelser i forbindelse med assisteret reproduktion - retsinformation.dk [Internet]. [cited 2017 May 17]. Available from: https://www.retsinformation.dk/Forms/R0710.aspx?id=172755#ide681ffa8-817c-4e1e-96ff-5ce8c2fa0a2a

10. Steward RG, Lan L, Shah AA, Yeh JS, Price TM, Goldfarb JM, et al. Oocyte number as a predictor for ovarian hyperstimulation syndrome and live birth: An analysis of 256,381 in vitro fertilization cycles. Fertil Steril. 2014;101(4):967–73.

11. Baker VL, Brown MB, Luke B, Conrad KP. Association of number of retrieved oocytes with live birth rate and birth weight: An analysis of 231,815 cycles of in vitro fertilization. Fertil Steril. 2015;103(4):931–938.e2.

12. Bdolah Y, Zemet R, Aizenman E, Lossos F, Abram TB, Shufaro Y. Frozen-Thawed embryo transfer success rate is affected by age and ovarian response at oocyte aspiration regardless of blasto- mere survival rate. J Bras Reprod Assist. 2015;19(4):210–5.

13. Danish Fertility Society Annual Reports 1997-2016 [Internet]. [cited 2017 May 17]. Available from: www.fertilitetsselskab.dk/images/2017/dfs1997-2016 oversigt_140317.pdf

14. Kupka MS, D'Hooghe T, Ferraretti AP, De Mouzon J, Erb K, Castilla JA, et al. Assisted reproductive technology in Europe, 2011: Results generated from European registers by ESHRE. Hum Reprod. 2015;31(2):233–48.

15. Dyer S, Chambers GM, De Mouzon J, Nygren KG, Zegers-Hochschild F, Mansour R, et al. International committee for monitoring assisted reproductive technologies world report: Assisted reproductive technology 2008, 2009 and 2010†. Hum Reprod. 2016;31(7):1588–609.

16. Vaegter KK, Lakic TG, Olovsson M, Berglund L, Brodin T, Holte J. Which factors are most predictive for live birth after in??vitro fertilization and intracytoplasmic sperm injection (IVF/ICSI) treatments? Analysis of 100 prospectively recorded variables in 8,400 IVF/ICSI single-embryo transfers. Fertil Steril. 2017;107(3):641–648.e2.

17. Brodin T, Hadziosmanovic N, Berglund L, Olovsson M, Holte J. Comparing four ovarian reserve markers - Associations with ovarian response and live births after assisted reproduction. Acta Obstet Gynecol Scand. 2015;94(10):1056–63.

18. Ferraretti AP, La Marca A, Fauser BCJM, Tarlatzis B, Nargund G, Gianaroli L. ESHRE consensus on the definition of 'poor response to ovarian stimulation for in vitro fertilization: The Bologna criteria. Hum Reprod. 2011;26(7):1616–24.

19. Polyzos NP, Nwoye M, Corona R, Blockeel C, Stoop D, Haentjens P, et al. Live birth rates in Bologna poor responders treated with ovarian stimulation for IVF/ICSI. Reprod Biomed Online. 2014;28(4):469–74.

20. Chai J, Lee VCY, Yeung TWY, Li RWH, Ho PC, Ng EHY. Live birth and cumulative live birth rates in expected poor ovarian responders defined by the Bologna criteria following IVF/ICSI treatment. PLoS One. 2015;10(3):1–10.

21. Ke H, Chen X, Liu YD, Ye DS, He YX, Chen SL. Cumulative live birth rate after three ovarian stimulation IVF cycles for poor ovarian responders according to the bologna criteria. J Huazhong Univ Sci Technol - Med Sci. 2013;33(3):418–22.

22. Yang Y, Sun X, Cui L, Sheng Y, Tang R, Wei D, et al. Younger poor ovarian response women achieved better pregnancy results in the first three IVF cycles. Reprod Biomed Online. 2016;32(5):532–7.

23. Daya S. Life table (survival) analysis to generate cumulative pregnancy rates in assisted reproduction: Are we overestimating our success rates? Hum Reprod. 2005;20(5):1135–43.

24. Smeenk JMJ, Verhaak CM, Stolwijk AM, Kremer JAM, Braat DDM. Reasons for dropout in an in vitro fertilization/intracytoplasmic sperm injection program. Fertil Steril. 2004;81(2):262–8.

25. Malizia B, Hacker M, Penzias A. Cumulative Live-Birth Rates after In Vitro Fertilization. N Engl J Med. 2009;360:236–43.

26. Toftager M, Bogstad J, Bryndorf T, Løssl K, Roskær J, Holland T, et al. Risk of severe ovarian hyperstimulation syndrome in GnRH antagonist versus GnRH agonist protocol: RCT including 1050 first IVF/ICSI cycles. Hum Reprod. 2016;31(6):1253–64.

27. Papanikolaou EG, Pozzobon C, Kolibianakis EM, Camus M, Tournaye H, Fatemi HM, et al. Incidence and prediction of ovarian hyperstimulation syndrome in women undergoing gonadotropin-releasing hormone antagonist in vitro fertilization cycles. Fertil Steril. 2006;85(1):112–20.

28. Malchau SS, Henningsen AA, Loft A, Rasmussen S, Forman J, Nyboe Andersen A, et al. The long-term prognosis for live birth in couples initiating fertility treatments. Hum Reprod. 2017;1–11.

Supplemental data paper II

Supplemental Table SI, Age at first treatment, cumulative live birthrates and incidence of OHSS, by missing and valid information in number of aspirated oocytes, Denmark 2002-2011

	Missing information on aspirated oocytes		Valid information on aspirated oocytes	
1st fresh cycle				
N, total	9470	95% CI[1]	21,016	95% CI[1]
Age (years), mean ±SD	33.1 ±4.85	[33.0–33.2]	33.1±4.90	[33.0–33.2]
CLBR[2] 1st complete cycle (%)[3]	26.2	[25.4–27.1]	26.4	[25.8–27.0]
OHSS[4] first cycle (%)[3]	1.7	[1.5–2.0]	1.7	[1.5–1.9]
2nd fresh cycle				
N, total	5472	95% CI[1]	12,010	95% CI[1]
Age (years), mean ±SD	33.3±4.8	[33.2–33.4]	33.4±4.9	[33.3–33.5]
CLBR[2] 1st complete cycle(%)[3]	27.0	[25.8–28.2]	25.7	[24.9–26.5]
OHSS[4] first cycle (%)[3]	1.4	[1.2–1.8]	1.2	[1.0–1.4]
3rd fresh cycle				
N, total	3123	95% CI[1]	6565	95% CI[1]
Age (years), mean ±SD	33.6±4.8	[33.4–33.8]	33.7±4.9	[33.6–33.8]
CLBR[2] 1st complete cycle (%)[3]	24.9	[23.4–26.4]	22.9	[21.9–24.0]
OHSS[4] first cycle (%)[3]	0.8	[0.5–1.1]	1.1	[0.9–1.4]
4th fresh cycle				
N, total	1414	95% CI[1]	3151	95% CI[1]
Age (years), mean ±SD	33.4±4.5	[33.2–33.6]	33.8±4.8	[33.6–33.9]
CLBR[2] 1st complete cycle (%)[3]	21.8	[19.7–24.0]	20.0	[18.7–21.3]
OHSS[4] first cycle (%)[3]	1.0	[0.6–1.7]	0.8	[0.6–1.2]

[1]95% confidence intervals of mean or proportion. [2]Cumulative livebirthrates. [3]Percent in non-censored cases. [4]Ovarian hyperstimulation syndrome.

Supplemental Table SII, Non-ART related live births in women starting their 1st to 4th ART cycle, Denmark 2006-2011

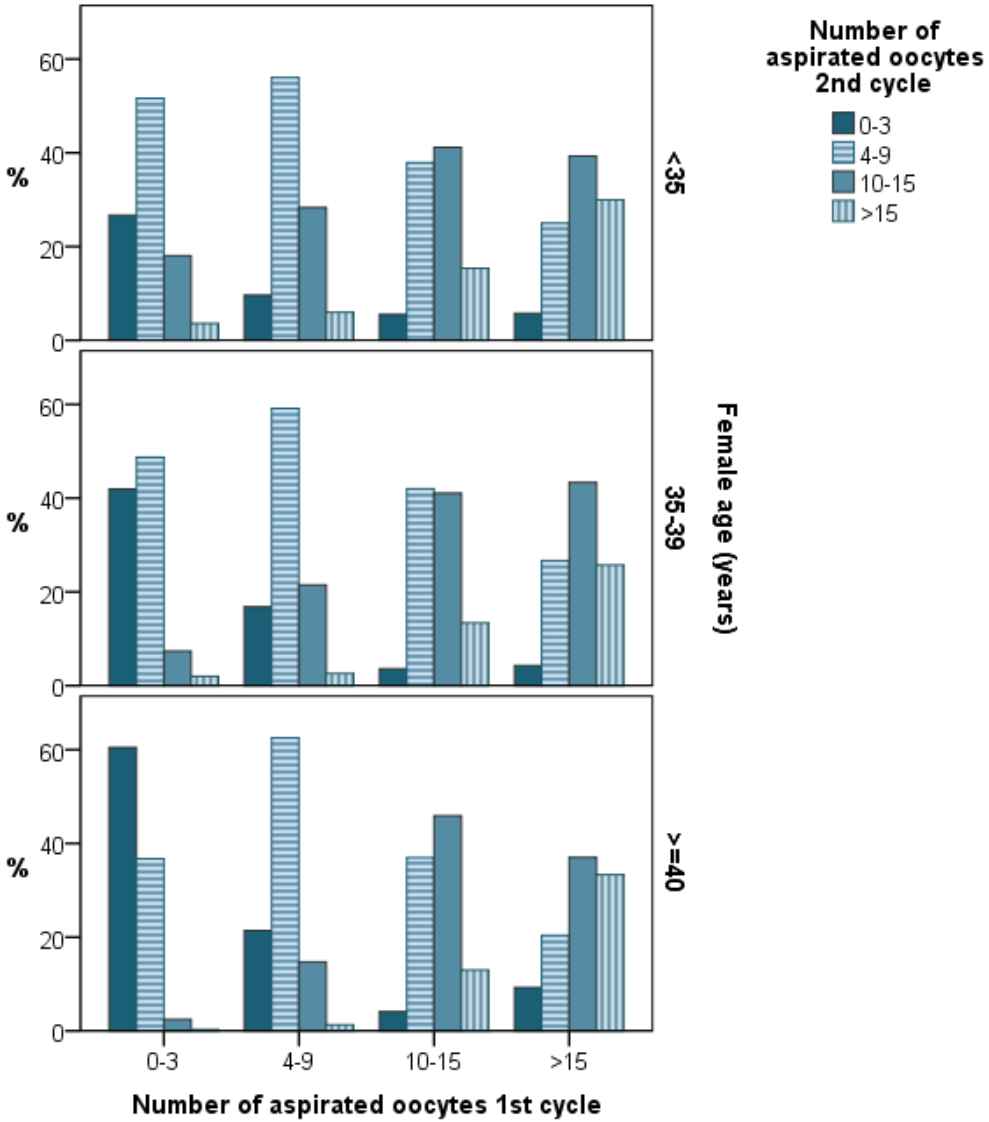
	1st complete ART cycle	2nd complete ART cycle	3rd complete ART cycle	4th complete ART cycle	4 cumulated complete cycles
All women starting treatment, N total	18,924	10,528	5657	2544	18,924
Censored, N women	428	272	186	78	964
Women with complete follow-up[1], N (%)	18,496 (100)	10,256 (100)	5471 (100)	2466 (100)	17,960 (100)
All non-ART livebirths[2], N (%)	919 (5.0)	429 (4.2)	292 (5.3)	126 (5.1)	1766 (9.8)
Natural conception, N (%)	766 (4.1)	383 (3.7)	238 (4.4)	95 (3.8)	1482 (8.2)
Insemination treatment, N (%)	153 (0.8)	46 (0.4)	54 (1.0)	31 (1.3)	284 (1.6)

[1]All women with at least on year follow-up at time of their last treatment in the 1st to 4th complete cycle [2]Conceived naturally or with insemination treatment

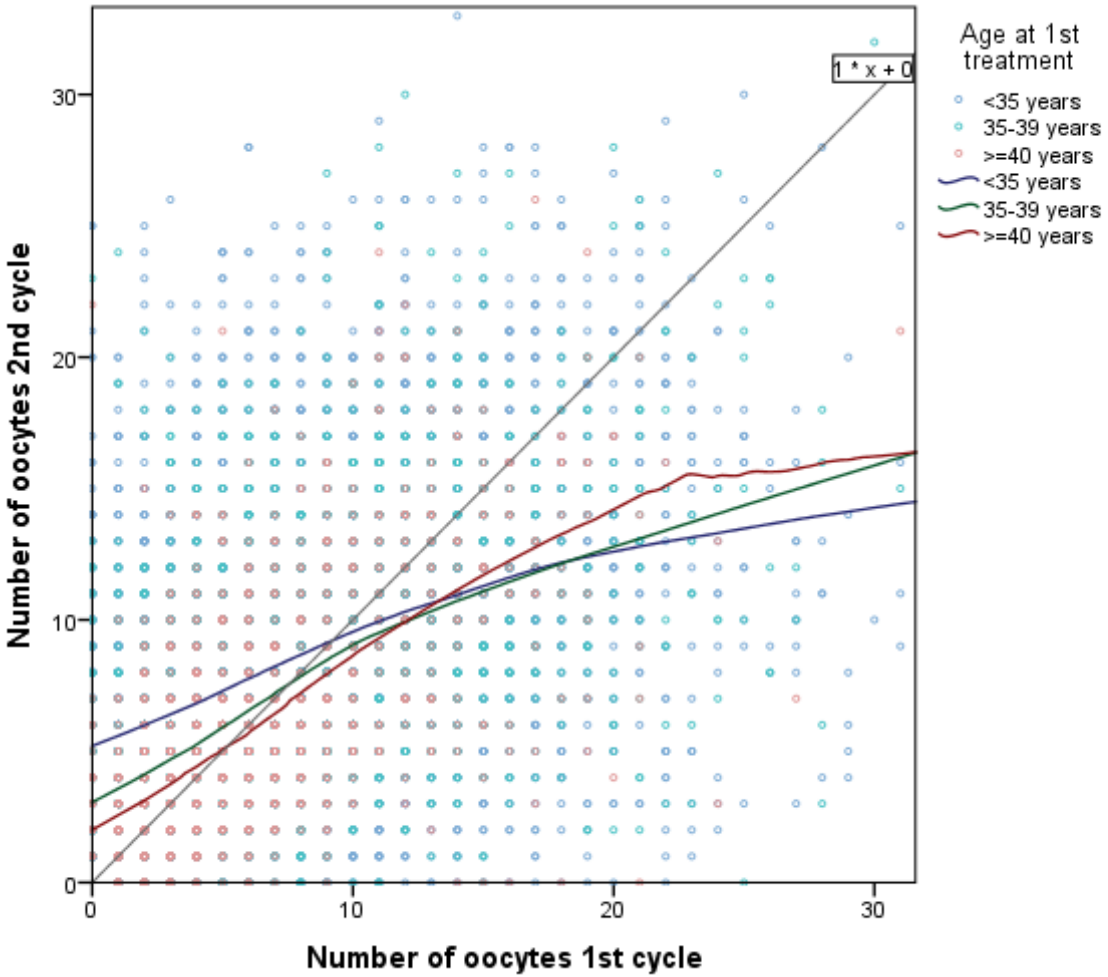
Supplemental Table SIII Incidence of ovarian hyper-stimulation syndrome in the 1st to 4th fresh ART-cycle, by number of aspirated oocytes, Denmark 2002-2011

No of retrieved oocytes	N, total	OHSS % [95% CI]
First fresh cycle		
0-3	3970	0.2% [0.09–0.4]
4-9	9791	1.1% [0.9–1.3]
10-15	5279	2.5% [2.1–2.9]
>15	2026	5.5% [4.6–6.6]
Second fresh cycle		
0-3	1824	0.2% [0.09–0.5]
4-9	5841	0.9% [0.7–1.2]
10-15	3321	1.6% [1.2–2.1]
>15	1032	3.4% [2.5–4.7]
Third fresh cycle		
0-3	1031	0.0% [0.0–0.4]
4-9	3191	0.8% [0.5–1.1]
10-15	1812	1.5% [1.0–2.2]
>15	536	3.7% [2.4–5.7]
Fourth fresh cycle		
0-3	498	0.2% [0.04–1.0]
4-9	1533	0.7% [0.4–1.3]
10-15	857	1.2% [0.6–2.1]
>15	266	1.5% [0.6–2.1]

Supplemental Figure S1, Number of aspirated oocytes in 2nd fresh cycle by number of aspirated oocytes in the 1st fresh cycle and female age



Supplemental figure S2, Number of aspirated oocytes in the 2nd cycle by number of aspirated oocytes in the first cycle and female age



Paper III

Perinatal outcomes in 6,338 singletons born after intrauterine insemination in Denmark, 2007 to 2012: the influence of ovarian stimulation

Sara Sofia Malchau, M.D.,[a,b] Anne Loft, M.D.,[a] Anna-Karina Aaris Henningsen, M.D.,[a] Anders Nyboe Andersen, M.D., D.M.Sci.,[a] and Anja Pinborg, M.D., D.M.Sci.[b]

[a] Fertility Clinic, Rigshospitalet, University of Copenhagen, Copenhagen; and [b] Department of Obstetrics and Gynecology, Hvidovre Hospital, University of Copenhagen, Hvidovre, Denmark

Objective: To study perinatal outcomes in singletons born after intrauterine insemination (IUI) compared with children born after in vitro fertilization (IVF), intracytoplasmic sperm injection, and spontaneous conception (SC), and to assess predictors of poor outcome in singletons born after IUI, exploring the effect of ovarian stimulation.
Design: National cohort study, 2007–2012.
Setting: Danish national registries.
Patient(s): Four thousand two hundred twenty-eight singletons born after insemination with partner semen (IUI-H) and 1,881 singletons born after insemination with donor semen.
Intervention(s): None.
Main Outcome Measure(s): Preterm birth (PTB), low birth weight (LBW), small for gestational age (SGA).
Result(s): Children born after IUI-H had higher risks of PTB, LBW, and SGA vs. SC singletons (adjusted odds ratios [aOR] 1.3; 95% confidence interval (CI), 1.1–1.5; 1.4; 95% CI, 1.2–1.7; and 1.4; 95% CI, 1.2–1.6), respectively. Compared with IVF, risk of SGA was similar, but risks of PTB and LBW were lower (aOR 0.6; 95% CI, 0.5–0.8; and 0.8; 95% CI, 0.6–0.9). Compared with intracytoplasmic sperm injection, no differences were found. For children born after IUI with donor semen, results were similar to those for IUI-H. Risks of LBW and SGA were higher in IUI singletons born after ovarian stimulation with clomiphene citrate, compared with natural-cycle IUI (aOR 1.5; 95% CI, 1.1–2.1 and 1.6; 95% CI, 1.1–2.4). Treatment with follicle-stimulating hormone vs. natural-cycle IUI did not seem to affect perinatal outcomes.
Conclusion(s): Singletons born after IUI had higher risk of adverse perinatal outcomes compared with SC children, similar to ICSI, but favorable outcomes compared with IVF. Stimulation with clomiphene citrate was associated with higher risk of SGA compared with natural-cycle IUI, but follicle-stimulating hormone treatment did not seem to be associated with adverse outcomes.
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Key Words: Intrauterine insemination, perinatal outcome, clomiphene citrate, ovarian stimulation, small for gestational age, preterm birth

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Intrauterine insemination (IUI) is widely used in Denmark and accounts for more than half of all fertility treatments conducted nationally (1). In Denmark, use of assisted reproductive technology (ART) has been recorded in the national ART register since 1994, and registration of IUI treatments has been mandatory since 2007. In Europe, reproductive technologies are recorded in national registers in 34 countries, whereas data on IUI are only available from 21 countries (2). A limited number of studies have investigated perinatal outcomes in children born after IUI and found increased risks compared

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Reprint requests: Sara Sofia Malchau, M.D., Department of Obstetrics and Gynecology, Hvidovre Hospital, Kettegård allé 30, DK - 2650 Hvidovre, Denmark (E-mail: smalchau@hotmail.com).

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with spontaneously conceived (SC) children (3–6). A few small, predominantly clinic-based studies have compared IUI to in vitro fertilization (IVF), and the results point toward either comparable or lower risks of adverse perinatal outcome in IUI vs. IVF (7–9).

Increased risks of adverse perinatal outcome in singletons born after ART compared with SC children have been observed in several descriptive studies (10). The slightly poorer outcomes in ART singletons are linked to maternal age and primiparity, subfertility, vanishing twins, fresh vs. frozen embryo transfer, and blastocyst transfer. Moreover, ovarian stimulation and laboratory procedures have also been suggested as explanatory factors (10–13). Understanding the relative contribution of the various factors is important for minimizing the effect of iatrogenic causes and for assessing the safety of fertility treatments and the continuous advancements in assisted reproduction. Considering the potential influence of culture media, culture time, and other laboratory factors in ART, a study on infant outcome after IUI may be suitable for assessing the effect of parental factors and ovarian stimulation.

This study, based on a national cohort of children born after IUI, aims to assess the obstetric and perinatal outcomes in children born after IUI compared with children born after IVF, those born after intracytoplasmic sperm injection (ICSI), and those who were SC. Further, we aim to explore predictors of poor perinatal outcome in children born after IUI, assessing whether outcomes are influenced by ovarian stimulation, cause of infertility, and use of partner or donor semen.

MATERIALS AND METHODS

The Danish ART Register

All initiated treatment cycles are recorded in the mandatory ART register, and through the personal social security number, data were cross-linked to the medical birth register and hospital discharge register, to obtain information on live births as well as obstetric and perinatal complications. The ART register includes data on body mass index (BMI), smoking status, cause of infertility, partner/donor semen, and type of medication used during fertility treatment.

All children born after IUI in Denmark from 2007 to 2012 were identified in the Danish ART register and cross-linked to the medical birth register (n = 7,900: singletons n = 6,338; twins n = 1,517; triplets n = 45). Two control cohorts of all children born after IVF (singletons n = 4,135) and ICSI (singletons n = 3,635), 2007–2012, were also identified in the 2 registers (ART and medical birth register). Children born after oocyte donation, preimplantation genetic diagnosis, percutaneous epididymal/testicular sperm aspiration, and frozen embryo transfer were excluded. The third control group, which consisted of all SC singletons, 2008–2011 (n = 229,749), were identified in the medical birth register.

We excluded SC children born in 2007 and 2012 for the following reasons: in 2007, the ART register was converted from paper-based registration to online registration, and during 2007, the electronic data registration was incomplete, in particular the IUI cycles, as the registration of IUI cycles was initiated concomitantly with introduction of the electronic register. Hence, some of the treatment cycles in 2007 were not registered, and some of the children conceived after fertility treatments in 2007 appear as SC children. Thus, to avoid bias, we excluded SC children from this year. The reason for excluding the year 2012 in the SC children cohort was that the ART registrations for 2012 were not yet completed at the time data were extracted from the registers, which again categorized children conceived after fertility treatment into the SC cohort.

Data on cause of infertility, use of partner/donor semen, duration of infertility, and type of medication used during fertility treatment were retrieved from the IVF register. Data on perinatal outcomes were extracted from the medical birth register. Diagnoses obtained from the hospital discharge register were: hypertensive disorders of pregnancy, placenta previa, cesarean section, induction of labor, and admittance to a neonatal intensive care unit.

According to Danish legislation, studies based solely on register data and with no personal contact/involvement of the participants do not require approval from an ethical committee. The study was approved by the Danish Data Protection Agency (CVR-FSEID 00000303) and the National Board of Health.

Statistics

The differences between study and control populations were analyzed in SPSS, version 15.0. Statistical significance was defined as $P<.05$. Differences of the means of continuous parametric data were analyzed with the use of analysis of variance and Bonferroni correction. For nonparametric data, medians and interquartile ranges (IQR) were reported, and differences were analyzed in SPSS with the Kruskal-Wallis test. The χ^2 test and Bonferroni correction were used to compare distributions between 2 groups.

The risks of being small for gestational age (SGA) and large for gestational age (LGA) were calculated in relation to the 10th percentile by Marsal's formula with the use of birth weight, child gender, and standard intrauterine growth curves for Scandinavia (14). Multivariate logistic regression analyses were performed to estimate risks of preterm birth (PTB, gestational age <37 weeks), low birth weight (LBW, birth weight <2,500 grams), SGA, and LGA. The analyses were adjusted for maternal age, parity, child gender, year of birth, smoking, maternal BMI, elective cesarean section, and induction of labor. All stillbirths and children with missing information on gestational age or birth weight, as well as children with a gestational age of <140 days or >308 days, and children with a birth weight <200 grams or >9,000 grams were excluded in the analyses of birth weight, gestational age, LBW, PTB, and SGA/LGA. In the stratified analysis, use of ovarian stimulation in the cycle leading to birth is assessed. Natural cycle IUI was defined as those who received no treatment with clomiphene citrate (CC) or follicle-stimulating hormone (FSH)–containing preparations. However, approximately 44% of women in the

TABLE 1

Maternal characteristics in IUI-H and IUI-D pregnancies compared to those from IVF, ICSI, and SC.

						P value					
	IUI-H	IUI-D	IVF	ICSI	SC	IUI-H vs. IVF	IUI-H vs. ICSI	IUI-H vs. SC	IUI-D vs. IVF	IUI-D vs. ICSI	IUI-D vs. SC
Singletons, N total	4,208	1,881	4,135	3,635	229,749						
Age (y), mean (±SD)	32.6 (±4.3)	34.4 (±4.4)	34.2 (±4.4)	33.4 (±4.3)	30.7 (±4.9)	<.001	<.001	<.001	1.0	<.001	<.001
Nulliparous, N (%)	2,657 (64.1)	1,287 (69.5)	2,719 (67.2)	2,329 (65.9)	97,692 (43.4)	.012	.10	<.001	.09	.04	<.001
BMI, median (IQR)	23.2 (21–27)	25.4 (22–28)	22.8 (21–26)	23.3 (21–27)	23.2 (21–27)	<.001	.27	.85	<.001	<.001	<.001
BMI >30, N (%)	509 (12.6)	302 (16.7)	349 (8.8)	385 (11.0)	27,401 (12.7)	<.001	.16	.84	<.001	<.001	<.001
Smokers, N (%)	180 (4.4)	205 (5.6)	205 (5.1)	140 (3.9)	24,214 (10.9)	.12	.38	<.001	.41	.04	<.001

Note: UI-H = intrauterine insemination with partner semen; IUI-D = intrauterine insemination with donor semen; SC = spontaneous conception; BMI = body mass index.

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natural-cycle group were treated with human choriogonadotropin (hCG) to trigger ovulation.

RESULTS

Of 6,338 singletons born after IUI, 4,208 (66.4%) were born after use of partner semen (IUI-H), and 1,881 (29.7%) after use of donor semen (IUI-D). Maternal characteristics are shown in Table 1 and Supplemental Table 1 (available online). Compared with the control groups, women giving birth after IUI-D were slightly older, and more of them were overweight than in the IUI-H group. For both IUI-H and IUI-D, median time to birth was shorter than in the IVF and ICSI groups. However, this information should be interpreted cautiously, as it was only available for one sixth of the total cohort. Perinatal and obstetric outcomes are shown in Table 2.

Among children born after IUI-H, mean birth weight and gestational age were lower than they were for SC children, higher than in the IVF group, and similar to those for infants born after ICSI. The incidence of placenta previa was higher than after SC, but lower compared with pregnancies after IVF and ICSI. The incidence of hypertensive disorders during pregnancy was higher compared with the SC group, but similar to that for pregnancies after IVF and ICSI.

Among children born after IUI-D, mean birth weight and gestational age were comparable to those for the SC children, but higher than in the IVF and ICSI groups. The incidence of placenta previa was similar compared with pregnancies after SC, but lower than seen in pregnancies after IVF and ICSI. The incidence of hypertensive disorders was higher compared with all three control groups. For infants born after either IUI-H or IUI-D, the frequency of admittance to a neonatal intensive care unit was higher than after SC, but less frequent than in the IVF group and similar compared with the ICSI group. Perinatal mortality did not differ between the IUI-H/IUI-D and control groups.

Crude and adjusted risks of LBW, PTB, SGA, and LGA for IUI-H and IUI-D are shown in Table 3. For both IUI-H and IUI-D, risks of LBW and SGA were higher compared with the SC group, lower compared with the IVF group, and similar compared with the ICSI group in the adjusted analyses. For IUI-H, risk of PTB was higher compared with that for SC children, lower compared with that for IVF, and similar to that for ICSI. For IUI-D, adjusted risk of PTB was similar to that for SC, lower than that for IVF, and similar to that for ICSI. Adjusted risks of SGA were higher compared with SC children, but similar in the IUI-H/IUI-D and IVF and ICSI groups.

Table 4 shows subanalyses of children born after IUI, stratified on type of medication used for ovarian stimulation, stratified on cause of infertility, and stratified on use of partner/donor semen. Among children born after IUI-H, 76% were conceived in a stimulated cycle with CC, FSH, or both. Only 36% of children born after IUI-D were conceived in a stimulated cycle. Overall, for singletons born after IUI, 1,744 (31.4%) were conceived in an unstimulated cycle, 1,357 (24.4%) were conceived after stimulation with CC, 1,117 (20.1%) were conceived after stimulation with FSH, and 1,340 (24.1%) were conceived after stimulation with CC and FSH in combination.

Subanalysis for crude and adjusted risks of LBW, PTB, and SGA for children born after IUI is shown in Table 4. The risks were similar for IUI-H vs. IUI-D, for idiopathic vs. male-factor infertility, and for anovulatory vs. male-factor infertility. However, in the crude and adjusted analysis, we found increased risk of LBW and SGA in the CC vs. natural-cycle group, and also an increased risk of LBW in the CC + FSH group vs. natural cycle. In contrast, the risks of LBW and SGA were similar after FSH vs. natural cycles. Mean birth weight and mean gestational age were lower in the IUI-H group than in the IUI-D group (3,434 ± 571 grams vs. 3,505 ± 589 grams, $P<.001$ and 277.5 ± 13.2 days vs. 278.5 ± 13.7 days, $P=.007$).

DISCUSSION

In 6,338 singletons born after IUI, we found increased obstetric and perinatal risks compared with SC children,

TABLE 2

Obstetric and perinatal outcomes in singletons born after IUI-H and IUI-D, compared with IVF, ICSI, and SC children.

						P value					
	IUI-H	IUI-D	IVF	ICSI	SC	IUI-H vs. IVF	IUI-H vs. ICSI	IUI-H vs. SC	IUI-D vs. IVF	IUI-D vs. ICSI	IUI-D vs. SC
Singletons, N total	4,208	1,881	4,135	3,635	229,749						
Birth weight (g), mean ± SD	3,434 ± 571	3,505 ± 590	3,356 ± 600	3,420 ± 574	3,515 ± 557	<.001	1.0	<.001	<.001	<.001	1.0
Gestational age (d), mean ± SD	277.5 ± 13.2	278.5 ± 13.7	275.8 ± 15.9	277.3 ± 13.8	278.7 ± 12.5	<.001	1.0	<.001	<.001	.04	1.0
LBW, N (%)	203 (4.9)	92 (5.0)	281 (6.9)	193 (5.4)	7,706 (3.4)	<.001	.317	<.001	<.01	.498	<.001
VLBW, N (%)	26 (0.6)	13 (0.7)	51 (1.3)	34 (1.0)	1,303 (0.6)	<.01	.11	.68	.10	.35	.48
PTB, N (%)	169 (4.1)	75 (4.0)	260 (6.4)	154 (4.3)	6,907 (3.1)	<.001	.61	<.001	<.001	.64	.08
VPTB, N (%)	24 (0.6)	15 (0.8)	58 (1.4)	41 (1.1)	1,651 (0.7)	<.001	.17	.75	.08	.90	.12
SGA, N (%)	156 (3.8)	72 (3.9)	156 (3.9)	119 (3.3)	6,164 (2.7)	.84	.31	<.001	.95	.29	<.001
LGA, N (%)	117 (2.8)	60 (3.2)	89 (2.2)	98 (2.7)	7,017 (3.1)	.07	.84	.29	.08	.30	.75
Placenta previa, N (%)	45 (1.1)	9 (0.5)	97 (2.3)	71 (2.0)	923 (0.4)	<.001	.004	<.001	<.001	<.001	.60
Hypertensive disorders, N (%)	234 (5.6)	140 (7.4)	198 (4.8)	185 (5.1)	8,577 (3.7)	.11	.36	<.001	<.001	<.001	<.001
Cesarean section, N (%)	1,165 (27.7)	489 (26.0)	1,141 (27.6)	932 (25.6)	45,799 (19.9)	.925	.16	<.001	.20	.77	<.001
Elective cesarean section, N (%)	420 (10.0)	160 (8.5)	465 (11.2)	361 (9.9)	19,503 (8.5)	.061	.94	.001	.001	.09	.98
Induction of labor, N (%)	559 (13.3)	287 (15.3)	557 (13.5)	484 (13.3)	26,633 (11.6)	.80	.97	.004	.06	.2	<.001
NICU, N (%)	442 (10.5)	212 (11.3)	500 (12.1)	358 (9.8)	17,993 (7.8)	.08	.34	<.001	.36	.10	<.001
Perinatal mortality, N (%)	45 (1.1)	16 (0.9)	61 (1.5)	39 (1.1)	2,080 (0.9)	.10	1.0	.27	.2	.43	.80

Note: LBW: <2,500 grams. VLBW: <1,500 grams. PTB: gestational age <37 weeks. VPTB: gestational age <32 weeks. NICU = neonatal intensive care unit; SC = spontaneous conception; UI-H = intrauterine insemination with partner semen; IUI-D = intrauterine insemination with donor semen; VLBW = very low birth weight; VPTB = very preterm birth. Hypertensive disorders are defined as: gestational hypertension, preeclampsia, hemolysis, elevated liver enzymes, low platelet count (HELLP), and eclampsia. Perinatal mortality is defined as stillbirths + deaths within first week of life.

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but lower risks than after IVF conception. Compared with children born after ICSI, there was no difference. In the stratified analysis, there were slightly increased adjusted odds ratios of SGA and LBW in children conceived in cycles stimulated with CC compared with children born after IUI in natural cycles.

The strengths of this study are the number of children born after IUI, and the detailed information on the cohorts, making it possible to stratify data according to origin of semen (donor vs. partner), cause of infertility, and type of ovarian stimulation. Further, we were able to adjust for known maternal confounders, such as smoking, BMI, age, and parity, as well as iatrogenic confounders, such as induction of labor and elective cesarean section. A higher incidence of elective cesarean section and medical induction of labor reduces GA in the newborn, which may affect analyses of PTB and SGA. As shown in Table 2, the incidences of these interventions are not comparable in the study and control populations. A higher incidence of these interventions could be due to a higher risk in ART pregnancies; in this case, it should be considered a mediator and no adjustment should be made. However, we considered that at least part of the cesarean sections and inductions were a result of more precautions are taken in ART pregnancies; therefore, we decided to adjust for them.

Our findings are consistent with an earlier Belgian study (3) that found adverse perinatal outcomes in children born after IUI with increased risks, compared with SC singletons, of PTB; very PTB (gestational age <32 weeks); LBW; very LBW (birth weight <1,500 grams); respiratory distress; intracranial bleeding; and transfer to a neonatal intensive care unit. The Belgian study was based on register data from all hospital deliveries in Flanders. Cases were not subdivided into IUI-H and IUI-D, and the effect of ovarian stimulation was not explored. Furthermore, odds ratios were not corrected for factors such as BMI, smoking, elective cesarean section, or induction of labor. In a Finnish study, Klemetti et al. (4) found increased risks of LBW, very LBW, PTB, and very PTB in 3,900 children born after ovarian stimulation with or without IUI compared with SC children. In addition, they had increased risk of asthma, allergy, diarrhea, and hospitalization. In an Australian study, Hoy et al. (15) studied 1,600 births after IUI-D and found no increase in PTB or LBW compared with SC children.

Only a few studies have compared children born after IUI with children born after IVF. Noujua-Huttunen et al. (7) compared perinatal outcome in 92 IUI-H singletons with 276 IVF singletons and found no significant difference in birth weight and gestational age between the groups. In a Belgian questionnaire study from 2005, De Sutter et al. (8) compared 126 children born after IUI to a matched control group of IVF children and found no difference between the groups. However, both studies are limited by small sample sizes. Wang et al. (16) assessed perinatal outcome of 1,015 children born after IUI and 1,019 children born after ART

TABLE 3

Risks of adverse perinatal outcomes in singletons born after IUI-H and IUI-D, compared to IVF, ICSI, and SC.

	IUI vs. IVF	IUI vs. ICSI	IUI vs. SC
IUI-H, crude			
LBW	0.691 [0.574–0.832]	0.902 [0.737–1.104]	1.457 [1.263–1.680]
PTB	0.621 [0.509–0.757]	0.944 [0.755–1.180]	1.345 [1.151–1.572]
SGA	0.976 [0.779–1.224]	1.135 [0.890–1.447]	1.393 [1.185–1.447]
LGA	1.293 [0.978–1.709]	1.030 [0.785–1.352]	0.905 [0.752–1.089]
IUI-H, adjusted			
LBW	0.751 [0.618–0.914]	0.933 [0.754–1.154]	1.435 [1.235–1.667]
PTB	0.632 [0.512–0.779]	0.948 [0.748–1.201]	1.264 [1.071–1.490]
SGA	1.118 [0.883–1.417]	1.245 [0.965–1.607]	1.394 [1.180–1.648]
LGA	1.096 [0.820–1.466]	0.917 [0.689–1.220]	0.966 [0.793–1.175]
IUI-D, crude			
LBW	0.701 [0.551–0.894]	0.916 [0.710–1.182]	1.479 [1.198–1.826]
PTB	0.615 [0.473–0.800]	0.935 [0.705–1.239]	1.332 [1.056–1.680]
SGA	1.010 [0.760–1.343]	1.174 [0.872–1.582]	1.441 [1.137–1.826]
LGA	1.491 [1.069–2.078]	1.188 [0.857–1.646]	1.044 [0.806–1.351]
IUI-D, adjusted			
LBW	0.714 [0.554–0.920]	0.886 [0.678–1.158]	1.359 [1.089–1.696]
PTB	0.591 [0.447–0.781]	0.885 [0.657–1.194]	1.177 [0.919–1.507]
SGA	1.077 [0.802–1.446]	1.199 [0.879–1.635]	1.338 [1.048–1.707]
LGA	1.314 [0.930–1.856]	1.098 [0.780–1.545]	1.157 [0.882–1.519]

Note: Values are odds ratio (crude) or adjusted odds ratio (adjusted), with 95% confidence intervals. Values were adjusted for year of birth, parity, maternal age, child gender, BMI, smoking, elective CS, and induction of labor. SC = spontaneous conception; UI-H = intrauterine insemination with partner semen; IUI-D = intrauterine insemination with donor semen.

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and found that both groups had increased risk of PTB compared with SC children, but the association was stronger in the ART group. No comparisons were made directly between IVF/ICSI and IUI. Interestingly, the risk of PTB did not rise with increasing time to pregnancy.

The perinatal outcomes among children born after IUI are less favorable than those after SC, but better than those for children born after IVF. This difference may be related to the fact that the IUI group overall is less reproductively challenged than the IVF group, with shorter duration of subfertility and a less severe reproductive problem. The treatment is less invasive, allowing fertilization in vivo, and the ovarian stimulation is milder. The twin rates, and possibly the vanishing twin phenomenon, are higher after IUI compared with SC pregnancies, but lower compared with ART pregnancies. The slight increase in hypertensive disorders in pregnancy and placenta previa seen in IUI compared with SC pregnancies may also have an effect on the perinatal outcome of the children.

Children born after IUI vs. after ICSI showed no significant differences in perinatal outcomes. Children born after ICSI have previously been described as having a lower risk of PTB and LBW compared to children born after IVF (10). The causal relationship behind this difference is difficult to determine. One theory is that female reproductive morbidity has a greater impact on perinatal outcome than male-factor infertility. This theory is consistent with the fact that approximately 55% of the ICSI cohort in this study were referred because of male-factor infertility, by far the highest prevalence among the study groups, most closely followed by IUI-D with 27.6% (Supplemental Table 1, available online). However, no differences were found between anovulation and male-factor infertility in terms of perinatal outcome.

TABLE 4

Crude and adjusted risks of PTB, LBW, and SGA in singletons born after IUI.

	CC vs. natural cycle	FSH vs. natural cycle	FSH + CC vs. natural cycle	Idiopathic vs. male factor	Anovulation vs. male factor	IUI-H vs. IUI-D
Crude						
LBW	1.496 [1.079–2.074]	0.875 [0.590–1.299]	1.556 [1.124–2.154]	1.253 [0.851–1.845]	1.041 [0.693–1.564]	1.015 [0.789–1.307]
PTB	1.151 [0.806–1.646]	0.817 [0.539–1.239]	1.288 [0.909–1.826]	1.359 [0.881–2.099]	1.134 [0.717–1.794]	0.990 [0.750–1.307]
SGA	1.605 [1.109–2.324]	1.154 [0.758–1.758]	1.366 [0.930–2.005]	0.939 [0.591–1.493]	0.801 [0.492–1.304]	1.034 [1.778–1.375]
Adjusted						
LBW	1.459 [1.032–2.064]	0.872 [0.577–1.317]	1.637 [1.164–2.300]	1.142 [0.752–1.734]	1.024 [0.656–1.599]	1.014 [0.775–1.326]
PTB	1.044 [0.710–1.537]	0.814 [0.528–1.255]	1.253 [0.867–1.813]	1.124 [0.709–1.782]	1.070 [0.652–1.757]	0.966 [0.716–1.303]
SGA	1.617 [1.110–2.385]	1.233 [0.800–1.901]	1.461 [0.983–2.172]	0.961 [0.594–1.555]	0.796 [0.479–1.324]	0.992 [0.736–1.337]

Note: Values are odds ratio (crude) and adjusted odds ratio (adjusted), with 95% confidence interval. For CC and FSH treatment, cases treated with CC and FSH were excluded. Adjusted values are adjusted for year of birth, parity, maternal age, child gender, BMI, smoking, elective CS, and induction of labor. CC = clomiphene citrate; FSH = follicle stimulating hormone; IUI-H = intrauterine insemination with partner semen; IUI-D = intrauterine insemination with donor semen.

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We also found a higher incidence of placenta previa in the IUI-H group compared to the SC group, but lower than in the IVF and ICSI groups. In the IUI-D group, the incidence of placenta previa was similar to that in the SC group. In a Japanese study from 2013, risk of placenta previa in IVF pregnancies has been associated with endometriosis and tubal disease (17). This finding may explain our results, since the IUI-H group had a lower prevalence of tubal disease and endometriosis compared to the IVF and ICSI groups.

Considering the many factors that affect perinatal outcome, we aimed to explore predictors of poor outcome in our stratified analysis. The risks of LBW, PTB, and SGA did not differ between male- and female- (anovulatory) factor infertility, or with use of donor or partner semen. This finding is interesting since the IUI-D and IUI-H groups are very different. The IUI-H group consists mainly of couples with a reproductive disease who usually inseminated in a stimulated cycle. The IUI-D group predominantly includes women without a reproductive disease (e.g., male-factor infertility, lesbian, or single women) who are inseminated in an unstimulated cycle. However, the IUI-D group is heterogeneous, as it also consists of couples with previous failed ART treatment, and a proportion of the IUI-D group did receive ovarian stimulation. Further, maternal age, primiparity, smoking, incidence of hypertensive disorders in pregnancy, and induction of labor is increased in IUI-D compared with IUI-H, equalizing outcome in the 2 groups.

The increased tendency to develop preeclampsia in pregnancies after IUI-D is described in previous studies and may be related to immunologic mechanisms, as the women have no previous exposure to the paternal semen (18–20). Even though the risks of LBW, PTB, and SGA were similar in the IUI-H and IUI-D group, we did find a lower mean birth weight and gestational age in the IUI-H pregnancies. The difference in birth weight and gestational age between the groups could in part be related to various degrees of subfertility and differences in maternal characteristics, considering the heterogeneity between and within the IUI-H and IUI-D groups. However, the slightly lower gestational age may not be clinically relevant, since it is not followed by increased risk of PTB and SGA, markers of short- and long-term morbidity in the child.

Our results indicate higher risks of LBW and SGA after treatment with CC. This relationship was also present in the group in which CC was combined with additional FSH administration, but not in the group who received FSH alone for ovarian stimulation. However, the results were not adjusted for subfertility, since the recording of information on duration of infertility was sparse. These data were reported by only some clinics, and not at all for single and lesbian women. Further, in the IVF register, "duration of infertility" is recorded rather imprecisely as the first year the couple attempted to conceive, but which month of that year is not specified. Exploring the effect of duration of infertility would have been interesting in this study. However, another consideration is that time to pregnancy is influenced by not only subfertility per se, but also how soon the couples seek help to conceive.

The effect of ovarian stimulation on perinatal outcome has been explored in a few earlier studies. In a Swedish study, Källén et al. (6) found increased risk of LBW and PTB in 4,307 children born after ovarian stimulation (IVF + ICSI excluded) compared with SC children. The risks decreased but remained significant after additional adjustment for duration of subfertility. The Swedish study did not specify the medication used for the ovarian stimulation.

Nakashima et al. (21) investigated birth weight in children born after IVF, exploring the effect of CC, FSH, and natural cycle. The study compared 981 children conceived after stimulation with CC and IVF to 610 children born after natural-cycle IVF, finding an increased risk of LBW with doubled adjusted odds ratio. In the same study, 1,237 children born after IVF following treatment with CC + FSH/human menopausal gonadotropin also had a higher risk of LBW compared with natural-cycle IVF. A third group of 322 children, conceived after ovarian stimulation with FSH/human menopausal gonadotropin alone (and IVF) did not have a higher risk of LBW compared to natural-cycle IVF. These results are thus similar to ours.

Hayashi et al. (22) compared 4,111 children born after ovarian stimulation (type of medication not specified) and timed intercourse to a matched control group of SC children. They also looked at 2,351 children born after natural-cycle IUI compared with another matched SC control group, finding increased risks of LBW, PTB, and SGA in both groups; however, risks were higher in the ovarian stimulation group. No comparisons were made between the 2 study populations.

Exploring the dose-effect of gonadotropins on birth weight, Griesinger et al. (23) investigated 32.000 IVF/ICSI singletons. After stratification on duration of stimulation, amount of gonadotropins administered and number of oocytes retrieved, there was no dose effect on birth weight. This finding is consistent with the current finding that stimulation with FSH had no association with SGA or LBW.

Our results do indicate, however, that stimulation with CC is a predictor of LBW and SGA. CC displaces endogenous estrogen from hypothalamic and pituitary estrogen receptor sites, thus resulting in negative feedback that leads to increased gonadotropin-releasing hormone secretion and increased endogenous FSH levels, which stimulate follicular growth and ovulation (24, 25). The anti-estrogenic effect of CC may be associated with endometrial thinning and affect cervical mucus (24, 26–28). Harita et al. (27) found decreased endometrial thickness, decreased endometrial growth rate, and increased serum estradiol levels, after CC treatment in 20 women with unexplained infertility, first observed in a natural cycle and then treated with CC. Further, CC treatment has been associated with decreased glandular density and an increased number of vacuolated cells in the endometrium of regularly ovulating women (29). It is unknown what consequence these adverse endometrial effects of CC have on implantation, placental development, and fetal growth, but they may be related to the findings of the current study.

In conclusion, IUI is associated with slightly higher obstetric and perinatal risks compared with those for SC children. Outcomes are favorable compared with those for

children born after IVF, and similar to those for children born after ICSI. Our data indicate that ovarian stimulation with CC is associated with higher risks of SGA and LBW compared with IUI in natural cycles. However, IUI following FSH treatment did not seem to increase risks of poor perinatal outcome compared with natural-cycle IUI.

REFERENCES

1. Danish society of fertility treatment. Available from: http://www.fertilitetsselskab.dk. Accessed December 1, 2013.
2. Ferraretti AP, Goossens V, Kupka M, Bhattacharya S, de Mouzon J, Castilla JA, et al. Assisted reproductive technology in Europe, 2009: results generated from European registers by ESHRE. Hum Reprod 2013;28:2318–31.
3. Ombelet W, Martens G, de Sutter P, Gerris J, Bosmans E, Ruyssinck G, et al. Perinatal outcome of 12,021 singleton and 3,108 twin births after non-IVF assisted reproduction: a cohort study. Hum Reprod 2006;21:1025–32.
4. Klemetti R, Sevon T, Gissler M, Hemminki E. Health of children born after ovulation induction. Fertil Steril 2010;93:1157–68.
5. Gaudoin M, Dobbie R, Finlayson A, Chalmers J, Cameron IT, Fleming R. Ovulation induction/intrauterine insemination in infertile couples is associated with low-birth-weight infants. Am J Obstet Gynecol 2003;188:611–6.
6. Källén B, Olausson PO, Nygren KG. Neonatal outcome in pregnancies from ovarian stimulation. Obstet Gynecol 2002;100:414–9.
7. Nuojua-Huttunen S, Gissler M, Martikainen H, Tuomivaara L. Obstetric and perinatal outcome of pregnancies after intrauterine insemination. Hum Reprod 1999;14:2110–5.
8. De Sutter P, Veldeman L, Kok P, Szymezak N, Van der Elst J, Dhont M. Comparison of outcome of pregnancy after intra-uterine insemination (IUI) and IVF. Hum Reprod 2005;20:1642–6.
9. Poon WB, Lian WB. Perinatal outcome of intrauterine insemination/clomiphene pregnancies represent an intermediate risk group compared with in vitro fertilisation/intracytoplasmic sperm injection and naturally conceived pregnancies. J Paediatr Child Health 2013;49:733–40.
10. Pinborg A, Wennerholm UB, Romundstad LB, Loft A, Aittomaki K, Söderström-Anttila V, et al. Why do singletons conceived after assisted reproduction technology have adverse perinatal outcome? Systematic review and meta-analyses. Hum Reprod Update 2013;19:87–104.
11. Romundstad LB, Romundstad PR, Von Düring A, Sunde A, Skjærven R, Gunell D, et al. Effects of technology or maternal factors on perinatal outcome after assisted fertilization: a population-based cohort study. Lancet 2008;372:737–43.
12. Messerlian C, Mclagan L, Basso O. Infertility and the risks of adverse pregnancy outcomes: a systematic review and meta-analysis. Hum Reprod 2013;28:125–37.
13. Kondapalli LA, Perales-Puchalt A. Low birth weight: Is it related to assisted reproductive technology or underlying infertility? Fertil Steril 2013;99:303–10.
14. Marsal K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. Intrauterine growth curves based on ultrasonically estimated foetal weights. Acta Paediatr 1996;85:843–8.
15. Hoy J, Venn A, Halliday J, Kovacs G, Waalwyk K. Perinatal and obstetric outcomes of donor insemination using cryopreserved semen in Victoria, Australia. Hum Reprod 1999;14:1760–4.
16. Wang JX, Norman RJ, Kristiansson P. The effect of various infertility treatments on the risk of preterm birth. Hum Reprod 2002;17:945–9.
17. Takemura Y, Osuga Y, Fujimoto A, Nagisa O, Tsutsumi R, Koizumi M, et al. Increased risk of placenta previa is associated with endometriosis and tubal factor infertility in assisted reproductive technology pregnancy. Gynecol Endocrinol 2013;29:113–5.
18. Dekker GA, Sibai BA. Etiology and pathogenesis of preeclampsia: current concepts. Am J Obstet Gynecol 1998;179:1359–75.
19. Need J, Bell B, Meffin E, Joner WR. Pre-eclampsia in pregnancies from donor inseminations. Reprod Immunol 1983;5:329–38.
20. Kho EM, McCowan L, North RA, Roberts CT, Chan E, Black MA. Duration of sexual relationship and its effect on preeclampsia and small for gestational age perinatal outcome. J Reprod Immunol 2009;82:66–73.
21. Nakashima A, Araki R, Tani H, Ishihara O, Kuwahara A, Irahara M, et al. Implications of assisted reproductive technologies on term singleton birth weight: an analysis of 25,777 children in the national assisted reproduction registry of Japan. Fertil Steril 2013;99:450–5.
22. Hayashi M, Nakai A, Satoh S, Matsuda Y. Adverse obstetric and perinatal outcomes of singleton pregnancies may be related to maternal factors associated with infertility rather than type of assisted reproductive procedure used. Fertil Steril 2012;98:922–8.
23. Griesinger G, Kolibianakis EM, Diedrich K, Ludwig M. Ovarian stimulation for IVF has no quantitative association with birth weight: a registry study. Hum Reprod 2008;23:2549–54.
24. Homburg R. Clomiphene citrate—end of an era? A mini review. Hum Reprod 2005;20:2043–51.
25. Kousta E, White DM, Franks S. Modern use of clomiphene citrate in induction of ovulation. Hum Reprod Update 2007;3:359–65.
26. Badawy A, Shokeir T, Allam AF, Abdelhady H. Pregnancy outcome after ovulation induction with aromatase inhibitors or clomiphene citrate in unexplained infertility. Acta Obstet Gynecol 2009;88:187–91.
27. Haritha S, Rajagopalan G. Follicular growth, endometrial thickness, and serum estradiol levels in spontaneous and clomiphene citrate-induced cycles. Int J Gynaecol Obstet 2003;81:287–92.
28. Gonen Y, Casper RF. Sonographic determination of a possible adverse effect of clomiphene citrate on endometrial growth. Hum Reprod 1990;5:670–4.
29. Sereepapong W, Suwajanakorn S, Triratanachat S, Sampatanukul P, Pruksananonda K, Boonkasemsanti W, et al. Effects of clomiphene citrate on the endometrium of regularly cycling women. Fertil Steril 2002;73:287–91.

SUPPLEMENTAL TABLE 1

Duration and cause of infertility in singleton IUI-H and IUI-D pregnancies compared with IVF and ICSI.

					P value			
	IUI-H	IUI-D	IVF	ICSI	IUI-H vs. IVF	IUI-H vs. ICSI	IUI-D vs. IVF	IUI-D vs. ICSI
N, total	4,208	1,881	4,135	3,635				
Time to birth (y), median (IQR)	2 (0–16)	2 (0–13)	3 (0–20)	3 (0–21)	<.001	<.001	<.001	<.001
Time to birth (y), N (%)								
>2	451 (44.2)	138 (45.7)	403 (72.7)	340 (72.4)	<.001	<.001	<.001	<.001
>3	146 (14.3)	72 (23.8)	234 (42.2)	189 (39.7)	<.001	<.001	<.001	<.001
Missing	3,187 (75.7)	3,581 (86.6)	3,159 (86.9)	1,579 (83.9)	<.001	<.001	.024	.012
Cause of infertility, N (%)								
Idiopathic	1,104 (26.2)	121 (6.4)	923 (22.3)	524 (14.4)	<.001	<.001	<.001	<.001
Anovulation	818 (19.4)	28 (1.5)	376 (9.1)	181 (5.0)	<.001	<.001	<.001	<.001
Tubal	52 (1.2)	5 (0.3)	635 (15.4)	169 (4.6)	<.001	<.001	<.001	<.001
Uterine	12 (0.3)	0 (0)	24 (0.6)	8 (0.2)	.16	.57	<.001	.16
Cervical	2 (0.0)	0 (0)	1 (0.0)	5 (0.1)	.18	.57	.50	.10
Ovarian	94 (2.2)	7 (0.4)	96 (2.3)	72 (2.0)	.44	.79	<.001	<.001
Male factor	481 (11.4)	519 (27.6)	513 (12.4)	2,020 (55.6)	.17	<.001	<.001	<.001
Other	1,575 (37.4)	1,134 (60.3)	1,535 (37.1)	558 (15.4)	.77	<.001	<.001	<.001
Endometriosis	30 (0.7)	3 (0.2)	195 (4.7)	73 (2.0)	<.001	<.001	<.001	<.001
Missing	70 (1.7)	67 (3.6)	32 (0.8)	98 (2.7)	<.001	.008	<.001	.074

Note: IUI-H = intrauterine insemination with partner semen; IUI-D = intrauterine insemination with donor semen.

Malchau. Perinatal outcomes after insemination. Fertil Steril 2014.

SUPPLEMENTAL TABLE 2

Incidence of singleton and multiple gestations in pregnancies conceived after IUI-H and IUI-D, compared with pregnancies conceived after IVF, ICSI, and SC.

	IUI-H	IUI-D	IVF	ICSI	SC	*P* value					
Deliveries, N (total)	4,804	2,019	4,888	4,341	233,118						
Singleton, N (%)	4,208 (87.3)	1,881 (93.2)	4,135 (84.6)	3,635 (83.8)	229,749 (98.6)	<.001	<.001	<.001	<.001	<.001	<.001
Twin, N (%)	583 (12.2)	137 (6.8)	747 (15.3)	701 (16.1)	3,330 (1.4)	<.001	<.001	<.001	<.001	<.001	<.001
Triplet, N (%)	14 (0.3)	1 (0.0)	6 (0.1)	5 (0.1)	39 (0.0)	.08	.08	<.001	.33	.37	.223

Note: IUI-H = intrauterine insemination with partner semen; IUI-D = intrauterine insemination with donor semen; SC = spontaneous conception.

Malchau. Perinatal outcomes after insemination. Fertil Steril 2014.