**Human Reproduction, Vol.34, No.1 pp. 171–180, 2019**
Advanced Access publication on December 12, 2018 doi:10.1093/humrep/dey341

human reproduction

**ORIGINAL ARTICLE** ***Reproductive epidemiology***

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

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

[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]University of Copenhagen, Department of Public Health, Section of Biostatistics, Øster Farimagsgade 5, DK-1014 KBH K, Denmark

*Correspondence address: Department of Obstetrics and Gynecology, Copenhagen University Hospital Hvidovre, Kettegård Allé 26, DK-2650, Hvidovre Denmark. E-mail: smalchau@hotmail.com

*Submitted on June 18, 2017; resubmitted on August 23, 2018; accepted on November 1, 2018*

**STUDY QUESTION:** Is the number of aspirated oocytes in the first 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 five 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, 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 one year follow-up from last treatment, 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 CLBRs attributable to ART treatment 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 attributable to non-ART related conception (natural conception or intrauterine insemination) 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 second and third cycle: When compared to women with 0–3 aspirated oocytes in the first cycle, the odds for live birth in the second and third cycle was 1.18 [1.07–1.30] for women with 4–9 aspirated oocytes in the first cycle, 1.41 [1.27–1.57] for women with 10–15 aspirated oocytes and 1.63 [1.42–1.88] for women with more than 15 aspirated oocytes. For women without a livebirth in the first and second cycle, the sum of aspirated oocytes predicted outcome in the third complete cycle. Women with a sum larger than six aspirated oocytes, had marked increased odds ratios for livebirth in the third complete cycle, compared to women with a sum of 0–6 oocytes in the first and second

fresh cycle. Incidence of hospital-admission due to OHSS was 1.7% in the first cycle, decreasing to 1.3% and 1.0% in the second and third cycles.

**LIMITATIONS, REASONS FOR CAUTION:** Although mandatory, there may be treatment-cycles not registered in the IVF-registry. Missing information in number of aspirated oocytes are most likely random losses of information. There were few observations in women with more than 15 aspirated oocytes and these birthrates should be interpreted cautiously. Information on gonadotrophin dose used for stimulation was not available, nor was information on dose adjustments in subsequent cycles.

**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.

**STUDY FUNDING/COMPETING INTEREST(S):** This study was unconditionally funded by Ferring Pharmaceuticals and ReproUnion. The funders had no role in the study design, data collection and interpretation, or decision to submit the work for publication. The authors have no conflicts of interest.

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

**Key words:** IVF/ICSI outcome / cumulative live birth rate / live birth rate / ovarian response / ART treatment / oocyte / natural conception / infertility prognosis

# Introduction

Fertility treatment is demanding. The couples face side effects, financial burdens and emotional strain (Sylvest *et al.*, 2016; Klitzman, 2017). 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 (Sunkara *et al.*, 2011; Briggs *et al.*, 2015; Drakopoulos *et al.*, 2015). 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 six complete cycles (6 fresh cycles with frozen–thawed transfers), compared to women with less than five aspirated oocytes in the first cycle (McLernon *et al.*, 2016). This may indicate that a low treatment response in the first cycle is likely to be repeated in later cycles. However, there are studies that describe a positive effect on the number of aspirated oocytes with an increased FSH start dose in the second cycle, in women with a low treatment response in the first cycle (Lashen *et al.*, 1998; Popovic-Todorovic *et al.*, 2004).

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 second and third 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 1 January 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 1 January 2002 to 31 December 2011. Follow-up on the births were available until 31 December 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 resulted in the first delivery after treatment-start and within the study period (prior to 31 December 2011), but regardless of time interval since first treatment. This means that women who received treatment in the early part of the study period had longer time to achieve a naturally conceived livebirth, compared to women who received treatment at a later point in the study period. 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 30 485 women and 94 025 fresh or frozen–

thawed 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 (Hermann, 2015). Further, the guideline recommends single embryo transfer if it was the first or second fresh transfer and if the woman was 37 years or below. There was a maximum of two embryos that could be transferred. 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. Cumulated live birth rates over several complete cycles are conservative estimates, based on observed livebirths without censoring of couples that discontinue treatments, assuming that they will not give birth. In contrast, in optimistic estimates it is assumed that couples who drop out of treatment, would have had the same chance of conceiving as the couples who continue, had they continued.

The livebirth rates in the first to fourth 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 first complete cycle was assessed with multivariable logistic regression analysis including the predictors: number of retrieved oocytes (linear spline with break points at 4, 10 and 15 oocytes), female age (linear spline with break points at 35 and 40 years) and cause of infertility (categorical). The number of observations in women with 0 aspirated oocytes or more than 20 aspirated oocytes was too small to be explored separately.

The association between number of aspirated oocytes in the first cycle and treatment success within the second and third complete cycles was likewise assessed with multivariable logistic regression analysis, including number of retrieved oocytes in the first cycle (categorical four categories), female age (linear spline), and cause of infertility as predictors.

The association between livebirth in the third complete cycle and the sum of retrieved oocytes in the first and the second fresh ART-cycle was assessed with multivariable logistic regression analysis including the sum of retrieved oocytes in first and second 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.

## Ethical 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%) were less than 35 years old at the time of their first treatment.

In this study we included 30 485 women and 94 025 fresh and frozen–thawed cycles (Fig. 1). 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 (Supplementary 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 Fig. 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 second 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 fourth 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 first treatment and non-ART birth was 20 months (interquartile range, IQR 13–31).

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 first 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.

## Frozen–thawed cycles

Out of all women starting the first fresh cycle, 14.5% had one or more frozen–thawed cycles before proceeding to the second fresh cycle. The proportion of frozen–thawed cycles was similar in women starting the second, third and fourth complete cycle. Among women who had

**Table I Background and treatment characteristics in women starting first to fourth fresh ART cycle, 2002–2011.**

| | First fresh ART cycle *N* (%) | Second fresh ART cycle *N* (%) | Third fresh ART cycle *N* (%) | Fourth fresh ART cycle *N* (%) |
|---|---|---|---|---|
| Starting ovarian stimulation | 30 485 (100) | 17 500[1] (100)[1] | 9697[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 | 512 (2.4) [5] | 156 (1.3) [5] | 74 (1.1)[5] | 34 (1.1)[5] |
| 1–3 | 3408 (16.2)[5] | 1668(13.9)[5] | 957 (14.6)[5] | 464 (14.7)[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] |
| N oocytes, mean ±SD | 8.2 ± 5.3 | 8.4 ± 4.9 | 8.3 ± 4.8 | 8.3 ± 4.9 |
| Ovarian Hyper stimulation syndrome | 517 (1.7) | 224 (1.3) | 95 (1.0) | 40 (0.9) |
| **Female age (y) at 1st treatment** | | | | |
| <35 | 19 828 (65.0) | 11 061 (63.2) | 5865 (60.5) | 2771 (60.6) |
| 35–40 | 7976 (26.2) | 4802 (27.4) | 2830 (29.2) | 1371 (30.0) |
| ≥40 | 2643 (8.7) | 1619 (9.3) | 993 (10.2) | 423 (9.3) |
| mean ± 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) |
| Idiopathic | 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]In all 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.

frozen–thawed cycles, 80.3% [79.1–81.5] had one FET cycle, 15.8% [14.7–17.0] had two FET cycles and 3.9% [3.3–4.5] had three or more FET cycles. The distribution was similar in cycles 2 through 4.

In the first complete cycle, a total of 9.7% [9.1–10.4] of ART-related livebirths were conceived in a frozen–thawed cycle. In the second complete cycle, 8.2% [7.4–9.1] of all livebirths were conceived after FET. In the third complete cycle, the proportion was 6.4% [5.4–7.5], and in the fourth cycle, it was 10.3% [8.5–12.4].

## Number of retrieved oocytes as a predictor of treatment outcome

Age-stratified live birthrates after the first, second, third and fourth complete cycle by number of aspirated oocytes in the corresponding cycle are shown in 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 first complete ART cycle, by number of aspirated oocytes, are shown in Fig. 2. CLBR increased with increasing number of oocytes up to 16 oocytes, after which the birthrate seems 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 1–3 aspirated oocytes, odds for livebirth increased with

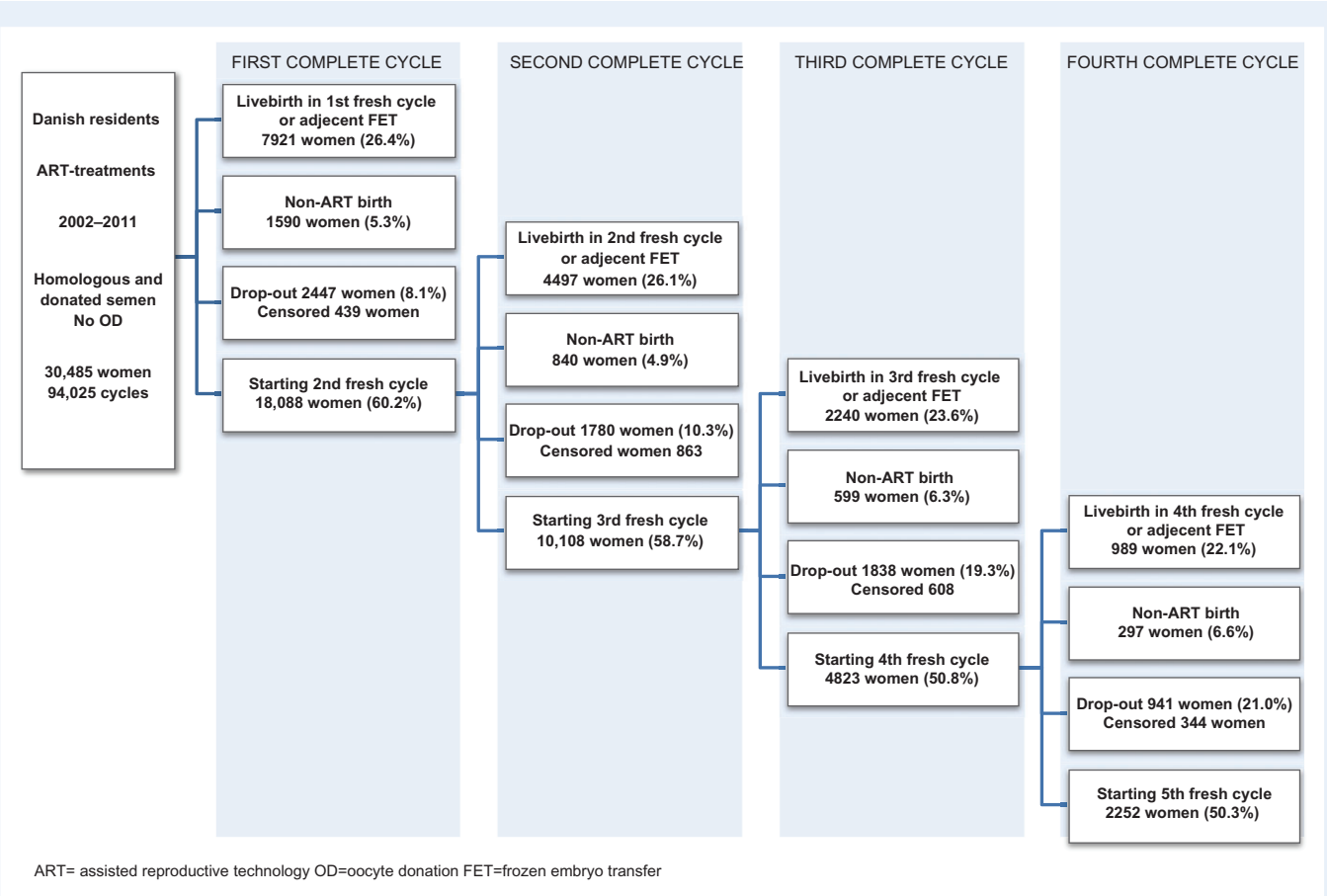


**Figure 1 Flow chart over included cases and their course of treatments, women followed until their first livebirth**. Cases are censored if follow-up is less than one year from start-date of fresh or frozen–thawed treatment cycle (FET), proportions are % of non-censored cases.

59.6% [40.9–80.7] for each added oocyte. In women with 4–9 aspirated oocytes, odds increased with 12.2% [10.0–14.5] for each added oocyte. In women with 10–15 aspirated oocytes, odds increased with 1.8% [−0.3–3.8] for each added oocyte, not statistically significant. In women with >15 aspirated oocytes, odds did not increase significantly by more oocytes, 0.0% [−2.2–1.9].

Adjusted odds for livebirth in the second or third 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 was associated with outcome in the second and third complete cycle. When compared to women with 0–3 aspirated oocytes in the first cycle, the odds for live birth in the second and third cycle was 1.18 [1.07–1.30] for women with 4–9 aspirated oocytes in the first cycle, 1.41 [1.27–1.57] for women with 10–15 aspirated oocytes and 1.63 [1.42–1.88] for women with more than 15 aspirated oocytes.

For women with no livebirth in the first and second complete cycles, the sum of aspirated oocytes in the first and second fresh cycle predicted outcome in the third complete cycle, as seen in Table IV.

Women with a sum of 0–6 aspirated oocytes, had marked decreased odds ratios for livebirth in the third complete cycle, compared to women with a larger sum of oocytes in the first and second fresh cycle.

For women with a sum of 0–6 oocytes in the first two cycles, the observed live birthrates in the third 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. The observed live birthrates in the third 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.

## Dose adjustments in subsequent cycles

In women who returned for a second fresh cycle (who did not have a livebirth in the first cycle), we assessed the difference in the number of aspirated oocytes between the first and second cycle, as shown in Fig. 3.

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 second 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 second cycle, respectively (Fig. 3).

## 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 second cycle, 1.0% [0.8–1.2] in the third cycle and 0.9% [0.6–1.2] in the fourth cycle (Table I). As expected the incidence of OHSS increased with higher number of aspirated oocytes (Supplemental Table SIII).

**Table II Live birthrates in first, second, third, and fourth 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 | 13 078 | | 5313 | | 1708 | |
| 1–3 | 1756 | 16.9 [15.2–18.8] | 1080 | 10.7 [9.0–12.7] | 517 | 5.4 [3.6–7.7] |
| 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 | 7310 | | 3227 | | 1071 | |
| 1–3 | 764 | 17.5 [14.9–20.4] | 580 | 10.9 [8.4–13.7] | 300 | 2.7 [1.2–5.2] |
| 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 | 3783 | | 1881 | | 648 | |
| 1–3 | 386 | 16.8 [13.2–21.0] | 351 | 9.1 [6.3–12.6] | 197 | 5.1 [2.5–9.1] |
| 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 | 1821 | | 905 | | 312 | |
| 1–3 | 200 | 16.0 [11.2–21.8] | 176 | 6.8 [3.6–11.6] | 79 | 3.8 [0.8–10.7] |
| 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.
[2]A total of two women had a livebirth in the first complete cycle, despite the record of 0 retrieved oocytes.

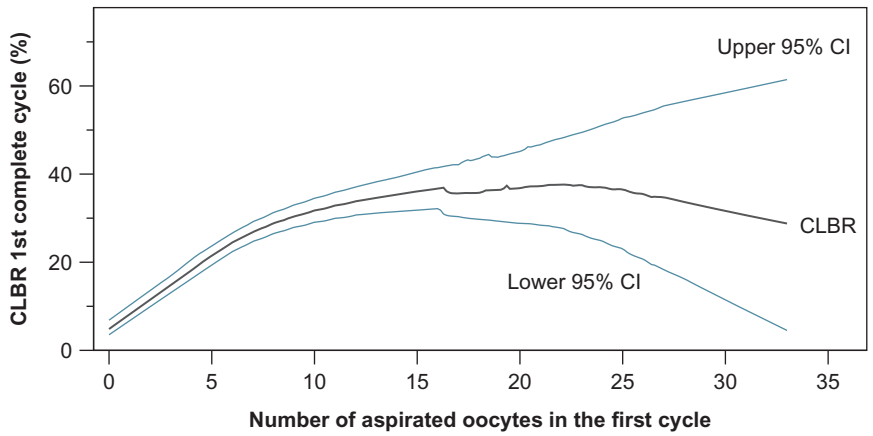


**Figure 2 Cumulative live birthrates (CLBR) with 95% confidence intervals in the first fresh ART cycle with possible adjacent frozen–thawed transfers, by number of aspirated oocytes, Denmark 2002–2011**

# 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 second and third 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 first and second cycle had decreased odds for livebirth in the third complete cycle. However, for women with 0–3 aspirated oocytes in the first cycle, the majority of women under 40 years of age at time of first treatment had an improved treatment response in the second cycle.

The observed birthrates in this study are consistent with findings in previous Swedish and US studies, reporting conservative estimates (the proportion of livebirth among all couples starting treatment, without censoring drop-outs) (Olivius *et al.*, 2002; Stern *et al.*, 2010; Luke *et al.*, 2012). However, since the study period, there have been advancements in fertility treatment and especially in frozen–thawed cycles, which have led to improved birthrates.

An association between number of aspirated oocytes and live birthrates has previously been demonstrated per fresh cycle (Sunkara *et al.*, 2011; Steward *et al.*, 2014; Baker *et al.*, 2015; Briggs *et al.*, 2015) as well as per complete cycle (Bdolah *et al.*, 2015; Drakopoulos *et al.*, 2015; Polyzos *et al.*, 2018). 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 (Sunkara *et al.*, 2011). Another large retrospective cohort study from the US, showed increasing live birthrates up to 15 oocytes (Steward *et al.*, 2014). 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 (Drakopoulos *et al.*, 2015). A large multicenter retrospective study showed progressively increasing CLBR with increasing number of oocytes, without a plateau (Polyzos *et al.*, 2018). These results were based on complete ART cycles, including contribution from vitrified frozen–thawed cycles. However, no confidence intervals for the CLBR in the different oocyte categories were reported. 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 (Kupka *et al.*, 2015; Danish Fertility Society Annual Reports, 1997–2016; Dyer *et al.*, 2016).

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 (Lashen *et al.*, 1998; Popovic-Todorovic *et al.*, 2004). A Danish retrospective study reported outcome in 385 patients returning for a second ART cycle (after an unsuccessful first cycle) and showed improved number of aspirated oocytes when rFSH start-dose was increased in the second ART cycle (Popovic-Todorovic *et al.*, 2004). 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 (Vaegter *et al.*, 2017). Another Swedish study showed

**Table III Adjusted[1] odds ratios for treatment related livebirth in the second or third complete cycle, by number of aspirated oocytes in the first cycle, Denmark 2002–2011.**

| Number of aspirated oocytes | Livebirth in the second or third complete cycle and number of aspirated oocytes in the first cycle aspiration AOR[1] [95% CI] |
|---|---|
| 0–3 | 1.0 (ref) |
| 4–9 | 1.18 [1.07–1.30] |
| 10–15 | 1.45 [1.27–1.58] |
| >15 | 1.63 [1.42–1.88] |

[1]AOR, adjusted odds ratio, including number of aspirated oocytes, female age and cause of infertility.
[2]One complete cycle defined as one fresh cycle and possible following frozen–thawed transfers

**Table IV Age-stratified live birthrates and adjusted[1] odds ratios (AOR) for livebirth after ART-conception in the third complete[2] ART-cycle, by number of aspirated oocytes in the first and second cycle, in women with no livebirth in the first or second complete cycle, Denmark 2002–2011.**

| Sum of aspirated oocytes first & second cycle | Observed live birthrate third complete cycle | | | |
|---|---|---|---|---|
| | Age <35 years % [95% CI] | Age 35–39 years % [95% CI] | Age ≥40 years % [95% CI] | Livebirths AOR [95% CI] |
| 0–6 | 21.5 [17.3–26.4] | 11.5 [8.2–16.0] | 5.6 [3.0–10.2] | 1.0 (ref) |
| 7–12 | 25.2 [22.5–28.1] | 17.4 [14.2–20.9] | 6.6 [3.6–10.8] | 1.34 [1.05–1.71] |
| 13–19 | 29.6 [27.0–32.3] | 19.1 [15.8–22.7] | 8.3 [4.2–14.4] | 1.61 [1.27–2.04] |
| 20–30 | 30.8 [27.9–33.8] | 20.0 [16.1–24.3] | 9.5 [5.1–17.0] | 1.71 [1.34–2.19] |
| >30 | 32.0 [26.0–38.5] | 22.7 [13.8–33.8] | 13.6 [2.9–34.9] | 1.84 [1.33–2.54] |

[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.

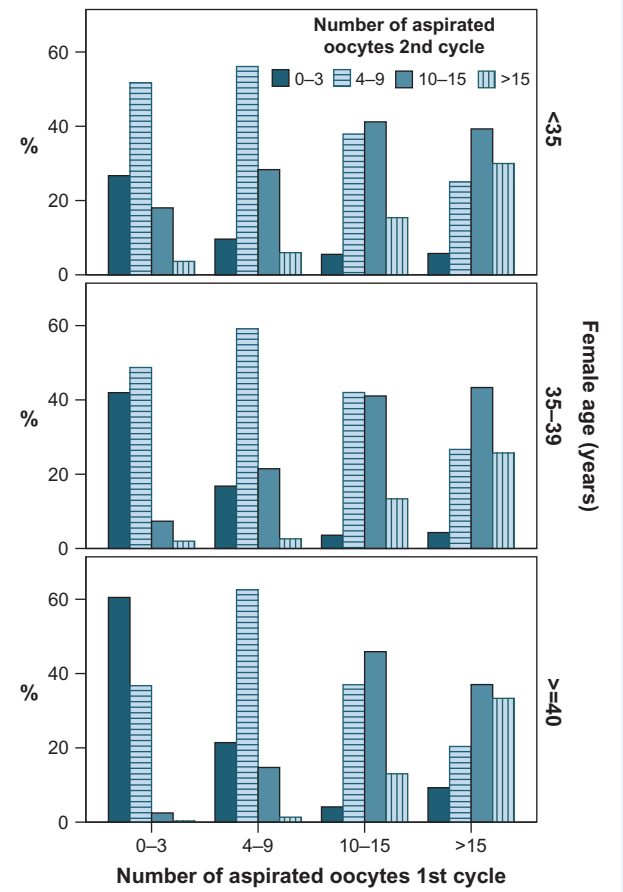


**Figure 3 Number of aspirated oocytes in second fresh cycle by number of aspirated oocytes in the first fresh cycle and female age**

that a combination of Anti-Müllerian hormone, age and antral follicle count best predicted ovarian response (Brodin *et al.*, 2015). The IVF register does 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 second cycle. For women aged below 35 years, three in four had an improved treatment response in the second 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 (Ferraretti *et al.*, 2011). 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 (Polyzos *et al.*, 2014). 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 are 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 (Chai *et al.*, 2015). 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 (Ke *et al.*, 2013; Yang *et al.*, 2016). 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 first cycle and birthrates in later cycles (McLernon *et al.*, 2016). The study report birthrates within three complete cycles of ~25% for women aged 40, who had IVF treatment, five eggs collected, no embryos frozen, and transferal of a single cleavage stage embryo in the first cycle (McLernon *et al.*, 2016). The predicted estimates from the UK-study are roughly consistent with our observed live birthrates in women aged 40 and over, with five 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 (Smeenk *et al.*, 2004; Daya, 2005; Malizia *et al.*, 2009).

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. 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) may be underestimated. Our results also showed increasing incidence of OHSS with increasing number of aspirated oocytes and the incidence of OHSS decreased with increasing cycle number. This may be explained by selection of the women continuing treatment: women with previous unsuccessful treatment 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.

## 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 (Hermann, 2015). 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 three cycles of insemination treatments (predominantly combined with ovulation induction/ovarian stimulation). For couples with anovulatory infertility, up to six cycles are offered. A previous study from this research group has shown that these recommendations are followed in the vast majority of cases (Malchau *et al.*, 2017). 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 first cycle and outcome in later cycles, may be due to frozen embryos originating from the first fresh cycle that were left over and not transferred until after oocyte pick-up in the second or third cycle. However, it is recommended by the Danish health authorities that all frozen embryos are used before proceeding to the next ovarian stimulation (Hermann, 2015).

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. There were missing data in number of aspirated oocytes. 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. Further, when number of aspirated oocytes is used as an explanatory variable and not an outcome variable, the main concern with missing data is reduced power.

# 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.

# Supplementary data

Supplementary data are available at *Human Reproduction* online.

# Acknowledgements

The authors gratefully thank the Danish fertility clinics, for reporting their treatment activity.

# Authors' roles

SSM, AAH, AL, JF, ANA, and AP contributed to the study design, analysis, data interpretation and further revised the manuscript. SSM was responsible for data-management and for writing the first draft of the manuscript. The final manuscript was approved by all co-authors.

# Funding

This study was partly and unconditionally funded by Ferring Pharmaceuticals and ReproUnion. The funders had no role in the study design, data collection and interpretation, or decision to submit the work for publication.

# Conflict of interest

The authors have no conflicts of interest.

# References

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**:931–938.e2. Elsevier Inc.

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 blastomere survival rate. *J Bras Reprod Assist* 2015;**19**:210–215.

Briggs R, Kovacs G, MacLachlan V, Motteram C, Gordon Baker HW. Can you ever collect too many oocytes? *Hum Reprod* 2015;**30**:81–87.

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**:1056–1063.

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**:1–10.

Danish Fertility Society Annual Reports 1997-2016 Available from: www.fertilitetsselskab.dk/images/2017/dfs1997-2016oversigt_140317.pdf.

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

Drakopoulos P, Blockeel C, Stoop D, Camus M, De Vos M, Tournaye H, Polyzos NP. 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**:370–376.

Dyer S, Chambers GM, De Mouzon J, Nygren KG, Zegers-Hochschild F, Mansour R, Ishihara O, Banker M, Adamson GD. International committee for monitoring assisted reproductive technologies world report: assisted reproductive technology 2008, 2009 and 2010†. *Hum Reprod* 2016;**31**:1588–1609.

Ferraretti AP, Marca La 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**:1616–1624.

Hermann M Vævsbekendtgørelsen - Vejledning om sundhedspersoners og vævscentres virksomhed og forpligtelser i forbindelse med assisteret reproduktion - retsinformation.dk. 2015;Available from: https://www.retsinformation.

dk/Forms/R0710.aspx?id=172755#ide681ffa8-817c-4e1e-96ff-5ce8c2fa0a2a. Assessed May 2017.

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**:418–422.

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 [Internet]* 2017;**12**:e0171939.

Kupka MS, D'Hooghe T, Ferraretti AP. Mouzon J De, Erb K, Castilla JA, Calhaz-Jorge C, Geyter C De, Goossens V. Assisted reproductive technology in Europe, 2011: results generated from European registers by ESHRE. *Hum Reprod* 2015;**31**:233–248.

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 [Internet]* 1998;**15**:438–443.

Luke B, Brown MB, Wantman E, Lederman A, Gibbons W, Schattman GL, Lobo RA, Leach RE, Stern JE. Cumulative birth rates with linked assisted reproductive technology cycles. *N Engl J Med* 2012;**366**:2483–2491.

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;**32**:1–11. Available from: https://academic.oup.com/humrep/article-lookup/doi/10.1093/humrep/dex096.

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

McLernon DJ, Steyerberg EW, Velde Te 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.

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.

Polyzos NP, Drakopoulos P, Parra J, Pellicer A, Santos-Ribeiro S, Tournaye H, Bosch E, Garcia-Velasco J. Cumulative live birth rates according to the number of oocytes retrieved after the first ovarian stimulation for in vitro fertilization/intracytoplasmic sperm injection: a multicenter multinational analysis including ~15,000 women. *Fertil Steril* 2018;**110**:661–670.e1. Elsevier Inc.

Polyzos NP, Nwoye M, Corona R, Blockeel C, Stoop D, Haentjens P, Camus M, Tournaye H. Live birth rates in Bologna poor responders treated with ovarian stimulation for IVF/ICSI. *Reprod Biomed Online* 2014;**28**:469–474. Reproductive Healthcare Ltd.

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**:842–849.

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**: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. Elsevier Ltd.

Steward RG, Lan L, Shah AA, Yeh JS, Price TM, Goldfarb JM, Muasher SJ. 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**:967–973. Elsevier Inc.

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**:1768–1774.

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

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**:641–648.e2. Elsevier Inc.

Yang Y, Sun X, Cui L, Sheng Y, Tang R, Wei D, Qin Y, Li W, Chen ZJ. Younger poor ovarian response women achieved better pregnancy results in the first three IVF cycles. *Reprod Biomed Online* 2016;**32**:532–537. Elsevier Ltd.