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ARTICLE

Pituitary suppression protocol among Bologna poor responders undergoing ovarian stimulation using corifollitropin alfa: does it play any role?

BIOGRAPHY

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KEY MESSAGE

New protocols using corifollitropin alfa among Bologna poor responders and various types of pituitary suppression do not significantly increase cumulative LBR. However, the number of cryopreserved embryos was significantly higher in the GnRH antagonist group, and compared to the long GnRH agonist, there was a significant reduction in terms of ovarian stimulation days and consumption.

ABSTRACT

Research question: Does the type of pituitary suppression protocol influence cumulative live birth rate (LBR) in Bologna poor responders treated with corifollitropin alfa (CFA)?

Design: Retrospective cohort analysis including poor responder patients fulfilling the Bologna criteria who underwent their first intracytoplasmic sperm injection cycle using a CFA-based ovarian stimulation protocol between 2011 and 2017. The starting dose of CFA was 150 µg. The primary outcome was cumulative LBR, defined as the first delivery of a live born resulting from the fresh and all the subsequent frozen embryo transfers.

Results: A total of 717 cycles were divided into three groups: A (gonadotrophin-releasing hormone [GnRH] antagonist protocol, $n = 407$), B (long GnRH agonist protocol, $n = 224$) and C (short GnRH agonist protocol, $n = 86$). Cumulative LBR did not significantly differ between groups (20.1% versus 17.4% versus 14.0%; $P = 0.35$). Significantly more patients in Group A had supernumerary embryos cryopreserved (28.3% versus 18.4% versus 11.6%; $P < 0.001$). Days of additional highly purified human menopausal gonadotrophin 300 IU injections following CFA were significantly different between Groups A, B and C (3 versus 5 versus 3 days; $P < 0.001$). Multivariate logistic regression analysis showed that the number of oocytes retrieved remained an independent predictive factor (odds ratio 1.23, 95% confidence interval 1.16–1.31) for cumulative LBR.

Conclusions: Poor responders according to the Bologna criteria in whom CFA is used for ovarian stimulation had comparable cumulative LBR, irrespective of the type of pituitary suppression. An increase in number of oocytes retrieved is an independent variable related to cumulative LBR.

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KEYWORDS
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INTRODUCTION

Poor ovarian response remains one of the most significant challenges in women undergoing ovarian stimulation, with a prevalence ranging from 9% to 24% (*Ubaldi et al., 2014*). Although the literature offers a wide range of protocols using different doses and types of gonadotrophins to manage this particular cohort of patients, to date there is no single efficient treatment for poor ovarian response. Hence, the search for the best ovarian stimulation protocol for patients identified as 'poor responders' continues (*De Placido et al., 2006; Ke et al., 2013; Polyzos et al., 2012, 2013b, 2014; Pu et al., 2011; Sunkara et al., 2014*).

Given the variability in the arbitrary definitions used for poor ovarian response until 2011 (*Polyzos and Devroey, 2011*), the European Society of Human Reproduction and Embryology (ESRHE) has developed a new definition, the so-called 'Bologna criteria', in order to refine this group of patients and overcome limitations in the interpretation of the relevant literature (*Ferraretti et al., 2011*). In spite of the uniform prognosis among Bologna criteria poor responders, concerns have been raised because heterogeneity among poor responders continues to exist with this definition, and because the prognostic effect of individual factors is still unclear, particularly in the young poor responder group (*Younis, 2012*).

In recent years, it has been postulated that corifollitropin alfa (CFA), a long-acting gonadotrophin, can have a benefit in poor ovarian responders, mainly due to its pharmacokinetic profile (*Polyzos et al., 2013b*). CFA reaches maximum serum concentrations 2 days after injection and the rapid increase in serum FSH concentration results in a significantly higher exposure of the small antral follicles to FSH during the early follicular phase, ensuring multi-follicular growth and stimulating growth of these follicles for an entire week (*Devroey et al., 2004; Fauser et al., 2010*). In normal responders this pharmacokinetic profile results in a significantly higher number of oocytes retrieved compared with recombinant FSH (rFSH) (*Devroey et al., 2004*).

In order to investigate the efficacy of this long-acting gonadotrophin in women with poor ovarian response according to the Bologna criteria, several studies have been conducted in this specific infertile population. Based on these results, CFA followed by the administration of highly purified human menopausal gonadotrophin (hp-HMG) in a GnRH antagonist protocol was found to be beneficial in terms of pregnancy rates in women younger than 40 years, whereas results remained poor in older women regardless of the protocol or the type of gonadotrophins used (*Polyzos et al., 2013a, 2013c, 2015*).

Taking into account the available evidence, the rationale for conducting the current retrospective study was to investigate whether the three most commonly used assisted reproductive technology (ART) protocols, in particular GnRH antagonist, long GnRH agonist and short GnRH agonist, combined with CFA, may influence the clinical outcome of Bologna poor responders in terms of cumulative LBR.

MATERIALS AND METHODS

This was a retrospective, single-centre cohort study including poor responder patients fulfilling the Bologna criteria and undergoing their first intracytoplasmic sperm injection (ICSI) cycle with CFA at the Centre for Reproductive Medicine, Universitair Ziekenhuis Brussel, Belgium, from January 2011 to March 2017. This study was approved by the Ethics Committee of Brussels University Hospital (approval B.U.N. 143201835510).

Patient eligibility criteria

Poor ovarian responders as defined by the Bologna criteria (*Ferraretti et al., 2011*): eligible women had to fulfil at least two of the three following criteria: (i) advanced maternal age (≥40 years) or any other risk factor for poor ovarian response (patients with genetic or acquired conditions); (ii) poor ovarian response (≤3 oocytes with a conventional stimulation protocol); (iii) abnormal ovarian reserve test (antral follicle count [AFC] <7 or anti-Müllerian hormone [AMH] <1.1 ng/ml). AMH was measured in a previous cycle, irrespective of the cycle day. The cut-off of AMH <1.1 ng/ml for prediction of poor response was used. AFC was measured on Day 2–4 of a previous cycle and the cut-off <7 was used to predict poor ovarian response.

With reference to the ovarian stimulation protocol, the population was divided into three categories: GnRH antagonist, long GnRH agonist and short GnRH agonist. The single-dose administration of 150 µg CFA was followed by daily injections of hp-HMG (300 IU/day) when necessary, until triggering of final oocyte maturation. Patients were triaged to the three stimulation protocols according to the physician's choice.

Inclusion criteria included age between 18 and 43 years, body mass index (BMI) of 17–35 kg/m^2, presence of both ovaries and absence of any untreated endocrine abnormality. No poor responders used any type of priming.

Patients who had undergone preimplantation genetic testing (PGT) were excluded, because in our centre PGT is only applied in patients with recurrent pregnancy loss and in normal responders with a favourable number of blastocysts. This practice is in accordance with a recently published Committee Opinion (*Penzias et al., 2018*). In Bologna poor ovarian response patients, the use of PGT is controversial because of the increased likelihood of having no embryo available for transfer per started cycle. Also excluded were conventional IVF cycles for fertility preservation and natural or modified natural IVF cycles (*van der Westerlaken et al., 2005*). Finally, only women who either delivered a baby or used all their embryos after their first stimulated cycle were included.

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. The minimum length of the follow-up period was 1 year.

GnRH antagonist protocol (Group A)

Ovarian stimulation was initiated on Day 2 or 3 of the menstrual cycle with a single subcutaneous injection of 150 µg CFA (Elonva®; MSD, Oss, the Netherlands). Pituitary down-regulation was performed with daily administration of GnRH antagonist (Orgalutran®; MSD) in a fixed protocol starting from Day 6 of the menstrual cycle onwards. If necessary,

daily injections of hp-HMG (Menopur®; Ferring, Saint-Prex, Switzerland) (300 IU) were given from Day 8 until the day of human chorionic gonadotrophin (HCG) trigger. In our centre, the fixed antagonist protocol is used for ovarian stimulation in normal, poor or high responders. Although a flexible regimen could have been a choice, there is evidence that fixed and flexible antagonist protocols are equally effective (*Al-Inany et al., 2016; Kolibianakis et al., 2015*).

Long GnRH agonist protocol (Group B)

Pituitary down-regulation was induced by daily subcutaneous injections of 0.1 mg triptorelin (Gonapeptyl®; Ferring) or buserelin nasal spray 0.60 mg daily (Suprefact®; Sanofi-Aventis, Germany) started on Day 21 of the pretreatment cycle (mid-luteal phase). Patients were treated with GnRH agonist for 2 weeks before the initiation of ovarian stimulation and pituitary suppression was continued during stimulation up to the day of HCG administration. After down-regulation was confirmed by low endogenous hormonal levels and ultrasound, a single subcutaneous injection of 150 µg CFA (Elonva®; MSD) was administered on stimulation day 1 (SD1) and a daily subcutaneous dose of hp-HMG (Menopur®; Ferring) (300 IU/day) was given, if necessary, from SD8 up to the day of HCG trigger.

Short GnRH agonist protocol (Group C)

On Day 2 of the menstrual cycle, daily subcutaneous injections of 0.1 mg triptorelin (Gonapeptyl®; Ferring) or buserelin nasal spray 0.60 mg daily (Suprefact®; Sanofi-Aventis, Germany) was given up to the day of HCG administration, as well as a single subcutaneous injection of 150 µg CFA commenced 1 day later (Elonva®; MSD). If necessary, from SD8 a daily subcutaneous dose of hp-HMG (Menopur®; Ferring) (300 IU) was given up to the day of HCG administration.

Ovulation triggering and luteal phase support (all groups)

Final oocyte maturation was triggered with either highly purified urinary or recombinant HCG (Pregnyl®; MSD; Ovitrelle®; Merck Serono Europe Ltd, London, UK). Cumulus–oocyte complexes (COC) were collected by transvaginal aspiration 36 h after the HCG administration, followed by the ICSI procedure (*Van Landuyt et al., 2005*).

Luteal phase support with intravaginal progesterone (Utrogestan®; Besins Iscovescu, Paris, France) was administered daily (3 times 200 mg per day) and was initiated on the day of oocyte retrieval or the day thereafter and continued for at least 7 weeks, in case of a positive pregnancy test that was performed 14 days after oocyte retrieval.

Embryo transfer (all groups)

Fresh embryo transfer was performed 3 or 5 days after oocyte retrieval with a maximum of three embryos transferred. The decision regarding the day of the embryo transfer was based on the policy of our centre, which was to expand the embryo culture to Day 5 in case of at least four embryos of top quality (at least 7 cells with maximum 10% fragmentation) or good quality (at least 6 cells with maximum 20% fragmentation) on Day 3.

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 dimethyl sulfoxide and ethylene glycol bis (succinimidyl succinate) as the cryoprotectants (Irvine Scientific Freeze Kit, Canada). 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 (*Van Landuyt et al., 2005*).

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 oestradiol. The number of embryos transferred in the frozen–thawed cycles complied with national regulations of Belgium and conformed to individual patient requests. The criteria for number of embryos to transfer are those according to the Belgian legislation, depending on the patient's age and the number of attempts (*Van Landuyt et al., 2006*).

The decision regarding the type of preparation for the frozen embryo transfer cycle was based on the physician's discretion and was related to the menstrual cycle pattern of the patient.

Primary outcome

The primary outcome parameter was cumulative LBR, defined as the LBR after one ART cycle with fresh and all subsequent frozen embryo transfer cycles until the achievement of a first live birth or until all embryos are exhausted (*Yovich et al., 2016*).

Live birth was defined as a complete expulsion or extraction from a woman of a product of fertilization, after 24 completed weeks of gestational age.

Women with frozen embryo(s), which had not been thawed by the end of the follow-up period, were excluded from cumulative live birth analyses.

Secondary outcomes

Secondary outcomes included duration of stimulation, the number of COC and metaphase II oocytes (MII), the maturation rate (defined as the number of MII oocytes divided by the number of COC retrieved), the fertilization rate (defined as the number of zygotes with two pronuclei divided by the number of MII oocytes), implantation rate (defined as the number of gestational sacs/number of embryos transferred) and the embryo utilization rate, defined as the total number of usable embryos (transferred and cryopreserved) per number of fertilized oocytes.

LBR was defined as the delivery of a live birth (>24 weeks of gestation) according to the criteria defined by the International Committee for Monitoring Assisted Reproductive Technology (ICMART) (*Zegers-Hochschild et al., 2017*).

Statistical analysis

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 chi-squared test/Fisher's exact test or Kruskal–Wallis test, as appropriate.

To identify characteristics that may be associated with the cumulative LBR,

TABLE 1 BASELINE CHARACTERISTICS OF THE THREE TREATMENT GROUPS

Characteristic	GnRH antagonist: Group A (*n* = 407)	Long GnRH agonist: Group B (*n* = 224)	Short GnRH agonist: Group C (*n* = 86)	*P*-value
Age (years)	36.8 ± 4.2	38.2 ± 3.7	37.6 ± 4.8	<0.001
BMI (kg/m^2)	24.9 ± 4.3	24.8 ± 4.9	24.7 ± 4.2	NS
AFC	4.2 ± 1.8	4.2 ± 1.9	4.5 ± 2.3	NS
AMH (ng/ml)	0.5 ± 0.3	0.4 ± 0.3	0.5 ± 0.5	0.003
Serum FSH (IU/l)	10.3 ± 4.5	9.5 ± 4.4	9.3 ± 4.7	NS
No. of previous attempts	2 (1–4)	2 (0–3)	1.5 (1–4)	NS

AFC = antral follicle count; AMH = anti-Müllerian hormone; BMI = body mass index; GnRH = gonadotrophin-releasing hormone; NS = not significant.

Data are mean ± SD or median (IQR).

Age:
Group A vs B: $P < 0.001$
Group A vs C: $P = 0.23$
Group B vs C: $P = 0.78$

AMH:
Group A vs B: $P < 0.001$
Group A vs C: $P = 0.23$
Group B vs C: $P = 0.77$

multivariate logistic regression analysis was performed with the cumulative LBR as the dependent variable and type of treatment as the main independent variable. The candidate variables were age, BMI, AMH, number of previous IVF attempts, stimulation units and number of COC.

All variables were simultaneously entered into the logistic regression model.

All statistical tests used a two-tailed α of 0.05. All analyses were performed using SPSS Statistics Version 24.0.

RESULTS

Baseline characteristics of the study population

Overall, a total of 717 ovarian stimulation cycles were analysed and divided into three groups: A (GnRH antagonist protocol, *n* = 407), B (long GnRH agonist protocol, *n* = 224) and C (short GnRH agonist protocol, *n* = 86). Baseline characteristics of the three groups are presented in TABLE 1. All groups were comparable regarding BMI, AFC and FSH. However, age was significantly different between Groups A and B (36.8 ± 4.2 versus 38.2 ± 3.7 years; $P < 0.001$) and AMH (0.5 ± 0.3 versus 0.4 ± 0.3 ng/ml; $P < 0.001$).

Cycle characteristics

The cycle characteristics are shown in TABLE 2. The number of patients who only used CFA without requirement of additional stimulation with 300 IU hp-HMG was significantly lower in Group B (GnRH long agonist) compared with Groups A and C (3.1% versus 9.9% versus 11.7%; $P = 0.002$).

There was a significant difference in the mean duration of stimulation among the three protocols ($P < 0.001$). Pairwise comparisons between the three groups showed that the duration of stimulation was significantly longer in Group B compared with Group A (12.0 ± 2.5 versus 10.0 ± 2.1 days; $P < 0.001$) and in Group B compared with Group C (12.0 ± 2.5 versus 10.3 ± 2.5 days; $P < 0.001$), but with no significant difference between Groups A and C (10.0 ± 2.1 versus 10.3 ± 2.5 days; $P = 0.14$).

There was a significant difference in the total gonadotrophin consumption among the three groups ($P < 0.001$). Pairwise comparisons showed that the total consumption of hp-HMG was significantly higher in Group B compared with Groups A and C (1500 ± 900 versus 900 ± 600 versus 900 ± 600; $P < 0.001$).

Reproductive outcome

The reproductive outcome parameters are shown in TABLES 3, 4 and 5. There were no differences between the groups in terms of number of COC, MII oocytes, maturation rates, fertilization rate, utilization rates and embryo quality on Day 3 and 5. Interestingly, more patients in Group A had cryopreserved supernumerary embryos compared with Group B and Group C: 28.3% versus 18.4% versus 11.6%; $P < 0.001$ and the average numbers of embryos cryopreserved for Groups A, B and C were 2.1 ± 1.4 versus 1.7 ± 0.7 versus 1.6 ± 0.6; $P = 0.002$. LBR after fresh embryo transfer were 16.5% versus 16.1% versus 9.3%; $P = 0.26$, the cumulative LBR per cycle were 20.1% versus

TABLE 2 CHARACTERISTICS OF THE RESPONSE TO STIMULATION

Stimulation	GnRH antagonist: Group A (*n* = 407)	GnRH agonist long: Group B (*n* = 224)	GnRH agonist short: Group C (*n* = 86)	Overall *P*-value	A vs B *P*-value	A vs C *P*-value	B vs C *P*-value
CFA, no additional hp-HMG	57 (9.9)	8 (3.1)	13 (11.7)	0.002	0.001	NS	0.001
Stimulation days	10.0 ± 2.1	12.0 ± 2.5	10.3 ± 2.5	<0.001	<0.001	NS	<0.001
Stimulation units of hp-HMG	900 ± 600	1500 ± 900	900 ± 600	<0.001	<0.001	NS	<0.001

CFA = corifollitropin alfa; GnRH = gonadotrophin-releasing hormone; hp-HMG = highly purified human menopausal gonadotrophin; NS = not significant.
Data are *n* (%) or mean ± SD.

TABLE 3 CHARACTERISTICS OF THE FRESH CYCLES INCLUDED AND EMBRYO DEVELOPMENT

	GnRH antagonist: Group A (*n* = 407)	GnRH long agonist: Group B (*n* = 224)	GnRH short agonist: Group C (*n* = 86)	*P*-value
COC	4.6 ± 3.2	4.3 ± 3.0	4.3 ± 3.1	NS
Metaphase II oocytes (MII)	3.7 ± 2.7	3.5 ± 2.7	3.2 ± 2.3	NS
Maturation rate (MII/COC), %	80.3	81.9	75.7	NS
Fertilization rate (2PN/MII), %	75.0	71.8	67.7	NS
Utilization rate (ET + cryo/2PN), %	66.4	70.8	71.8	NS
Top-quality embryos among patients with ET in fresh (D3+D5), %	69.2	73.1	70.5	NS
Number of embryos transferred in fresh cycle	1.6 ± 0.6	1.7 ± 0.7	1.6 ± 0.7	NS
Number of embryos cryopreserved	2.1 ± 1.4	1.7 ± 0.7	1.6 ± 0.6	0.002
Patients with supernumerary cryopreserved embryos, *n* (%)	115 (28.3)	40 (18.4)	10 (11.6)	<0.001

2PN = pronuclei zygotes; COC = cumulus-oocyte complexes; cryo = embryos cryopreserved; D = day; ET = embryo transfer; GnRH = gonadotrophin-releasing hormone; MII = metaphase II; NS = not significant.

Data are mean ± SD, unless otherwise stated.

Number of embryos cryopreserved:
Group A vs B: $P < 0.001$
Group A vs C: $P < 0.001$
Group B vs C: $P = 0.100$

Patients with supernumerary cryopreserved embryos
Group A vs B: $P = 0.02$
Group A vs C: $P = 0.01$
Group B vs C: $P = 0.10$

TABLE 4 EMBRYO TRANSFER, LIVE BIRTH RATES FOR FRESH CYCLES AND CUMULATIVE LBR

	GnRH antagonist: Group A (*n* = 407)	GnRH long agonist: Group B (*n* = 224)	GnRH short agonist: Group C (*n* = 86)	*P*-value
Single embryo transfer (%)	51.5	42.8	44.9	NS
Implantation rate (%)[a]	22	17	44	NS
LBR fresh, *n* (%)	67 (16.5)	36 (16.1)	8 (9.3)	NS
CLBR per cycle, *n* (%)	82 (20.1)	39 (17.4)	12 (14.0)	NS
CLBR-OPU, *n* (%)	82 (21.3)	39 (17.8)	12 (14.6)	NS

CLBR = cumulative live birth rates (fresh and all subsequent frozen embryo transfers until first live birth known or until all embryos are exhausted); GnRH = gonadotrophin-releasing hormone; LBR = live birth rates; NS = not significant; OPU = patients with actual mature oocytes injected.
[a] Implantation rate: number of gestational sacs/number of embryos transferred.

TABLE 5 NUMBER OF TRANSFER CYCLES TO ACHIEVE A LIVE BIRTH

	GnRH antagonist: Group A (*n* = 407)	GnRH long agonist: Group B (*n* = 224)	GnRH short agonist: Group C (*n* = 86)	*P*-value
Number of transfer cycles to achieve a live birth	1.2 ± 0.6	1.1 ± 0.4	1.5 ± 0.8	0.08

Data are expressed as mean ± SD.

GnRH = gonadotrophin-releasing hormone.

17.4% versus 14.0%; $P = 0.36$, and the cumulative LBR per oocyte retrieval was 21.3% versus 17.8% versus 14.6%; $P = 0.29$, for the three study groups.

Variables associated with cumulative LBR

Multivariable logistic regression analysis demonstrated that type of down-regulation protocol, age, AMH, BMI, number of previous attempts and stimulation units were not associated with cumulative LBR. The number of COC was the only variable that was significantly associated with cumulative LBR (odds ratio [OR] 1.23; 95% confidence interval [CI] 1.16–1.31). (Multivariable logistic regression analyses are shown in Supplementary TABLE 1.) (CLBR divided according to the age are shown in the Supplementary TABLE 2.)

DISCUSSION

This is one of the largest studies evaluating cumulative LBR in poor ovarian responders, using CFA in different types of pituitary suppression

protocols. According to these results, cumulative LBR in CFA cycles followed by hp-HMG were comparable between the different protocols. Nevertheless, patients in the GnRH antagonist group had significantly more supernumerary embryos cryopreserved compared with the other protocols. Moreover, this study demonstrates a higher gonadotrophin consumption and longer duration of stimulation with the long GnRH agonist compared with the other protocols.

The management of poor ovarian response remains one of the most significant challenges of ART. Numerous interventions have been proposed, yet few have been shown to be beneficial. Besides this, the lack of a standardized definition for poor responders has compromised the appraisal of the available evidence. Several studies comparing the GnRH antagonist versus short GnRH agonist (*Griesinger et al., 2006; Lainas et al., 2008*) or versus long GnRH agonist protocols (*Cheung et al., 2005; Pu et al., 2011; Roberto et al., 2005*) in poor responders showed conflicting and varied results. A recent randomized controlled trial comparing the three protocols in poor responders showed that the GnRH antagonists and the long GnRH agonist were comparable in terms of number of oocytes retrieved, as opposed to the short GnRH agonist protocol that led to inferior results (3.3 versus 4.4 versus 2.71; $P = 0.4$) (*Sunkara et al., 2014*). The inconsistencies in results could be attributed to the heterogeneity in the definition of poor responders between these studies.

The 'Bologna' criteria were introduced to define poor ovarian responders in order to overcome the systematically derived deficiencies by studies using 'random definitions' or a 'variety of definitions', hampering the external validity of the provided results (*Polyzos and Devroey, 2011; Polyzos and Tournaye, 2014*). *Polyzos et al. (2012)* explored the effect of natural cycle IVF in women with poor ovarian response according to the Bologna criteria (*Polyzos et al., 2012*). It is noteworthy that the exact mechanisms of ovarian ageing have not yet been completely elucidated (*Frydman, 2011; Younis, 2012*) and therefore the Bologna criteria may include heterogeneous subpopulations with different baseline characteristics and clinical prognosis. In this context, the POSEIDON (Patient-Oriented Strategies Encompassing Individualized Oocyte Number) classification of the 'low prognosis patient' was introduced, providing a more detailed stratification of patients into more homogenous subgroups (*Alviggi et al., 2016*); albeit this classification needs to be validated in further clinical trials (*Haahr et al., 2018*).

CFA, a compound that was recently introduced in clinical practice, may improve poor ovarian response better than daily administration of rFSH, because it has been shown to reach peak circulating levels within 2 days, whereas daily FSH does so only after 3–5 days (*Fauser et al., 2010*). This is advantageous for CFA as follicular recruitment is dependent on the starting dose and cannot be rectified by increasing FSH dose after 5 days. Previous studies in normal responders have demonstrated comparable results for CFA and daily rFSH in pregnancy rates with a potential increase in the number of oocytes retrieved (*Boostanfar et al., 2015; Devroey et al., 2009*). Although the use of new treatment molecules may be considered a promising approach for poor responders, a pilot study with the use of CFA in an antagonist protocol has shown that ongoing pregnancy rates are low in these women, similar to treatment with a short agonist protocol (*Polyzos et al., 2013b*). Moreover, although it has been shown that CFA followed by hp-HMG in a GnRH antagonist protocol may result in promising pregnancy rates in poor ovarian responders <40 years of age (*Polyzos et al., 2013a*), these findings were not replicated in a recent well-designed RCT (*Drakopoulos et al., 2017*). Similarly, another observational cohort study demonstrated that in women with a low AFC (less than 4), oocyte yield remains comparable between CFA and daily rFSH (*Yovich et al., 2018*).

In the current study, the number of supernumerary cryopreserved embryos was relatively small and differed slightly between groups. Therefore, it could be stated that such small differences in the number of supernumerary cryopreserved embryos may not have been translated to higher cumulative LBR (which remained comparable between groups). Our results are in line with a recent randomized control trial using CFA in Bologna poor ovarian responders, in which, although there was a significant difference in the number of frozen embryos [22 (28.6%) versus 10 (14.3%); $P = 0.04$] between groups, at the end there was no difference in the live birth in young poor responders (*Drakopoulos et al., 2017*).

Finally, our study demonstrated a higher gonadotrophin consumption and longer duration of stimulation with the long GnRH agonist protocol compared with the short agonist and antagonist protocols; these results were consistent with previous research (*Pandian et al., 2010; Sunkara et al., 2014*).

The major strength of this study relies on the large sample size, including Bologna poor responders treated with CFA and a fixed dose of hp-HMG. The primary outcome was cumulative LBR, which is the most meaningful outcome for each infertile patient. Nevertheless, limitations do exist and should be taken into consideration. This is a retrospective analysis inherent to risk of bias, evaluating the pituitary suppression protocol and not the type of gonadotrophins used (which was CFA in all cases); therefore, a control group in which patients would have used other gonadotrophins was not included. Patients were allocated to a specific stimulation protocol according to the physician's choice, which could have resulted in selection bias.

However, the use of CFA in ovarian stimulation protocols for Bologna poor responders is a relatively recent concept, based on preliminary studies (*Drakopoulos et al., 2017; Polyzos et al., 2015*). If we consider that previous studies did not show any significant difference in outcomes between protocols and that recent evidence does not suggest any benefit of one protocol over another in terms of ongoing pregnancy rates (*Lambalk et al., 2017; Sunkara et al., 2014*), we may consider that the physician's choice of the stimulation protocol is highly unlikely to have compromised the validity of our results in Bologna poor responders. Moreover, several baseline characteristics (age, AMH) differed significantly between groups, which illustrates that the use of Bologna criteria does not guarantee patient homogeneity in spite of the considerable sample size. Therefore, the higher number of cryopreserved embryos in the antagonist group might have been due to the younger age of women allocated to this protocol. Nevertheless, multivariable logistic regression analysis was performed,

adjusting for relevant confounders and showing that the number of COC retrieved was the only independent predictive factor for cumulative LBR. This finding is in line with previous studies in the general infertile population (*Drakopoulos et al., 2016*) and in poor responders (*Oudendijk et al., 2011*), highlighting that the number of oocytes retrieved is related to the chances of live birth, following a complete IVF cycle.

In conclusion, the treatment of poor ovarian responders still remains a challenge in clinical practice. This study provides robust evidence that Bologna poor ovarian responders using CFA and different types of pituitary ovarian suppression have comparable cumulative LBR. However, given the limitations of the study (retrospective design and patient heterogeneity) and although multivariate analysis was performed to adjust for all known confounders, it cannot be excluded that non-apparent sources of bias might still be present. Finally, it is important to note that the purpose of the study was to evaluate the different stimulation protocols, rather than the gonadotrophin used. Future randomized controlled trials are necessary to verify the present findings.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2018.12.030.

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