Human Reproduction, Vol.31, No.2 pp. 370–376, 2016
Advanced Access publication on January 2, 2016 doi:10.1093/humrep/dev316

human reproduction

ORIGINAL ARTICLE *Infertility*

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?

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Submitted on August 21, 2015; resubmitted on November 19, 2015; accepted on November 25, 2015

STUDY QUESTION: What is the impact of ovarian response on cumulative live birth rates (LBR) following utilization of all fresh and frozen embryos in women undergoing their first ovarian stimulation cycle, planned to undergo single embryo transfer (SET).

SUMMARY ANSWER: Cumulative LBR significantly increases with the number of oocytes retrieved.

WHAT IS KNOWN ALREADY: Several studies have addressed the issue of the optimal number of oocytes retrieved following controlled ovarian stimulation (COS) for IVF/ICSI and demonstrated that ovarian response is independently related to LBR following IVF/ICSI. The vast majority of studies pertained only to the outcome of the fresh IVF cycle and did not evaluate the cumulative LBR following the transfer of all fresh and frozen–thawed embryos after a single ovarian stimulation, which is the most meaningful outcome for the infertile patient.

STUDY DESIGN, SIZE, DURATION: This study is a large cohort analysis of retrospective data from January 2009 to December 2013 in a tertiary medical centre, at the Centre for Reproductive Medicine at the University Hospital of Brussels.

PARTICIPANTS/MATERIALS, SETTING, METHODS: This study included 1099 eligible consecutive women 18–40 years old undergoing their first IVF cycle and planned to undergo SET in their fresh cycle. All patients were treated with a conventional starting gonadotrophin dose of 150–225 IU recombinant FSH (rFSH) in a fixed GnRH antagonist protocol. Vitrification was used as cryopreservation method. To evaluate the impact of oocyte yield on fresh LBR and on cumulative LBR after utilization of all cryopreserved embryos, patients were categorized into four groups according to the number of oocytes retrieved: 1–3 (Group A), 4–9 (Group B), 10–15 (Group C) or >15 oocytes (Group D).

MAIN RESULTS AND THE ROLE OF CHANCE: Regarding LBR in the fresh IVF/ICSI cycles, unadjusted results did not show any significant difference when comparing either high (>15 oocytes) versus normal (10–15 oocytes) ($P = 0.65$), or normal (10–15) versus suboptimal (4–9 oocytes) responders ($P = 0.2$). LBR in the fresh cycles were significantly higher ($P < 0.05$) in high, normal and suboptimal responders when compared with the low ovarian responder group (1–3 oocytes). Moderate-severe ovarian hyperstimulation syndrome occurred in 11 out of 1099 patients (1%). The cumulative LBR significantly increased with the number of oocytes retrieved (χ^2 test for trend $P < 0.001$). High responders (>15 oocytes) demonstrated a significantly higher LBR not only versus poor (0–3 oocytes) ($P < 0.001$) and suboptimal (4–9) responders ($P < 0.001$), but also versus women with normal (10–15) ovarian response ($P = 0.014$). Finally, although suboptimal responders had a better outcome compared with poor ovarian responders ($P = 0.002$), this group had a significantly lower cumulative LBR compared with normal ovarian responders ($P = 0.02$). Multivariate logistic regression analysis showed that the ovarian response category remained an independent predictive factor ($P < 0.001$) for cumulative LBR.

LIMITATIONS, REASONS FOR CAUTION: This is a cohort analysis based on retrospective data collection. Despite our robust methodological approach, the presence of biases related to retrospective design cannot be excluded. High responders may inherently have had a better prognosis. In addition, we cannot provide any guidance for patients undergoing either multiple embryo transfers or treated with higher gonadotrophin doses.

WIDER IMPLICATIONS OF THE FINDINGS: Women undergoing COS for their first IVF/ICSI cycle and SET should be informed that, although the number of oocytes retrieved does not affect LBR in the fresh cycle, the higher the oocyte yield, the higher the probability to achieve a live birth after utilization of all cryopreserved embryos. Large cohort studies are needed in order to confirm our results of cumulative LBR in different ovarian stimulation settings with higher stimulation doses.

STUDY FUNDING/COMPETING INTERESTS: No external funding was used for this study. No conflicts of interest are declared.

Key words: ovarian response / single embryo transfer / live birth rate / cumulative live birth / ovarian stimulation

Introduction

Controlled ovarian stimulation (COS) is the key component of assisted reproductive technologies (ART) that shifted clinical practice from natural mono-follicular cycles to multi-follicular stimulated IVF treatment cycles. The increased number of follicles, and consequently the number of oocytes retrieved, improved pregnancy rates in women undergoing IVF/ICSI, not only by increasing the number of available embryos but also by allowing extended embryo culture and enabling the selection of the best quality embryo for transfer (Fauser *et al.*, 2005).

Several studies have addressed the issue of the optimal number of oocytes retrieved following COS for IVF/ICSI and demonstrated that ovarian response is independently related to live birth rates (LBR) following IVF/ICSI (Timeva *et al.*, 2006; van der Gaast *et al.*, 2006; Sunkara *et al.*, 2011; Baker *et al.*, 2015; Briggs *et al.*, 2015). Nevertheless, although all the above-mentioned trials pointed towards the same conclusion, suggesting an optimal number of between 8 and 18 oocytes, all of them included women undergoing different ovarian stimulation protocols, with diverse doses of gonadotrophins used, different numbers of embryos transferred and heterogeneous populations. Furthermore, the vast majority of studies pertained only to the outcome of the fresh IVF cycle and did not evaluate the cumulative LBR following the transfer or all fresh and frozen–thawed embryos after one ovarian stimulation protocol, which is the most meaningful outcome for the infertile patient.

Although results of the above-mentioned studies are homogenous enough to imply a strong relationship between the number of oocytes retrieved and LBR, it is well established that the magnitude of ovarian response is strictly related to the stimulation protocol used, type and dose of gonadotrophin and type of down-regulation, and patient's profile (Arce *et al.*, 2014). In addition, although LBR following a fresh IVF cycle is definitely a key outcome measure for treatment success, the cumulative LBR following a single ovarian stimulation cycle and utilization of all fresh and frozen–thawed embryos after one ovarian stimulation protocol until delivery, appears to be a more clinically relevant outcome.

The aim of the current study is to evaluate the cumulative LBR following utilization of all fresh and frozen embryos in women undergoing their first ovarian stimulation cycle, with a single embryo transfer (SET) in the fresh cycle.

Materials and Methods

This is a retrospective cohort study including all consecutive women attending the Centre for Reproductive Medicine of the University Hospital of Brussels, Belgium from January 2009 to December 2013. The study was approved by the institutional review board of our hospital (B.U.N. 143201525181).

Patients' eligibility criteria

Eligible patients were considered all consecutive women between 18 and 40 years old undergoing their first IVF cycle, who were planned to undergo SET in the fresh cycle. Eligible patients were only those treated with a conventional starting gonadotrophin dose of 150–225 IU recombinant FSH (rFSH) in a fixed GnRH antagonist protocol.

Patients were excluded from the study if they had planned to undergo ovarian stimulation for preimplantation genetic diagnosis or preimplantation genetic screening (PGS), oocyte donation, and social or medical freezing of oocytes. In addition, women who were planned to undergo natural cycle IVF/ICSI were excluded, given that in such cases no ovarian stimulation was used, while women with cycle cancellation or zero oocytes retrieved were also excluded from the analysis. Finally, we included only women who either delivered a baby or used all their embryos after their first stimulated cycle.

Women who fulfilled the above mentioned criteria but, for unknown reasons, still had frozen embryos remaining or who had transferred the remaining embryos to another IVF unit, while not delivering a live born following their stimulated IVF/ICSI cycle, were excluded from the analysis in order to minimize the risk of misclassification bias (Fig. 1). The minimum length of the follow up period was 2 years.

Treatment protocol

IVF and fresh embryo transfer

Patients received daily injections of 150–225 IU of rFSH starting on day 2 or 3 of their menstrual cycle, followed by a daily dose of 0.25 mg of GnRH antagonist in a fixed protocol starting 6 days later. All patients were considered as potential normo-responder women based on ovarian reserve markers and clinicians' discretion and thus the starting stimulation dose ranged between 150 and 225 IU rFSH per day. Cycle monitoring was performed through serum estradiol (E_2), progesterone and LH assessments, and serial transvaginal ultrasound examinations. Ovulation triggering was performed with the administration of 10 000 IU hCG as soon as three follicles of 17 mm diameter were observed. Oocyte retrieval took place 36 h later. In case of risk for ovarian hyperstimulation syndrome (OHSS) (based on $\geq$18 follicles $>$11 mm diameter on the day of final oocyte maturation triggering) (Papanikolaou *et al.*, 2006), triggering of ovulation was performed either by administration of GnRH agonist followed by a 'freeze all' policy or by GnRH agonist combined with modified luteal support (Devroey *et al.*, 2011; Humaidan *et al.*, 2013). However, although this was the policy of our centre since July 2009, agonist triggering was not considered the choice of triggering in case of patients at risk of OHSS treated prior to this period.

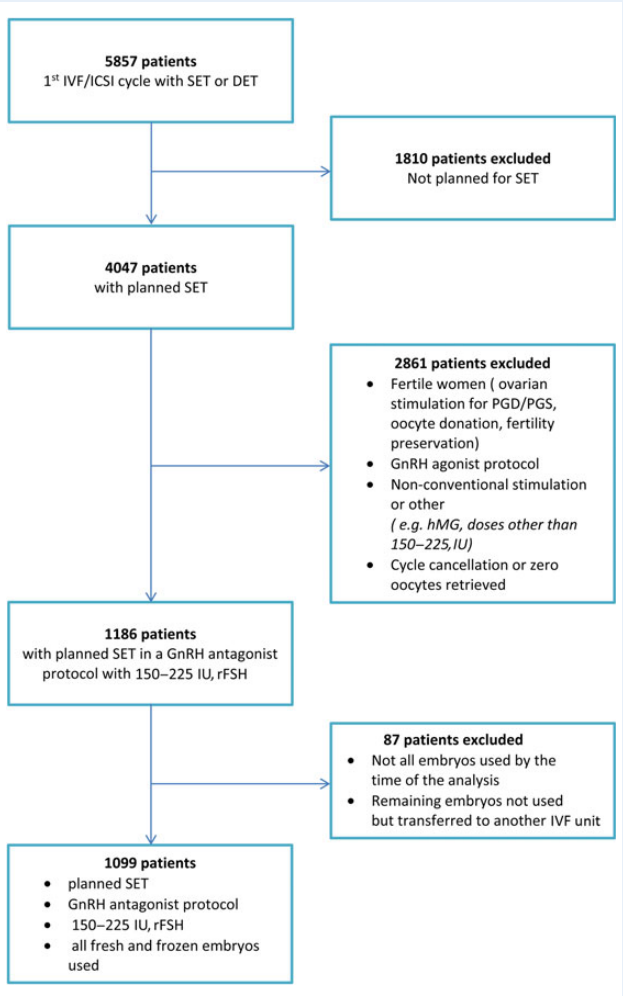

Figure 1 Flowchart of patient selection. SET, single embryo transfer; DET, double embryo transfer; PGS, preimplantation genetic screening; rFSH, recombinant FSH.

Collected oocytes were inseminated either via conventional IVF or ICSI or via IVF/ICSI, embryos were cultured up to Day 3 or Day 5 following oocyte retrieval and SET was performed under ultrasound guidance. The decision regarding the day of the embryo transfer was mostly based on the policy of our centre, which was to expand the embryo culture to Day 5 in case of at least 4 embryos of top (at least 7 cells with maximum 10% fragmentation) or good quality (at least 6 cells with maximum 20% fragmentation) on Day 3. Vaginal progesterone tablets 200 mg (Utrogestan®) three times daily were provided for luteal phase support from the day after oocyte retrieval until 7 weeks of pregnancy.

Cryopreservation and thawing-warming procedure

Supernumerary embryos (or all embryos in case of a freeze all policy) were vitrified on Day 3 or Day 5 by closed vitrification using closed blastocyst vitrification high security straws (Cryo Bio System, Paris, France) combined with dimethylsulphoxide and ethylene glycol bis (succinimidyl succinate) as the cryoprotectants (Irvine ScientificR Freeze Kit, Canada) (Van Landuyt *et al.*, 2011). Day 3 embryos that reached the 6-cell stage with <20% fragmentation were classified as good quality embryos and were cryopreserved. Blastocyst quality was categorized as excellent (AA), good (AB, BA, BB), fair (BC, CB) or poor (CC) based on trophectoderm and inner cell mass quality scores. Only good quality embryos were cryopreserved.

Frozen–thawed embryo transfer

Frozen–thawed cycles were performed through either a natural cycle, with or without hCG triggering, or through an artificial cycle by the use of estradiol. The number of embryos transferred (one or two) in the frozen/thawed cycles complied with national regulations of Belgium and conformed to individual patient requests. The decision regarding the type of preparation for the frozen embryo transfer (FET) cycle was based on the physicians discretion and was related to the menstrual cycle pattern of the patient.

Ovarian response categories

To evaluate the impact of oocyte yield, patients were categorized into four groups according to the number of oocytes retrieved: 1–3 (Group A), 4–9 (Group B), 10–15(Group C) or >15 oocytes (Group D). The specific categorization was based on previous consensus papers (Ferraretti *et al.*, 2011) and more recent evidence suggesting that ovarian response categories should be considered as poor, suboptimal, normal and high responders (Polyzos and Sunkara, 2015).

Main outcome measures

The primary outcome was the cumulative LBR defined as the delivery of a live born (>24 weeks of gestation) in the fresh or in the subsequent frozen–thawed cycles in relation to the ovarian response category. Only the first delivery was considered in the analysis.

The secondary outcome was the live birth following the fresh IVF/ICSI cycle only, in relation to the ovarian response category.

Statistical analysis

In order to ascertain patients' baseline characteristics and important aspects of the treatment outcome among ovarian response groups, outcomes were analysed by ovarian response category. Continuous data are presented as mean ± SD and categorical data are described by number of cases, including numerator and denominator, and percentages. Categorical data and continuous data that did not show a normal distribution were analysed by Pearson's χ^2 test/Fisher exact test or Kruskal–Wallis test as appropriate.

To identify characteristics that may be associated with the cumulative LBR, multivariate logistic regression analysis was performed with the cumulative live birth as the dependent variable and oocyte group as the main independent variable. Univariate regression analyses were performed to identify candidate factors that predict the cumulative LBR. The candidate variables were response category, fertilization rate, age, BMI, indication of infertility, duration of stimulation, day of SET and type of insemination method. Variables showing a tendency of association with cumulative LBR ($P < 0.25$) in the univariate analysis were included in the multivariate model. Thus, response category ($P < 0.001$), fertilization rate ($P < 0.001$), age ($P = 0.01$), day of SET ($P = 0.05$) and type of insemination method ($P = 0.09$) were included as covariates in the final model. All independent variables were simultaneously entered into the logistic regression model. The Hosmer–Lemeshow goodness-of-fit test, assessed the goodness-of-fit of the normal regression models. The likelihood of cumulative live birth after IVF is presented as an odds ratio (OR) with SE and 95% confidence interval (CI).

All statistical tests used a two-tailed α of 0.05. All analyses were performed using STATA 13.0 (StataCorp. Stata Statistical Software: Release 13. College Station, TX, USA).

Table I IVF outcome for groups of women with different ovarian response.

	Ovarian response groups				
	Group A 1–3 oocytes *n* = 83	**Group B 4–9 oocytes *n* = 471**	**Group C 10–15 oocytes *n* = 327**	**Group D >15 oocytes *n* = 218**	***P*-value**
Age (years)	32.8 (3.9)	31.6 (4.1)	30.5 (3.8)	30.3 (3.5)	<0.001[a]
BMI (kg/m^2)	24.7 (4.9)	23.9 (4.8)	23.2 (4.3)	22.9 (3.7)	0.11[a]
Indication of IVF					
Male	42 (7.4%)	222 (39.3%)	175 (30.1%)	126 (22.3%)	0.2[b]
Endometriosis	6 (18.8%)	16 (50%)	5 (15.6%)	5 (15.6%)	
Tubal	7 (8.3%)	39 (46.4%)	25 (29.8)	13 (15.5%)	
Ovulatory	6 (7.6%)	35 (44.3%)	23 (29.1%)	15 (19%)	
Unexplained	22 (6.5%)	159 (46.9%)	99 (29.2%)	59 (17.4%)	
Duration of stimulation	9.2 (2.2)	9.3 (1.8)	8.9 (1.6)	8.9 (1.3)	0.018[a]
Insemination method					
IVF	12 (9.7%)	55 (44.3%)	38 (30.7%)	19 (15.3%)	0.6[b]
ICSI	59 (7.8%)	322 (42.5%)	219 (28.9%)	158 (20.8%)	
IVF + ICSI	12 (5.5%)	94 (43.3%)	70 (32.3%)	41 (18.9%)	
Fertilization rate	60.64% (34.6)	63.4% (24.7)	60.5% (20.3)	56.9% (20.5)	0.003[a]
Oocytes retrieved	2.3 (0.7)	6.6 (1.6)	12.1 (1.7)	22 (7.6)	<0.001[a]
Day of embryo transfer in the fresh cycle					
Day 3	43 (10.9%)	189 (48%)	113 (28.7%)	49 (12.4)	<0.001[b]
Day 5	22 (3.6%)	241 (38.9%)	205 (33.1%)	152 (24.5%)	
No embryo transfer in the fresh cycle	19 (22.9%)	45 (9.6%)	11 (3.4%)	24 (11%)	<0.001[b]
Moderate-severe OHSS	0	0	2 (0.6%)	9 (4.1%)	<0.001[c]
Live birth in the fresh cycle*	14 (16.9%)	140 (29.7%)	111 (33.4%)	70 (32.1%)	0.02[b]
Cumulative live birth*	18 (21.7%)	187 (39.7%)	165 (50.5%)	134 (61.5%)	<0.001[b]

OHSS, ovarian hyperstimulation syndrome.
[a]Kruskal–Wallis test. Values are mean (SD).
[b]Pearson χ^2 test. Values are number (percentage).
[c]Fisher exact test. Values are number (percentage).
*Pairwise comparisons with Bonferroni correction.
Live birth in the fresh cycle
>15 oocytes versus 10–15 oocytes: $P = 0.65$
10–15 oocytes versus 4–9 oocytes: $P = 0.20$
4–9 oocytes versus 1–3 oocytes: $P = 0.016$
Cumulative live birth
>15 oocytes versus 10–15 oocytes: $P = 0.014$
10–15 oocytes versus 4–9 oocytes: $P = 0.02$
4–9 oocytes versus 1–3 oocytes: $P = 0.002$

Results

Patient characteristics according to number of oocytes retrieved

Overall, our analysis included 1099 patients treated with a fixed GnRH antagonist protocol and planned SET. Among them, 504 (45.9%) patients achieved a live birth and 595 (54.1%) did not.

The patients' baseline characteristics are presented in Table I. Comparisons between the four groups did not reveal any significant difference for BMI, indication of IVF treatment, insemination method and day of fresh embryo transfer. The age, the duration of stimulation, the fertilization rate, the number of oocytes retrieved and the day of fresh embryo transfer differed significantly between ovarian response categories. For the calculation of the fertilization rate of all patients, the dominator was the total number of cumulus-oocyte-complexes retrieved, irrespective of the insemination method used.

LBR in the fresh IVF/ICSI cycles

LBRs in the fresh cycle according to the ovarian response category are presented in Table I.

Overall, 99 patients did not have a fresh embryo transfer either because of low response and lack of good embryos to transfer (64 patients) or because of high response and freezing of all embryos due to high risk of OHSS (35 patients).

As shown, no significant difference in LBR in the fresh cycle was identified when comparing either high (>15 oocytes) versus normal (10–15

Table II Multivariate logistic regression with odds ratios for cumulative live birth.

Cumulative live birth	Odds ratio	SE	95% confidence interval	*P*-value
Response category				
0–3 oocytes*	1	–	–	<0.001
4–9 oocytes	2.4	0.7	1.3–4.4	
10–15 oocytes	3.5	1.1	1.9–6.7	
>15 oocytes	5.6	2	3.1–11.6	
Fertilization rate	1.01	0.003	1.006–1.018	<0.001
Day 5 of fresh embryo transfer	0.9	0.13	0.73–1.27	0.8
Age	0.9	0.02	0.9–1.01	0.3
Insemination method				
ICSI	1	–	–	0.4
IVF	0.8	0.2	0.5–1.2	
IVF + ICSI	1.1	0.2	0.8–1.2	

*Reference category.

oocytes) ($P = 0.65$), or normal (10–15) versus suboptimal (4–9 oocytes) responders ($P = 0.2$). However, LBRs were significantly higher ($P < 0.05$) between high, normal and suboptimal responders when compared with the low ovarian responder group (1–3 oocytes).

Overall, 11 out of 1099 patients (1%) presented with moderate-severe OHSS. The classification of OHSS was based on Golan criteria (Golan *et al.*, 1989). In particular, 9 out of 218 patients (4.1%) in the >15 oocyte category and 2 out of 327 patients (0.6%) in the 10–15 oocyte category presented with moderate-severe OHSS. Among them, all but one were triggered with hCG and their stimulation cycle started before July 2009. Only one patient out of 1099 had OHSS following fresh embryo transfer and modified luteal support. In total, three patients received GnRH agonist and modified luteal support.

Cumulative LBRs including live births from fresh and frozen–thawed embryos

The cumulative LBR significantly increased with the number of oocytes retrieved, χ^2 test for trend, $P < 0.001$ (Table II).

As expected, poor responders demonstrated significantly lower cumulative LBR compared with all the other ovarian response categories.

However, high responders (>15 oocytes) demonstrated a significantly higher LBR not only versus poor (0–3 oocytes) ($P < 0.001$) and suboptimal (4–9) responders ($P < 0.001$), but also versus women with normal (10–15) response ($P = 0.014$) (Table I footnote).

Finally, although suboptimal responders had a better outcome compared with poor ovarian responders ($P = 0.002$), this group had significantly lower LBR compared with normal ovarian responders ($P = 0.02$) (Table I footnote).

Multivariate logistic regression analysis for cumulative LBR

Table II presents the estimated ORs with the SEs and 95% CIs between cumulative LBR and response category. The 0–3 oocytes category is used as a reference. After adjustment for fertilization rate, age, day of fresh embryo transfer and insemination method, the response category remained an independent predictive factor ($P < 0.001$) for cumulative LBR. The ORs for cumulative LBR increased from 2.4 (1.3–4.4) in the 4–9 oocytes category to 3.5 (1.90–6.7) and 5.99 (3.1–11.6) in the 10–15 oocytes and >15 oocytes category, respectively. The fertilization rate had a significant effect on cumulative LBR. That was not the case for age, day of fresh embryo transfer and insemination method.

Discussion

Our study demonstrates a robust positive relationship between ovarian response, as reflected by the number of oocytes retrieved, and cumulative LBR in women undergoing their first IVF/ICSI cycle using the GnRH antagonist protocol and planned SET. Compared with all other ovarian response categories, low ovarian responders demonstrated a significantly lower LBR in their first fresh IVF cycle and lower cumulative LBR following the utilization of all embryos. Nevertheless, substantially more interesting is that, although LBR in the fresh IVF/ICSI were comparable in all the other ovarian response categories (sub-optimal, normal and high responders), cumulative LBR after the use of all fresh and frozen embryos significantly increased with the number of oocytes retrieved. In this regard, high responders demonstrated the best outcome compared with all other ovarian response categories, while suboptimal responders appear to form a distinct ovarian response category, given that their cumulative LBR is significantly worse compared with the normal responders and significantly better compared with the poor ovarian response patients.

Two large registry analyses evaluating LBR following a fresh IVF/ICSI cycle have been published to date, demonstrating that LBRs either reach a plateau (Steward *et al.*, 2014) or even decline when more than 15–20 oocytes are retrieved (Sunkara *et al.*, 2011). However, both studies analysed their results per cycle and not per patient, and all patients were included irrespective of their IVF/ICSI cycle rank. Our study, aiming to overcome such methodological shortcomings, included only women undergoing their first IVF/ICSI cycle and demonstrated that in these patients LBRs following a fresh IVF/ICSI cycle are comparably high when retrieving 4–9, 10–15 or >15 oocytes. On the contrary, as far as OHSS rates are concerned, women in the high response category are at risk of OHSS if hCG instead of GnRH agonist triggering is selected for final oocyte maturation.

Over the last decade, there has been a major debate regarding the ideal number of oocytes needed following ovarian stimulation for IVF/ICSI, with several previous studies suggesting that high ovarian response may not only impair implantation rates (Valbuena *et al.*, 2001; Joo *et al.*, 2010) but also may increase chromosomally abnormal embryos (Baart *et al.*, 2007; Haaf *et al.*, 2009). However, others claimed that although high response may lead to an increased proportion of immature oocytes, pregnancy outcomes in high responders are not impaired (Kok *et al.*, 2006). Our study adds further to the currently available evidence by suggesting that although fertilization rate seems to decrease in high responders, high response not only does not impair LBR but, on the contrary, increases cumulative LBR following the use of fresh and frozen–thawed embryos.

Although two recent studies have also evaluated pregnancy outcome following the use of cryopreserved embryos in subsequent cycles (Fatemi *et al.*, 2013; Arce *et al.*, 2014), patients in both studies were followed only for a certain period and not until either having a live birth or until all their

frozen embryos were used. In this regard, we cannot exclude that the final results might be underestimated in the higher oocyte yield groups, taking into account that these women are highly likely to have more embryos cryopreserved after their stimulated cycle. Only one previous retrospective cohort study (Ji *et al.*, 2013) adequately addressed the issue of cumulative LBR following the use of all frozen–thawed embryos, with results similar to our analysis. Nevertheless, this study included only young women (aged 18–34 years) with a very low BMI, introducing a potential selection bias. In addition, all women in the Ji *et al.* (2013) study were treated with a long GnRH agonist protocol with varying doses of stimulation and multiple embryos transferred. Furthermore, although adjusted LBR rates were reported, the authors did not include in their regression model one of the strongest confounders, the number of embryos transferred. Our work differs in that it pertains to a relatively homogenous population of infertile women in which such biases have either been eliminated or results were adjusted for.

In spite of the numerous efforts taken to avoid biases in our study some limitations have to be recognized. The major limitation is associated with its retrospective design. Consequently, despite the study population being a clearly defined homogenous group of women, confounding bias may still exist and may affect our conclusions. In this regard, it is likely that the patients with a greater number of oocytes retrieved were those with a better prognosis. In addition, although our data support that in women treated with COS and SET a high ovarian response may result in a higher cumulative LBR, we cannot provide any guidance for patients undergoing either multiple embryo transfer or are treated with higher gonadotrophin doses. In this regard, large cohort studies are urgently needed in order to confirm our results of cumulative LBR in different ovarian stimulation settings with higher stimulation doses. Finally, although the study design was fairly robust regarding the fresh cycles, FET cycle preparation was not consistent among the whole population. Nevertheless, FET with natural or artificial preparation with or without GnRH down-regulation has been shown to be equally effective (Ghobara and Vandekerckhove, 2008; Glujovsky *et al.*, 2010; van de Vijver *et al.*, 2014).

In conclusion, our findings clearly demonstrate interesting and clinically relevant elements for infertility counselling. Women undergoing COS for their first IVF/ICSI cycle and planned SET should be informed that, although the number of oocytes retrieved does not affect LBR in the fresh cycle, the higher the oocyte yield the higher the probability to achieve a live birth after utilization of all cryopreserved embryos. This is indeed very promising especially if we consider that we only focused on the first live birth and conclusions may be somewhat conservative. Hence we may have underestimated the value of a higher egg number, taking into account that some couples may have had a second child from their 1st IVF cycle, but our study end-point was the first live birth. Nevertheless, we should mention that the risk of OHSS increases in case of high ovarian response in the fresh cycle. However, the use of the antagonist stimulation protocol combined with the GnRH agonist trigger followed by a 'freeze all' policy significantly reduces, if not eliminates, OHSS in the majority of at risk patients who have fresh transfer.

Acknowledgements

The authors would like to thank Walter Meul for his invaluable help during data collection and abstraction. In addition, we would like to thank the statistician Dimitris Stogiannis for the statistical brainstorming.

Authors' roles

N.P.P. and D.S. are responsible for the concept design. P.D. scrutinized patients' files and performed the statistical analysis. P.D. and N.P.P. wrote the manuscript. C.B., D.S., M.C., M.d.V. and H.T. contributed to the interpretation of the results and editing of the manuscript.

Funding

No external funding was used for this study.

Conflict of interest

None declared.

References

Arce JC, Andersen AN, Fernandez-Sanchez M, Visnova H, Bosch E, Garcia-Velasco JA, Barri P, de Sutter P, Klein BM, Fauser BC. Ovarian response to recombinant human follicle-stimulating hormone: a randomized, antimullerian hormone-stratified, dose-response trial in women undergoing in vitro fertilization/intracytoplasmic sperm injection. *Fertil Steril* 2014; **102**:1633–1640 e1635.

Baart EB, Martini E, Eijkemans MJ, Van Opstal D, Beckers NG, Verhoeff A, Macklon NS, Fauser BC. Milder ovarian stimulation for in-vitro fertilization reduces aneuploidy in the human preimplantation embryo: a randomized controlled trial. *Hum Reprod* 2007;**22**:980–988.

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 e932.

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

Devroey P, Polyzos NP, Blockeel C. An OHSS-Free Clinic by segmentation of IVF treatment. *Hum Reprod* 2011;**26**:2593–2597.

Fatemi HM, Doody K, Griesinger G, Witjes H, Mannaerts B. High ovarian response does not jeopardize ongoing pregnancy rates and increases cumulative pregnancy rates in a GnRH-antagonist protocol. *Hum Reprod* 2013;**28**:442–452.

Fauser BC, Devroey P, Macklon NS. Multiple birth resulting from ovarian stimulation for subfertility treatment. *Lancet* 2005;**365**:1807–1816.

Ferraretti AP, La Marca A, Fauser BC, Tarlatzis B, Nargund G, Gianaroli L, Definition EwgoPOR. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. *Hum Reprod* 2011;**26**:1616–1624.

Ghobara T, Vandekerckhove P. Cycle regimens for frozen-thawed embryo transfer. *Cochrane Database Syst Rev* 2008: CD003414.

Glujovsky D, Pesce R, Fiszbajn G, Sueldo C, Hart RJ, Ciapponi A. Endometrial preparation for women undergoing embryo transfer with frozen embryos or embryos derived from donor oocytes. *Cochrane Database Syst Rev* 2010: CD006359.

Golan A, Ron-el R, Herman A, Soffer Y, Weinraub Z, Caspi E. Ovarian hyperstimulation syndrome: an update review. *Obstet Gynecol Surv* 1989; **44**:430–440.

Haaf T, Hahn A, Lambrecht A, Grossmann B, Schwaab E, Khanaga O, Hahn T, Tresch A, Schorsch M. A high oocyte yield for intracytoplasmic sperm injection treatment is associated with an increased chromosome error rate. *Fertil Steril* 2009;**91**:733–738.

Humaidan P, Polyzos NP, Alsbjerg B, Erb K, Mikkelsen AL, Elbaek HO, Papanikolaou EG, Andersen CY. GnRHa trigger and individualized luteal phase hCG support according to ovarian response to stimulation: two

prospective randomized controlled multi-centre studies in IVF patients. *Hum Reprod* 2013;**28**:2511–2521.

Ji J, Liu Y, Tong XH, Luo L, Ma J, Chen Z. The optimum number of oocytes in IVF treatment: an analysis of 2455 cycles in China. *Hum Reprod* 2013; **28**:2728–2734.

Joo BS, Park SH, An BM, Kim KS, Moon SE, Moon HS. Serum estradiol levels during controlled ovarian hyperstimulation influence the pregnancy outcome of in vitro fertilization in a concentration-dependent manner. *Fertil Steril* 2010;**93**:442–446.

Kok JD, Looman CW, Weima SM, te Velde ER. A high number of oocytes obtained after ovarian hyperstimulation for in vitro fertilization or intracytoplasmic sperm injection is not associated with decreased pregnancy outcome. *Fertil Steril* 2006;**85**:918–924.

Papanikolaou EG, Pozzobon C, Kolibianakis EM, Camus M, Tournaye H, Fatemi HM, Van Steirteghem A, Devroey P. Incidence and prediction of ovarian hyperstimulation syndrome in women undergoing gonadotropin-releasing hormone antagonist in vitro fertilization cycles. *Fertil Steril* 2006; **85**:112–120.

Polyzos NP, Sunkara SK. Sub-optimal responders following controlled ovarian stimulation: an overlooked group? *Hum Reprod* 2015;**30**:2005–2008.

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.

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.

Timeva T, Milachich T, Antonova I, Arabaji T, Shterev A, Omar HA. Correlation between number of retrieved oocytes and pregnancy rate after in vitro fertilization/intracytoplasmic sperm infection. *Scientific World Journal* 2006;**6**:686–690.

Valbuena D, Martin J, de Pablo JL, Remohi J, Pellicer A, Simon C. Increasing levels of estradiol are deleterious to embryonic implantation because they directly affect the embryo. *Fertil Steril* 2001;**76**:962–968.

van de Vijver A, Polyzos NP, Van Landuyt L, De Vos M, Camus M, Stoop D, Tournaye H, Blockeel C. Cryopreserved embryo transfer in an artificial cycle: is GnRH agonist down-regulation necessary? *Reprod Biomed Online* 2014;**29**:588–594.

van der Gaast MH, Eijkemans MJ, van der Net JB, de Boer EJ, Burger CW, van Leeuwen FE, Fauser BC, Macklon NS. Optimum number of oocytes for a successful first IVF treatment cycle. *Reprod Biomed Online* 2006; **13**:476–480.

Van Landuyt L, Stoop D, Verheyen G, Verpoest W, Camus M, Van de Velde H, Devroey P, Van den Abbeel E. Outcome of closed blastocyst vitrification in relation to blastocyst quality: evaluation of 759 warming cycles in a single-embryo transfer policy. *Hum Reprod* 2011; **26**:527–534.