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Live birth rates after modified natural cycle compared with high-dose FSH stimulation using GnRH antagonists in poor responders

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STUDY QUESTION: Do live birth rates differ between modified natural cycles (MNCs) and cycles using high-dose follicle stimulating hormone (HDFSH) with gonadotrophin-releasing hormone (GnRH) antagonist in poor responder patients?

SUMMARY ANSWER: Live birth rates are significantly higher in MNC compared with HDFSH GnRH antagonist cycles in poor responder patients.

WHAT IS KNOWN ALREADY: Previous data on the efficiency of MNC in poor responders are very limited and suggest that MNC *in vitro* fertilization (IVF) does not offer a realistic solution for parenthood in these patients, since live birth rates are disappointingly low. To date, no studies exist comparing MNC with HDFSH stimulation protocols in poor responders.

STUDY DESIGN, SIZE, DURATION: The present retrospective study included 161 MNCs (106 women in the MNC group) and 164 HDFSH antagonist cycles (136 women in the HDFSH group) performed between January 2008 and December 2013 at Eugonia Assisted Reproduction Unit. The patients included in the study had to fulfill the Bologna criteria for the definition of poor ovarian response.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Irrespective of their age, poor responder patients should have a diminished ovarian reserve as shown by low antral follicle count ($\leq$5) and increased basal FSH ($>$12 IU/l), and one or more previous failed IVF cycles in which $\leq$3 oocytes were retrieved using a high gonadotrophin dose. Analysis was performed by adjusting for the non-independence of the data.

MAIN RESULTS AND THE ROLE OF CHANCE: The probability of live birth was significantly higher in the MNC when compared with the HDFSH group (OR: 4.01, 95% CI: 1.14–14.09), after adjusting for basal FSH, female age and cause of infertility, variables which were shown to be associated with the probability of live birth in univariable analysis. MNCs were characterized by significantly lower total gonadotrophin dose (490.0 $\pm$ 35.2 IU versus 2826.1 $\pm$ 93.4 IU, $P < 0.001$), lower estradiol concentrations (237.5 $\pm$ 12.3 pg/ml versus 487.3 $\pm$ 29.8 pg/ml, $P < 0.001$), fewer follicles present on the day of hCG (1.9 $\pm$ 0.1 versus 3.2 $\pm$ 0.2, $P < 0.001$), fewer oocytes retrieved (1.1 $\pm$ 0.01 versus 2.4 $\pm$ 0.1, $P < 0.001$), fewer oocytes fertilized (0.7 $\pm$ 0.1 versus 1.4 $\pm$ 0.1, $P < 0.001$), fewer embryos transferred (0.7 $\pm$ 0.1 versus 1.4 $\pm$ 0.1, $P < 0.001$), fewer good-quality embryos available (0.5 $\pm$ 0.1 versus 0.8 $\pm$ 0.1, $P < 0.001$) and fewer good-quality embryos transferred (0.5 $\pm$ 0.05 versus 0.8 $\pm$ 0.1, $P < 0.001$) compared with the HDFSH group. However, the proportion of cycles with at least one good-quality embryo transferred per started cycle was similar between the two groups compared (62.5, 95% CI: 52.7–72.3 versus 62.7, 95% CI: 53.0–72.5, respectively).

LIMITATIONS, REASONS FOR CAUTION: This is a retrospective comparison between MNC and HDFSH GnRH antagonist protocols in a large group of poor responder patients according to the Bologna criteria. Although the two groups compared were not imbalanced for all basic characteristics and multivariate analysis were performed to adjust for all known confounders, it cannot be excluded that non-apparent sources of bias might still be present. Future randomized controlled trials are necessary to verify the present findings.

WIDER IMPLICATIONS OF THE FINDINGS: Both MNC and HDFSH antagonist protocols offer very low chances of live birth in poor responder patients who fulfill the Bologna criteria. However, MNC-IVF is a more patient-friendly approach, with a higher probability of live birth compared with the HDFSH antagonist protocol. In this respect, the current data might be of help in counseling such patients, who do not wish to undergo oocyte donation, prior to abandoning treatment altogether and/or proceeding to adoption.

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Key words: poor responders / modified natural cycle / Bologna criteria / GnRH antagonist protocol / live birth

Introduction

Poor responders are women who show reduced ovarian response to gonadotrophin stimulation, mainly due to a diminished ovarian reserve, resulting in the retrieval of a low number of oocytes and low pregnancy rates (Pellicer *et al.*, 1998; Tarlatzis *et al.*, 2003).

Until recently, there was no universal definition of poor responders and this was usually confirmed retrospectively after administration of a standard ovarian stimulation regimen (Tarlatzis, *et al.*, 2003), by taking into consideration the number of developed follicles and/or the number of oocytes retrieved (Ben-Rafael *et al.*, 1994; Surrey and Schoolcraft, 2000; Kligman and Rosenwaks, 2001; Tarlatzis, *et al.*, 2003). However, a recent consensus introduced specific criteria (Bologna criteria) for the definition of poor responders (Ferraretti *et al.*, 2011), with the aim of making feasible comparisons between different studies on poor responders. Nevertheless, it is accepted that the Bologna criteria includes a spectrum of a rather heterogeneous population of women in terms of ovarian reserve and pregnancy prognosis, already necessitating revision (Ferraretti and Gianaroli, 2014; Venetis, 2014).

The efficacy of various ovarian stimulation protocols in poor responders, using either gonadotrophin-releasing hormone (GnRH) agonists or antagonists, has been reviewed, without, however, conclusive results (Loutradis *et al.*, 2008; Kyrou *et al.*, 2009; Venetis *et al.*, 2010; Polyzos and Devroey, 2011), although it has been supported that the use of growth hormone (Kolibianakis *et al.*, 2009) or transdermal testosterone (Bosdou *et al.*, 2012) are associated with an increased probability of live birth.

The modified natural cycle (MNC) has been an option for the treatment of poor responders for several years and there is some evidence to suggest that it might be associated with higher implantation rates compared with conventional ovarian stimulation protocols (Bassil *et al.*, 1999; Morgia *et al.*, 2004). MNC can minimize the increased incidence of a premature luteinizing hormone (LH) surge and cycle cancellation associated with the natural cycle IVF (Pelinck *et al.*, 2002), while maintaining a patient-friendly approach. A renewed interest in its use in the treatment of poor responders has emerged in recent years (Elizur *et al.*, 2005; Kadoch *et al.*, 2011; Polyzos *et al.*, 2012). However, with the lack of available data, it is still not known whether it is preferable to treat poor responders by an MNC or a high-dose follicle stimulating hormone (HDFSH) protocol using GnRH antagonists.

Considering the increasing demand for donated oocytes, the various ethical and/or religious issues associated with oocyte donation, and the scarcity of available donors, the need to provide alternative low-cost treatment options using the couples own genetic material is evident. For this reason, the need to evaluate the use of MNC compared HDFSH protocols using GnRH antagonists in these patients is justified.

The aim of the present study was to assess whether live birth rates differ between the MNC and HDFSH stimulation using GnRH antagonists in poor responder patients who fulfill the Bologna criteria.

Materials and Methods

Study design and patient population

The present retrospective study analyzed 106 poor responders who underwent 161 MNCs and 136 poor responders who underwent 164 HDFSH GnRH antagonists cycles between January 2008 and December 2013 at the Eugonia Assisted Reproduction Unit (Fig. 1). All patients included in the current study had to fulfill the Bologna criteria (Ferraretti, *et al.*, 2011) for definition of poor ovarian response. Women not fulfilling the Bologna criteria for poor ovarian response as well as women who underwent pure natural cycle IVF were excluded from the present study.

All patients had been fully informed that, based on current evidence, pregnancy rates are very low for poor responders undergoing IVF using their own oocytes. The choice of treatment protocol (MNC or HDFSH), with the lack of relevant data was decided after discussion with the couple, during which oocyte donation was also offered as an option but was rejected.

MNC and HDFSH protocol using GnRH antagonists

Before initiation of treatment, all patients had a vaginal ultrasound, to ensure that no follicles >10 mm were present on Day 2 of the cycle, as well as a basal hormonal profile, including assessment of FSH, LH, estradiol and progesterone levels.

In the MNC group, patient monitoring, using vaginal ultrasound and blood tests for LH, estradiol, and progesterone, started on Day 6 of the cycle, and was repeated every 2 days thereafter. When a follicle with a mean diameter of 14 mm was present at ultrasound, 150 IU of recombinant FSH (rFSH) (Gonal-F, Merck Serono, Geneva, Switzerland) and 0.25 mg of the GnRH antagonist Cetrorelix (Cetrotide, Merck Serono, Geneva, Switzerland) were initiated concomitantly and continued daily thereafter until and including the day of human chorionic gonadotrophin (HCG) administration. HCG 10 000 IU (Pregnyl; MSD, Oss, The Netherlands) was administered as soon as the mean follicular diameter was at ≥ 16 mm.

In the flexible GnRH antagonist protocol, recombinant FSH (rFSH) (follitropin alpha) was initiated (Gonal-F, Merck Serono, Geneva, Switzerland) on Day 2 or 3 of cycles. Administration of rFSH continued daily thereafter until and including the day of hCG administration. Daily administration of

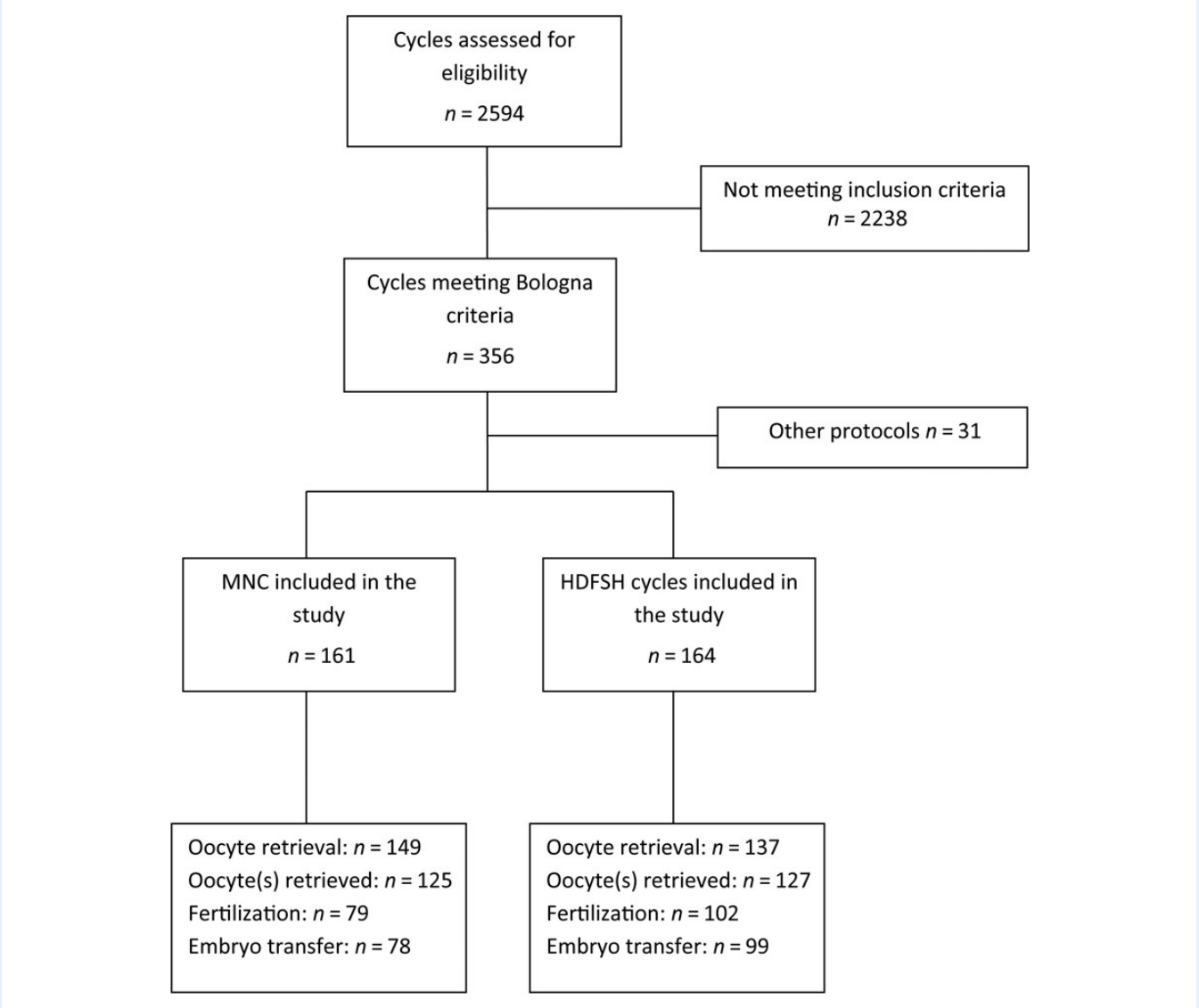

Figure 1 Flow diagram of the study.

0.25 mg of Cetrorelix (Cetrotide, Merck Serono, Geneva, Switzerland) was initiated in a flexible manner when a follicle with a mean diameter of 14 mm was present at ultrasound and/or serum LH levels reached >10 IU/l, as previously described (Lainas *et al.*, 2005;, 2008). Additional treatment with GnRH antagonist continued daily thereafter until and including the day of hCG administration. The starting dose of rFSH was 300 IU/day for all patients treated with the flexible GnRH antagonist protocol. This dose was adjusted during ovarian stimulation, depending on the ovarian response, as assessed by estradiol levels and ultrasound, up to a maximum of 450 IU. Triggering of final oocyte maturation was performed using 10 000 IU hCG (Pregnyl, Organon, The Netherlands) when at least 1–2 follicles reached a mean diameter of ≥16 mm.

Oocyte retrieval, laboratory procedures and luteal phase support

In both patients groups, oocyte retrieval was performed by transvaginal ultrasound-guided double lumen needle aspiration 35–36 h after hCG administration. Repeated follicular flushing was performed when the oocyte was not retrieved in the first aspirate. Intracytoplasmic sperm injection (ICSI) was used instead of conventional IVF in cases of suboptimal semen characteristics, but also in cases with fertilization failure or a high percentage of polyspermy using conventional IVF in a previous cycle (Vicdan and Isik, 1999). Embryo transfer was performed on Day 2 or 3 after oocyte retrieval, depending on the Unit's daily schedule, under ultrasound guidance.

Embryos were assessed based on morphological criteria (number, size and shape of blastomeres, degree of fragmentation, multinucleation, appearance of cytoplasm), and were categorized into four grades [grade 1 (highest) to grade 4 (lowest)]. Embryos with four cells on Day 2 and embryos with 6–8 cells on Day 3, and <20% fragmentation were regarded as good-quality embryos (grades 1 and 2) (Baczkowski *et al.*, 2004). Up to three embryos and up to four embryos were allowed to be transferred in women <40 years and ≥40 years old, respectively, according to the Greek law of reproduction technologies.

The luteal phase was supplemented with daily vaginal administration of 600 mg natural micronized progesterone in three separate doses (Utrogestan; Besins, Brussels, Belgium), starting 1 day after oocyte retrieval and continued until 7 weeks of gestation if pregnancy was achieved.

Ultrasound and laboratory assays

All ultrasound measurements were performed using a 7.5 or 6 or 5 MHz vaginal probe (Sonoline Adara, Siemens). FSH, LH, estradiol and progesterone levels were measured using an Immulite analyzer and commercially available kits (DPC, Los Angeles, CA, USA). Analytical sensitivity were 0.1 mIU/ml for FSH, 0.1 mIU/ml for LH, 15 pg/ml for estradiol and 0.2 ng/ml for progesterone. Intra- and inter-assay precision at the concentrations of most relevance to the current study (expressed as coefficients of variation) were 2.6 and 5.8% for FSH, 5.9 and 8.1% for LH, 6.3 and 6.4% for estradiol and 7.9 and 10% for progesterone.

Outcome measures

The primary outcome measure was live birth, defined as the delivery of a live infant after 24 weeks of gestation. Secondary outcome measures included positive hCG, clinical pregnancy (presence of gestational sac with fetal heart beat detection at 7 weeks of gestation), ongoing pregnancy (presence of gestational sac with fetal heart beat detection at 12 week of gestation), cycle cancellation, proportion of cycles with oocyte(s) retrieved, proportion of cycles with fertilization, proportion of cycles with embryo transfer, proportion of cycles with at least one good-quality embryo transferred, number of oocytes retrieved, number of oocytes fertilized, number of embryos transferred, number of good-quality embryos, total dose of gonadotrophins.

Statistical analysis

All analyses were performed by adjusting for the non-independence of data. Comparisons between MNC and HDFSH cycles and between cycles that did or did not result in live birth, were performed using univariable linear regression with clustered robust standard errors and univariable binary logistic regression with clustered robust standard errors for continuous and binary variables, respectively. To calculate odds ratio for live birth adjusted for potential confounders, multivariable logistic regression with robust clustered standard errors was performed. Statistical analysis was carried out with STATA v 13 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP). Statistical significance was considered at $P < 0.05$.

Results

The study included 106 poor responders who underwent a total of 161 MNC, and 136 poor responders who underwent 164 cycles with a HDFSH flexible GnRH antagonist protocol (Fig. 1). Similar patient characteristics and baseline hormone levels were present between the MNC and the HDFSH groups, as shown in Table I.

Cycle characteristics and embryological data of cycles resulting in oocyte retrieval are shown in Table II. MNCs were characterized by significantly lower total gonadotrophin dose ($P < 0.001$), estradiol concentrations ($P < 0.001$), follicles present on the day of hCG ($P < 0.001$), oocytes retrieved ($P < 0.001$), oocytes fertilized ($P < 0.001$), embryos transferred ($P < 0.001$), good-quality embryos available ($P < 0.001$) and good-quality embryos transferred ($P = 0.003$) compared with HDFSH GnRH antagonist cycles (Table II).

A significantly lower proportion of MNCs were cancelled compared with HDFSH antagonist cycles ($P = 0.020$) (Table III). The proportion of cycles with retrieved oocyte(s) per started cycle was similar between the two groups compared. However, the proportions of cycles with 2PN oocytes per started cycle ($P = 0.028$) as well as that of cycles reaching embryo transfer per started cycle ($P = 0.036$) were significantly lower in the MNC when compared with the HDFSH antagonist group (Table III). On the other hand, the proportion of cycles with at least one good-quality embryo transferred per started cycle was similar between the two groups compared (Table III).

Table I Baseline characteristics of poor responders treated by MNC or HDFSH GnRH antagonist protocol.

	MNC	HDFSH antagonist
	Mean ± RSE[a] 95% CI	
Age (years)	41.3 ± 0.4 40.4 – 42.1	40.7 ± 0.3 40.0 – 41.3
Body mass index (kg/m^2)	22.8 ± 0.4 22.0 – 23.5	23.4 ± 0.4 22.6 – 24.3
Duration of infertility (years)	3.2 ± 0.2 2.7 – 3.6	3.7 ± 0.3 3.1 – 4.3
Number of previous attempts	3.5 ± 0.1 3.3 – 3.8	3.2 ± 0.1 2.9 – 3.4
Basal FSH (IU/l)	23.5 ± 1.1 21.3 – 25.7	21.4 ± 1.2 19.1 – 23.7
Basal LH (IU/l)	10.3 ± 0.6 9.1 – 11.6	9.2 ± 0.6 8.1 – 10.3
Basal estradiol (pg/ml)	34.5 ± 2.2 30.1 – 38.9	33.6 ± 1.3 31.0 – 36.2
Basal progesterone (ng/ml)	0.6 ± 0.1 0.5 – 0.7	0.6 ± 0.1 0.5 – 0.7
Antral follicle count	2.6 ± 0.1 2.4 – 2.8	2.8 ± 0.1 2.7 – 3.0
Cause of infertility	% (*n*)[b] 95% CI	
Poor ovarian reserve	9.3 (11) 4.4 – 14.3	7.9 (12) 3.4 – 12.4
Poor ovarian reserve + male factor	13.7 (14) 6.7 – 20.6	17.7 (28) 11.6 – 23.8
Poor ovarian reserve + endometriosis	5.6 (6) 1.7 – 9.5	8.5 (13) 3.9 – 13.2
Poor ovarian reserve + advanced maternal age (≥40 years)	60.9 (65) 50.7 – 71.0	54.3 (65) 45.5 – 63.1
Poor ovarian reserve + tubal factor	10.6 (10) 4.7 – 16.4	11.6 (18) 6.7 – 16.5

FSH, follicle stimulating hormone; LH, luteinizing hormone; MNC, modified natural cycle; HDFSH, high-dose FSH; GnRH, gonadotrophin-releasing hormone.
[a]Univariable linear regression with robust clustered standard errors (RSE).
[b]Univariable logistic regression with clustered RSE.

Primary outcome measure

Live birth was achieved in 12 MNCs (7.5%, 95% CI: 3.1–11.8) and in 5 HDFSH antagonist cycles (3.1%, 95% CI: 0.4–5.7). Positive hCG, clinical pregnancy and ongoing pregnancy were achieved in 20 (12.4%, 95% CI: 7.3–17.5), 14 (8.7%, 95% CI: 4.1–13.3) and 12 (7.5%, 95% CI: 3.1–11.8) MNCs, respectively, and in 19 (11.6%, 95% CI: 6.9–16.3), 15 (9.1%, 95% CI: 4.9–13.4) and 6 (3.7%, 95% CI: 0.8–6.5) HDFSH antagonist cycles, respectively. Twin birth was observed in 1 MNC (8.3%, 95% CI: 0–24.6) and in 1 HDFSH antagonist cycle (20.0%,

Table II Cycle characteristics and embryological data of patients who proceeded to oocyte retrieval in the MNC and the HDFSH GnRH antagonist groups.

	MNC	HDFSH antagonist	*P*
	Mean ± RSE[a] 95% CI		
LH on day of hCG (IU/l)	13.6 ± 1.5 10.6 – 16.6	9.6 ± 1.0 7.5 – 11.6	0.030
Estradiol on day of hCG (pg/ml)	237.5 ± 12.3 213.3 – 261.8	487.3 ± 29.9 428.4 – 546.1	0.001
Progesterone on day of hCG (ng/ml)	0.6 ± 0.1 0.43 – 0.72	0.6 ± 0.0 0.48 – 0.63	0.832
Endometrial thickness (mm)	9.6 ± 1.4 6.9 – 12.2	8.6 ± 0.2 8.2 – 9.1	0.503
Total gonadotrophin dose (IU)	490.9 ± 35.2 421.5 – 560.3	2826.1 ± 93.7 2641.7 – 3010.4	0.001
Number of follicles on hCG day	1.9 ± 0.1 1.7 – 2.1	3.2 ± 0.2 2.8 – 3.6	0.001
Number of oocytes retrieved	1.1 ± 0.1 1.0 – 1.2	2.4 ± 0.1 2.1 – 2.6	0.001
Number of oocytes fertilized (2PN)	0.7 ± 0.1 0.6 – 0.9	1.4 ± 0.1 1.2 – 1.6	0.001
Fertilization rate	58.8 ± 4.3 50.4 – 67.2	56.8 ± 3.2 50.4 – 63.2	0.707
Number of embryos transferred	0.7 ± 0.1 0.6 – 0.9	1.4 ± 0.1 1.2 – 1.5	0.001
Number of good-quality embryos on embryo transfer day	0.6 ± 0.1 0.5 – 0.7	0.9 ± 0.1 0.8 – 1.1	0.001
Number of good-quality embryos transferred	0.5 ± 0.1 0.4 – 0.6	0.8 ± 0.1 0.7 – 1.0	0.003
Proportion of good-quality embryos per oocyte retrieved	44.4 ± 4.4 35.7 – 53.1	38.5 ± 3.4 31.7 – 42.5	0.290
	% (95% CI)[b]		
Fertilization method			
IVF	38.4 (27.6 – 49.1)	31.5 (22.6 – 40.4)	0.331
ICSI	61.6 (50.9 – 72.3)	68.5 (59.6 – 77.5)	
Embryo transfer day			
Day 2	71.1 (61.2 – 80.9)	82.8 (75.5 – 90.1)	0.059
Day 3	28.9 (19.1 – 38.8)	17.2 (9.9 – 24.5)	

FSH, follicle stimulating hormone; LH, luteinizing hormone; MNC, modified natural cycle; HDFSH, high-dose FSH; GnRH, gonadotrophin-releasing hormone; hCG, human chorionic gonadotrophin; PN, pronuclei; IVF, in-vitro fertilization; ICSI, intracytoplasmic sperm injection.
[a]Linear regression with clustered robust standard errors (RSE).
[b]Logistic regression with clustered RSE.

95% CI: 0–56.2). No high-order pregnancies (≥3) were observed in either group.

By comparison of patient characteristics in cycles that did and did not result in live birth (Table IV), significant differences were found regarding female age ($P < 0.001$), basal FSH ($P < 0.001$) and cause of infertility ($P < 0.001$) in the two protocols compared. Using binary logistic regression analysis controlling for the confounding effect of the aforementioned variables, the probability of live birth was significantly higher in the MNC group (OR: 4.01, 95% CI: 1.14–14.09, $P = 0.030$) (Table V).

Repeating the above analysis by controlling in addition for the number of embryos transferred or for the number of good-quality embryos transferred, which were significantly different between the two groups compared (Table II), did not change the direction of the results obtained (Table VI). MNC was still associated with a higher probability of live birth although the effect of the number of embryos transferred ($P = 0.009$) or that of the number of good-quality embryos transferred ($P < 0.001$) was also significantly associated with the probability of live birth (Table VI). Two live births occurred in women above 40 years of age: one at the age of 41 (HDFSH group: 1.1%, 95% CI: 1.5–7.6) and one at the age of 43 years (MNC group: 1.0%, 95% CI: 1.4–6.9). The lowest age at which live births were achieved were 34 and 36 years in the two groups compared, respectively. The highest basal FSH level associated with subsequent live birth was 25 IU/l.

Table III Progression of MNC and HDFSH GnRH antagonist cycles from cycle initiation to embryo transfer.

	MNC (n = 161)	HDFSH (n = 164)	P^a
	% (n) 95% CI		
Cycle cancellation % per started cycle	7.5 (12) 3.1–11.8	16.5 (27) 10.7–22.2	0.020
Due to no follicle development	5.0 (8) 1.2–8.7	13.4 (22) 8.6–18.3	0.018
Due to premature follicle rupture	2.5 (4) 0.1–4.9	3.1 (5) 0.5–5.6	0.751
Cycles with retrieved oocyte(s) % per cycle reaching oocyte retrieval	83.9 (125) 77.4–90.4	92.7 (127) 88.4–97.0	0.028
Cycles with retrieved oocyte(s) % per started cycle	77.6 (125) 70.8–84.5	77.4 (127) 70.8–84.1	0.967
Cycles with 2PN oocytes per cycle with oocytes retrieved	63.2 (79) 54.5–71.9	80.3 (102) 73.4–87.2	0.003
Cycles with 2PN oocytes per started cycle	49.1 (79) 40.7–57.5	62.2 (102) 54.3–70.1	0.027
Cycles with embryo transfer per cycle with 2PN oocytes	68.4 (78) 59.5–77.4	72.8 (99) 64.9–80.7	0.471
Cycles with embryo transfer per started cycle	48.5 (78) 40.8–56.1	60.4 (99) 52.4–68.3	0.036
Cycles with at least one good-quality embryo transferred per started cycle	62.5 (60) 52.7–72.3	62.7 (69) 53.0–72.5	0.974
Cycles with at least one good-quality embryo transferred per cycle with ET	82.8 (60) 75.2–90.3	80.3 (69) 73.0–87.6	0.646

MNC, modified natural cycle; HDFSH, high-dose FSH; PN, pronuclei; embryo transfer, embryo transfer.
[a]Logistic regression with clustered robust standard error.

Discussion

The present study suggests that MNC-IVF is associated with a four times higher probability of live birth, and significantly lower gonadotrophin consumption compared with the HDFSH GnRH antagonist protocol in Bologna criteria poor responders, after adjusting for the confounding effect of basal FSH, cause of infertility and female age. To the best of our knowledge, this is the first study to examine the effectiveness of an MNC compared with an HDFSH GnRH antagonist protocol in patients who fulfill the Bologna criteria for the definition of poor ovarian response.

To date, no randomized controlled trials (RCTs) exist comparing MNC with HDFSH or other conventional ovarian stimulation protocols in poor responders. The only relevant RCT (Morgia *et al.*, 2004) comparing natural cycle against flare-up FSH protocol in poor responders undergoing IVF suggested that the probability of clinical pregnancy is similar between the two strategies (6.1 versus 6.9% per cycle, respectively).

Published data on the effectiveness of MNC in poor responders are very limited. Two available studies on the use of MNC in poor responders (Kolibianakis *et al.*, 2004; Kedem *et al.*, 2014) showed that MNC-IVF does not offer a realistic solution for parenthood in these patients, since live birth rates were disappointingly low. Higher pregnancy rates compared with the current study have also been reported with the use of MNC (Elizur *et al.*, 2005) in poor responders (9.6% clinical pregnancy per cycle). These, however, might be explained by the lower mean FSH of the patients included in that study (FSH $<$ 10 IU/l) who were not fulfilling the Bologna criteria.

Any conclusions from the current study should be viewed with caution, due to its retrospective nature. Although the two groups compared were not imbalanced for all basic characteristics (Table I) and a significant effort has been made to eliminate all known sources of systematic error through multivariable analysis (Tables V and VI), there might still exist non-apparent sources of bias confounding the comparison of live birth rates between the two strategies for treating poor responders. Based on the results of the current retrospective study, an RCT comparing the MNC with the HDFSH antagonist protocol in this patient population appears to be worth performing.

It is not clear, based on the results of the current study, what is the explanation of the higher probability of live birth in the MNC group as compared with the HDFSH group, in the presence of fewer oocytes retrieved, fewer oocytes fertilized and fewer embryos transferred. Nevertheless, it could be hypothesized that the higher probability of live birth in the MNC group might be explained by differences in endometrial receptivity when compared with the HDFSH group. In this respect, it has been shown that the estradiol and progesterone receptor expression in stimulated cycles on the day of hCG administration is similar to that present during the first days of the luteal phase in natural cycles (Papanikolaou *et al.*, 2005). Thus transfer in the HDFSH cycles might have occurred, in the current study, with a suboptimal endometrium when compared with the transfer after MNC. High doses of gonadotrophins, such as

Table IV Baseline characteristics in cycles that did or did not result in live birth after treatment by either MNC or HDFSH antagonist protocol.

	No live birth, *N* = 308	Live birth, *N* = 17	*P*
	Mean ± RSE 95% CI[a]		
Age (years)	41.2 ± 0.3 40.6 – 41.7	37.6 ± 0.5 36.5 – 38.7	<0.001
Body mass index (kg/m^2)	23.1 ± 0.3 22.6 – 23.7	22.3 ± 0.6 21.1 – 23.5	0.202
Duration of infertility (years)	3.5 ± 0.2 3.0 – 3.9	3.2 ± 0.4 2.4 – 4.1	0.647
Number of previous attempts	3.3 ± 0.1 3.1 – 3.5	3.9 ± 0.4 3.2 – 4.7	0.114
Basal FSH (IU/l)	22.7 ± 0.8 21.0 – 24.4	17.4 ± 1.0 15.5 – 19.3	<0.001
Basal LH (IU/l)	9.9 ± 0.5 9.0 – 10.7	8.3 ± 0.8 6.8 – 9.8	0.088
Basal estradiol (pg/ml)	34.0 ± 1.4 31.4 – 36.7	33.9 ± 3.3 27.4 – 40.4	0.968
Basal progesterone (ng/ml)	0.6 ± 0.0 0.5 – 0.7	0.5 ± 0.1 0.4 – 0.7	0.320
Antral follicle count	2.7 ± 0.1 2.6 – 2.9	2.9 ± 0.3 2.4 – 3.5	0.394
Cause of infertility[b]	% (95% CI)[b]		
Poor ovarian reserve only	6.5 (3.5 – 9.5)	47.1 (22.1 – 72.1)	<0.001
Poor ovarian reserve + male factor	15.3 (10.7 – 19.8)	23.5 (4.6 – 42.5)	
Poor ovarian reserve + endometriosis	6.8 (3.7 – 9.9)	11.8 (0 – 26.2)	
Poor ovarian reserve + advanced maternal age (≥40 years)	60.1 (53.4 – 66.7)	11.8 (0.0 – 27.2)	
Poor ovarian reserve + tubal factor	11.4 (7.4 – 15.3)	5.9 (0 – 17.1)	
Fertilization method			
IVF	34.9 (27.7 – 42.1)	35.3 (11.8 – 58.8)	0.974
ICSI	65.1 (57.9 – 72.4)	64.7 (41.2 – 88.2)	
Day of embryo transfer			
Day 2	23.7 (17.1 – 30.3)	6.7 (0 – 18.5)	0.135
Day 3	76.3 (69.8 – 82.8)	93.3 (81.5 – 100.0)	

FSH, follicle stimulating hormone; LH, luteinizing hormone; MNC, modified natural cycle; HDFSH, high-dose FSH; hCG, human chorionic gonadotrophin; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection.
[a]Simple linear regression with clustered robust standard error (RSE) adjusted for multiple observations.
[b]Univariable logistic regression with clustered RSE adjusted for multiple observations.

those used in the HDFSH antagonist group, result in significantly higher E2 levels on the day of hCG administration and have been associated with decreased endometrial receptivity (Devroey *et al.*, 2004; Horcajadas *et al.*, 2007). Moreover, the considerably milder approach of the MNC may result in an oocyte of better quality and higher developmental competence, and thus to the transfer of a viable embryo, as opposed to antagonist protocols that employ high gonadotrophin dosages (Reyftmann *et al.*, 2007). This is further supported by the fact that the proportion of cycles with at least one good-quality embryo transferred was similar between the MNC and the HDFSH groups (Table III), while the odds ratio for the probability of live birth increased in favor of the MNC group after adjusting for the number of embryos transferred or the number of good-quality embryos transferred (Table VI).

Despite the superiority of the MNC regarding the probability of live birth when compared with the HDFSH antagonist protocol, the results of the current study cannot be used to promote MNC in poor responders fulfilling the Bologna criteria as a method offering a realistic chance of parenthood. Nevertheless, it is generally accepted that patients should not be denied treatment and instead they should be counseled extensively about the chances of a successful outcome using their own genetic material. In this respect, the current data might be of help in counseling such patients, who do not wish to undergo oocyte donation, prior to abandoning treatment altogether and/or proceeding to adoption.

MNC appears to offer a more patient-friendly approach associated with a significantly lower consumption of gonadotrophins apparently

Table V Multivariable logistic regression with robust clustered standard errors (RSE) estimating odds ratio for live birth adjusted for variables found to have a significant association on live birth in univariable logistic regression analysis (Table IV).

	Odds ratio (95% CI)	Robust SE	*P*
HDFSH (ref)			
MNC	4.01 (1.14–14.09)	2.57	0.030
Age	0.93 (0.74–1.18)	0.11	0.545
Basal FSH	0.87 (0.80–0.93)	0.03	<0.001
Cause of infertility			
Poor ovarian reserve only (ref)			
Poor ovarian reserve + male factor	0.12 (0.03–0.45)	0.08	0.002
Poor ovarian reserve + endometriosis	0.17 (0.03–1.03)	0.16	0.054
Poor ovarian reserve + advanced maternal age (≥40 years)	0.02 (0.00–0.35)	0.03	0.007
Poor ovarian reserve + tubal factor	0.04 (0.00–0.40)	0.05	0.006

HDFSH, High-dose FSH; MNC, modified natural cycle.
Model $P = 0.001$. Pseudo-$R^2 = 0.31$.

Table VI Odds ratio for live birth after multivariable logistic regression with robust clustered standard errors adjusting for female age, basal FSH, cause of infertility, plus number of embryos transferred (Model 1) or number of good-quality embryos transferred (Model 2).

Model 1	**Odds ratio (95% CI)**	**Robust SE**	***P***
HDFSH (ref)			
MNC	15.12 (1.94–117.24)	15.80	0.009
Age	0.94 (2.33–10.68)	0.13	<0.001
Basal FSH	0.87 (0.79–0.96)	0.04	0.005
Cause of infertility			
Poor ovarian reserve only (ref)			
Poor ovarian reserve + male factor	0.09 (0.02–0.39)	0.07	0.001
Poor ovarian reserve + endometriosis	0.09 (0.01–0.98)	0.11	0.048
Poor ovarian reserve + advanced maternal age (≥40 years)	0.01 (0.00–0.22)	0.02	0.003
Poor ovarian reserve + tubal factor	0.03 (0.00–0.36)	0.04	0.005
Number of embryos transferred	4.99 (1.95–10.68)	1.94	0.009
Model 2	**Odds ratio**	**Robust SE**	***P***
HDFSH (ref)			
MNC	8.50 (1.89–38.11)	6.50	0.005
Age	0.89 (0.69–1.16)	0.12	0.395
Basal FSH	0.86 (0.78–0.97)	0.05	0.010
Cause of infertility			
Poor ovarian reserve only (ref)			
Poor ovarian reserve + male factor	0.09 (0.02–0.44)	0.07	0.002
Poor ovarian reserve + endometriosis	0.24 (0.29–2.09)	0.27	0.199
Poor ovarian reserve + advanced maternal age (≥40 years)	0.03 (0.00–0.47)	0.03	0.014
Poor ovarian reserve + tubal factor	0.05 (0.01–0.38)	0.05	0.014
Number of good-quality embryos transferred	4.54 (2.16–9.55)	1.72	<0.001

HDFSH, high-dose FSH; MNC, modified natural cycle.
Model 1: $P = 0.000$; pseudo-$R^2 = 0.43$.
Model 2: $P = 0.001$; pseudo-$R^2 = 0.43$.

leading to a lower cost of FSH/GnRH analogue treatment when compared with HDFSH antagonist protocol. Nevertheless, a formal cost-effectiveness analysis has not been performed in the current study, and is worth pursuing in this difficult category of patients.

In conclusion, the current study suggests that MNC-IVF offers a higher probability of live birth than ovarian stimulation with HDFSH and GnRH antagonists in poor responders fulfilling the Bologna criteria who do not wish to undergo oocyte donation.

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Authors' roles

T.G.L. had the original idea, provided general supervision of the study, participated in the study design, acquisition, analysis and interpretation of data, writing and revision of the manuscript, and performed clinical work. I.A.S. participated in the acquisition, analysis and interpretation of data, writing and revision of the manuscript, and performed embryology work. C.A.V. contributed in the statistical analysis and interpretation of data, and participated in the revision of the manuscript. G.T.L. participated in the study design, acquisition and analysis of data and writing of the manuscript. I.Z.Z. participated in the acquisition of data and performed clinical work. B.C.T. participated in the interpretation of data and revision of the manuscript. E.M.K. performed the statistical analysis and interpretation of data, and participated in the writing revision and supervision of the study. All authors read and approved the final manuscript.

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Conflict of interest

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