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ORIGINAL ARTICLE *Reproductive endocrinology*

The BISTIM study: a randomized controlled trial comparing dual ovarian stimulation (duostim) with two conventional ovarian stimulations in poor ovarian responders undergoing IVF

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STUDY QUESTION: Is the total number of oocytes retrieved with dual ovarian stimulation in the same cycle (duostim) higher than with two consecutive antagonist cycles in poor responders?

SUMMARY ANSWER: Based on the number of total and mature oocytes retrieved in women with poor ovarian response (POR), there is no benefit of duostim versus two consecutive antagonist cycles.

WHAT IS KNOWN ALREADY: Recent studies have shown the ability to obtain oocytes with equivalent quality from the follicular and the luteal phase, and a higher number of oocytes within one cycle when using duostim. If during follicular stimulation smaller follicles are sensitized and recruited, this may increase the number of follicles selected in the consecutive luteal phase stimulation, as shown in non-randomized controlled trials (RCT). This could be particularly relevant for women with POR.

STUDY DESIGN, SIZE, DURATION: This is a multicentre, open-labelled RCT, performed in four IVF centres from September 2018 to March 2021. The primary outcome was the number of oocytes retrieved over the two cycles. The primary objective was to demonstrate in women with POR that two ovarian stimulations within the same cycle (first in the follicular phase, followed by a second in the luteal phase) led to the retrieval of 1.5 (2) more oocytes than the cumulative number of oocytes from two consecutive conventional stimulations with an antagonist protocol. In a superiority hypothesis, with power 0.8 alpha-risk 0.05 and a 35% cancellation rate, 44 patients were needed in each group. Patients were randomized by computer allocation.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Eighty-eight women with POR, defined using adjusted Bologna criteria (antral follicle count $\leq$5 and/or anti-Müllerian hormone $\leq$1.2 ng/ml) were randomized, 44 in the duostim group and 44 in the conventional (control) group. HMG 300 IU/day with flexible antagonist protocol was used for ovarian stimulation, except in luteal phase stimulation of the duostim group. In the duostim group, oocytes were pooled and inseminated after the second retrieval, with a freeze-all protocol. Fresh transfers were performed in the control group, frozen embryo transfers were performed in both control and duostim groups in natural cycles. Data underwent intention-to-treat and per-protocol analyses.

MAIN RESULTS AND THE ROLE OF CHANCE: There was no difference between the groups regarding demographics, ovarian reserve markers, and stimulation parameters. The mean (SD) cumulative number of oocytes retrieved from two ovarian stimulations was

not statistically different between the control and duostim groups, respectively, 4.6 (3.4) and 5.0 (3.4) [mean difference (MD) [95% CI] +0.4 [−1.1; 1.9], $P = 0.56$]. The mean cumulative numbersof mature oocytes and total embryos obtained were not significantly different between groups. The total number of embryos transferred by patient was significantly higher in the control group 1.5 (1.1) versus the duostim group 0.9 (1.1) ($P = 0.03$). After two cumulative cycles, 78% of women in the control group and 53.8% in the duostim group had at least one embryo transfer ($P = 0.02$). There was no statistical difference in the mean number of total and mature oocytes retrieved per cycle comparing Cycle 1 versus Cycle 2, both in control and duostim groups. The time to the second oocyte retrieval was significantly longer in controls, at 2.8 (1.3) months compared to 0.3 (0.5) months in the duostim group ($P < 0.001$). The implantation rate was similar between groups. The cumulative live birth rate was not statistically different, comparing controls versus the duostim group, 34.1% versus 17.9%, respectively ($P = 0.08$). The time to transfer resulting in an ongoing pregnancy did not differ in controls 1.7 (1.5) months versus the duostim group, 3.0 (1.6) ($P = 0.08$). No serious adverse events were reported.

LIMITATIONS, REASONS FOR CAUTION: The RCT was impacted by the coronavirus disease 2019 pandemic and the halt in IVF activities for 10 weeks. Delays were recalculated to exclude this period; however, one woman in the duostim group could not have the luteal stimulation. We also faced unexpected good ovarian responses and pregnancies after the first oocyte retrieval in both groups, with a higher incidence in the control group. However, our hypothesis was based on 1.5 more oocytes in the luteal than the follicular phase in the duostim group, and the number of patients to treat was reached in this group (N = 28). This study was only powered for cumulative number of oocytes retrieved.

WIDER IMPLICATIONS OF THE FINDINGS: This is the first RCT comparing the outcome of two consecutive cycles, either in the same menstrual cycle or in two consecutive menstrual cycles. In routine practice, the benefit of duostim in patients with POR regarding fresh embryo transfer is not confirmed in this RCT: first, because this study demonstrates no improvement in the number of oocytes retrieved in the luteal phase after follicular phase stimulation, in contrast to previous non-randomized studies, and second, because the freeze-all strategy avoids a pregnancy with fresh embryo transfer after the first cycle. However, duostim appears to be safe for women. In duostim, the two consecutive processes of freezing/thawing are mandatory and increase the risk of wastage of oocytes/embryos. The only benefit of duostim is to shorten the time to a second retrieval by 2 weeks if accumulation of oocytes/embryos is needed.

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Key words: dual ovarian stimulation / duostim / IVF / luteal phase stimulation / oocyte accumulation / poor ovarian reserve / poor ovarian responder

Introduction

In IVF, the chance of a live birth is correlated with the number of oocytes retrieved. Each additional oocyte increases the chance of a live birth, and women with poor ovarian response (POR) have lower chances of conceiving compared to women with normal or high ovarian *reserve*, even after several IVF cycles. The timing of events is also an important matter, as the reserve and the quality of oocytes decline with age. Even a short period of postponing IVF decreases the chance of a life birth, especially in older women (Bhattacharya *et al.*, 2021). Delayed childbearing increases the risk of POR, and older women are of particular attention as they are growing in number worldwide (Choi *et al.*, 2022). However, to date, there is no validated treatment or add-on to improve the prognosis for POR women. The ESHRE guideline group on ovarian stimulation urges for more research in this group of patients (The ESHRE Guideline Group on Ovarian Stimulation *et al.*, 2020).

Two or three waves of follicular development occur in the same cycle (Baerwald *et al.*, 2003a,b). Several studies have shown the ability to obtain oocytes with equivalent quality in the follicular and the luteal

phase, with different stimulation protocols reviewed in Massin (2017), even after a previous ovarian stimulation in the follicular phase. The equivalent competence of oocytes was demonstrated either by a similar euploid blastocyst rate (Ubaldi *et al.*, 2016) or by an equivalent implantation and live birth rate with safety for the children (Kuang *et al.*, 2014b; Chen *et al.*, 2015). Therefore, in all the published studies, a higher number of oocytes were obtained with dual stimulation in a single cycle (duostim) compared to a single conventional cycle, as shown in the review from Glujovsky *et al.* (2020) (MD 3.35, 95% CI 2.54–4.15, moderate-quality evidence). This appears as an interesting option in case of a short time for medical or personal reasons (such as urgent fertility preservation or cost), but the counterpart in women actively trying to conceive is the need for a freeze-all strategy and postponed transfer.

In the hypothesis that during stimulation smaller follicles are recruited and sensitized, this may increase the selection of follicles available on the second stimulation. In fact, until recently, only retrospective, and non-randomized prospective studies have been performed to evaluate the increase of the number of oocytes in the second stimulation for women with POR. Most of the studies in the more recent review from Polat *et al.* (2021) tend to show a slight increase in the number of oocytes in the luteal phase after a follicular stimulation (Kuang *et al.*, 2014a; Vaiarelli *et al.*, 2018). Only one small observational study reported more oocytes in the follicular than in the luteal phase with duostim (5.3 versus 3.8, respectively) (Bourdon *et al.*, 2020). A greater increase (4.8 versus 6.6, respectively) was shown in the larger observational study (N = 827) reported by Vaiarelli *et al.* (2020). In POR women this potentializing may be of great interest, as two stimulations in the same cycle could give a higher number of oocytes in a shorter time compared to two consecutive conventional stimulations. However, conflicting results remain: in a recent retrospective study, Li *et al.* (2022) report a comparable cumulative number of oocytes retrieved with duostim versus two consecutive mild stimulations in a POR population (3.0 versus 3.4, respectively, $P = 0.15$); and in a more recent randomized controlled trial (RCT), Cerrillo *et al.* (2022) report, in women aged ≥38 years with low ovarian reserve markers, a lower cumulative number of oocytes retrieved with duostim versus two antagonist cycles (9.2 versus 13.4, respectively, $P = 0.01$). A similar number of euploid blastocysts (0.6 versus 0.8, respectively, $P = 0.45$) were obtained but the RCT was stopped early; in this RCT, the number of oocytes retrieved in the luteal phase and follicular phase was similar (5.1 versus 4.1, respectively, $P = 0.23$). These data need to be confirmed, particularly in the context of immediate desire for a pregnancy and not the accumulation of embryos.

The primary objective of the present study was to compare the cumulative number of oocytes retrieved in women with POR using duostim versus two consecutive conventional cycles. Secondary objectives included stimulation parameters, time to the second oocyte retrieval and live birth, and cumulative clinical pregnancy and live birth rates.

Materials and methods

Design

This is a multicentre, open-labelled RCT, performed in four IVF centres in France from September 2018 to March 2021 (trial registration number: ID-RCB 2017-A00498-45).

Participants

Women with POR were defined with adjusted Bologna criteria (Ferraretti *et al.*, 2011; Ferraretti and Gianaroli, 2014), i.e. at least two of the three following criteria: advanced maternal age (≥40 years); ≤3 oocytes in previous IVF; and antral follicle count (AFC) ≤ 5 and/or anti-Müllerian hormone (AMH) ≤ 1.1 ng/ml (this criterion was mandatory). These criteria correspond to the Poseidon criteria (Poseidon Group (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number) *et al.*, 2016) group 3 (<35 years) and group 4 (≥35 years). POR women enrolled in an IVF or ICSI programme, aged from 20 to 41 years, with BMI from 19 to 32 kg/m^2, with no more than two previous IVF cycles were recruited. Women with amenorrhoea, FSH ≥20 IU/L or AFC ≤1 and women with a partner with an extremely severe sperm anomaly or sperm donor use were excluded. After presentation of the research and obtaining signed informed consent, patients were included on the same day of planning of the IVF cycle by their physician who performed the randomization and allocation. Patients were randomized using a computer system independent of the physician, stratified by age <35 and ≥35 years and by centre. Patients and physicians were not blinded to group assignment.

Interventions

All women were pre-treated with 17β-oestradiol (Provames®—Norgine, France, Rueil-Malmaison) 4 mg/day starting 7 days before expected date of menses, followed by a flexible antagonist protocol in the first stimulation cycle (control and duostim Group OK). HMG FertistartKit® (IBSA, Lugano, Switzerland) 300 IU/day at fixed dose was used for ovarian stimulation. Antagonist (Orgalutran®—Organon, Amsterdam, Netherlands) 0.25 mg/day was started when the leading follicle was ≥14 mm and/or oestradiol ≥500 pg/mL. Recombinant HCG (Ovitrelle®—Merck KGaA, Darmstadt, Germany) 250 µg was used to trigger ovulation. Oocyte retrieval was performed 36 h later. In the control group, ICSI was performed with a fresh transfer with vaginal luteal support, 600 mg of natural progesterone (Progestan®—Besins Healthcare, Monaco), and supernumerary embryos were cryopreserved and consecutively transferred before the second ovarian stimulation. The first cycle was consecutively followed by a second similar cycle if no pregnancy was achieved.

In the duostim group, all the mature oocytes were vitrified after the first oocyte retrieval, and the second ovarian stimulation cycle started the day after, in the luteal phase, with FertistartKit® 300 IU/day. No antagonist was used in the luteal phase and natural progesterone (Progestan® 400 mg/day) was started after 7 days of stimulation until triggering with Ovitrelle® 250 µg, to avoid menstrual bleeding. On the day of the second oocyte retrieval, the cryopreserved and fresh oocytes were pooled to perform ICSI, and all embryos were frozen. All the frozen/thawed embryo transfers were performed in the consecutive spontaneous cycle with a modified natural cycle (600 mg of Progestan® started the day before embryo transfer).

As defined in the registered protocol, women in the duostim group with an unexpected fair response (six or more oocytes) were dropped out of the study to have the chance of fresh embryo transfer. To avoid bias, the women in the control group with six or more oocytes were also dropped out the study. Stimulation protocols are presented in Supplementary Fig. S1.

Oocyte vitrification was performed in a close system (High security straw from Cryo Bio System®, L'Aigle, France), according to the IrvineScientific protocol (Fujifilm®, Santa Ana, CA, USA), after 3 h incubation at 37 °C—6% CO_2 followed by decoronization with hyaluronidase (Fertipro®, Beernem, Belgium). The IrvineScientific protocol was used for the thawing process. All the embryos were cleavage stage embryos (Day 2 or 3).

Sample size calculation

The primary objective was to demonstrate that two ovarian stimulations within the same cycle (first in the follicular phase, followed by a second in the luteal phase) led to the mean (SD) retrieval of 1.5 (2) more oocytes than the cumulative number of oocytes from two consecutive conventional stimulations with antagonist protocol, in women with POR. The hypothesis was based on the publication of Kuang *et al.* (2014a) reporting 1.7 (1.0) oocytes in follicular phase versus 3.5 (3.2) in the luteal phase, i.e. +1.8 oocytes in the luteal phase stimulation in duostim. In a superiority hypothesis, with power 0.8 and alpha-risk 0.05, the number of patients needed was 28 per group. We anticipated a high cancellation rate of 35% in this population (no response or unexpected, good response—six or more oocytes—drop out, pregnancy after first cycle with conventional protocol). According to this, 44 patients were needed in each group, 88 women in total. The CONSORT flowchart is presented in Fig. 1, showing that 44 women were randomized to the control group (conventional protocol) and 44 to the duostim group.

Statistical analysis

The baseline variables are described in a comparative way between the two groups. Quantitative variables are described by their mean and SD values. Categorical variables are described as counts and percentages. The analysis is presented by intention to treat (all the women who started the first ovarian stimulation) and, when notified, per protocol (women who completed two oocyte retrievals). The primary outcome is the number of oocytes retrieved over the two cycles. When the patient had a missing value for the number of oocytes in one of the cycles, this value was replaced by zero. Student's *t*-test was performed to compare the MD between the two treatment groups. Other endpoints were compared by their means and proportions. A chi-square or Fisher test was performed to compare the proportions. The significance level was 5%.

The additional analyses consisted of studying an association between the treatment and the occurrence of pregnancy by a survival analysis. The survival curves of the two groups in relation to the occurrence of pregnancy were compared by a log-rank test. A Cox model was then performed to quantify the association (hazard ratio with its 95% CI) between treatment and the occurrence of pregnancy. Because the proportional hazard assumption was not valid for the survival curve in

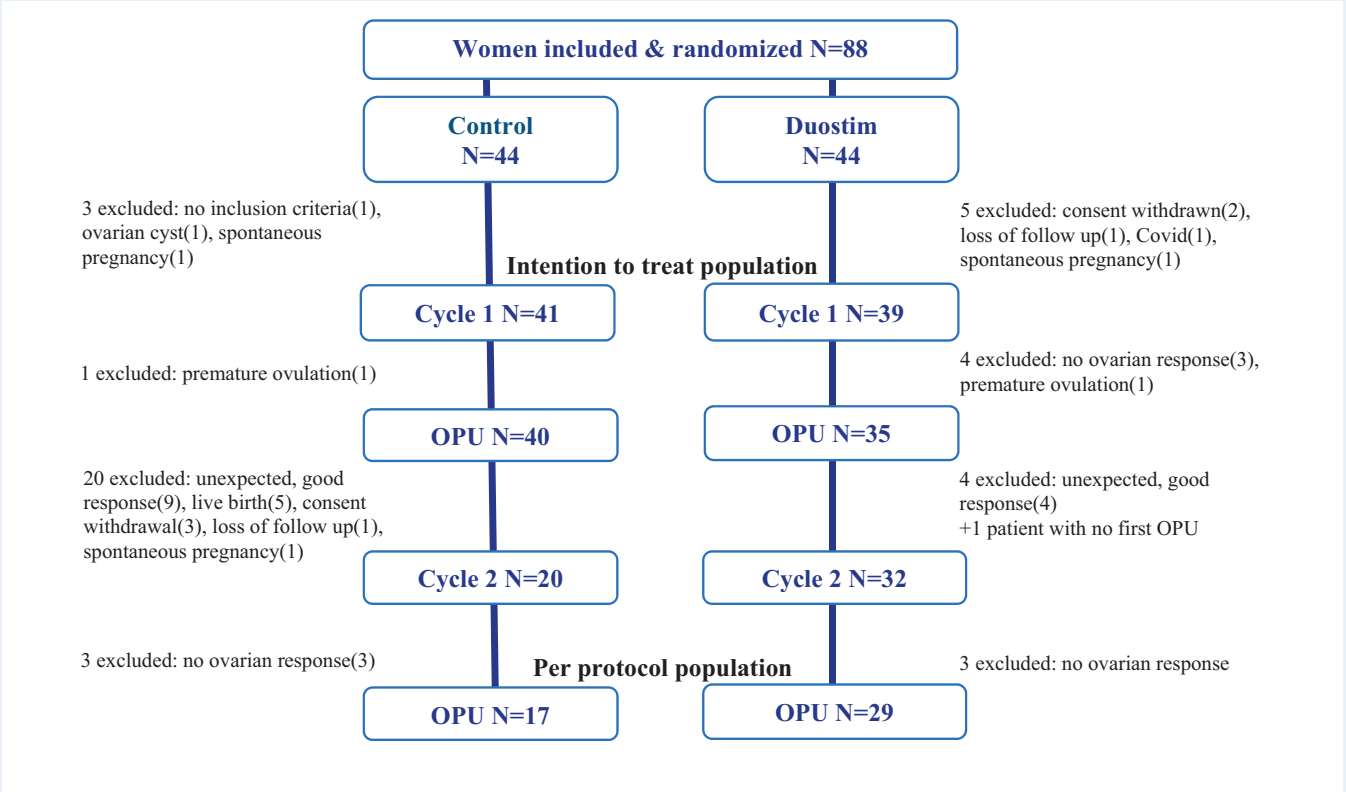

Figure 1. Consort flow chart of the BISTIM study. BISTIM is a randomized controlled trial comparing dual ovarian stimulation (duostim) with two conventional ovarian stimulations in women with poor ovarian response undergoing IVF. OPU: oocyte pick-up (retrieval). For an extended flow chart, with embryo transfer and live birth data, see Supplementary Fig. S2.

our population (hazard curves crossed) and the conclusion of hazard ratio and log-rank test could be biased, we also performed the restricted mean survival difference method (RMST). This method overcomes some limitations of the hazard ratio when the main assumptions of the survival analysis are not met. In our case, the RMST difference (ΔRMST) will represent the mean absolute difference in time between two groups relative to the time to pregnancy.

All analyses were performed with the R statistical software package (R Foundation for Statistical Computing, Vienna).

Results

There was no difference between the groups regarding demographics and ovarian reserve markers (AFC and AMH), presented in Table I.

There was no difference between the groups for ovarian stimulation parameters (days of stimulation, total dose of gonadotrophins used). The mean (SD) number of cumulative oocytes retrieved from two ovarian stimulations was not statistically different between the duostim and the control groups, 5.0 (3.4) and 4.6 (3.4) (MD [95% CI] +0.4 [−1.1; 1.9], $P=0.56$), respectively, in intention-to-treat analysis, and 4.7 (3.0) and 5.4 (2.9) (MD +0.7 [−1.1; 2.6], $P=0.43$), respectively, in per-protocol analysis. Ovarian stimulation outcomes per cycle and per cumulative cycles are presented in Table II. The mean (SD) cumulated number of embryos was not significantly different between the duostim and the control groups, 2.0 (2.3) and 2.7 (2.6) (MD [95% CI] −1.7 [−1.8; 0.3], $P=0.18$), respectively, in intention-to-treat analysis, and 2.3 (2.3) and 2.7 (2.4) (MD −0.4 [−1.9; 1.0], $P=0.53$), respectively in per-protocol analysis. However, the total number of useable embryos (transferred, fresh, or frozen) was significantly lower in the duostim group 0.9 (1.1) versus the control group 1.5 (1.1) ($P=0.03$) (MD [95% CI] −0.6 [−1.1; −0.1], $P=0.03$), respectively, in intention-to-treat analysis but does not reach statistical difference in per-protocol analysis, 1.2 (1.2) and 1.6 (1.1) (MD −0.4 [−1.1; 0.2], $P=0.25$), respectively. After two cumulative cycles, 53.8% of women in the duostim group and 78% in the control group had at least one transfer ($P=0.02$) in intention-to-treat analysis (64.3% and 82.4%, $P=0.20$, respectively, in per protocol). No serious adverse event was reported in the study.

Table I Demographic data of participants in randomized controlled trial comparing dual ovarian stimulation with two conventional ovarian stimulations in women with poor ovarian response.

Mean (SD)	Control group N = 41	Duostim group N = 39
Age (years)	35.7 (3.9)	35.5 (3.4)
BMI (kg/m²)	23.7 (3.5)	24.5 (3.8)
Primary infertility	28 (68.3%)	20 (51.3%)
Cause of infertility		
Female	19 (46.3%)	21 (53.8%)
Male	7 (17.1%)	5 (12.8%)
Idiopathic	15 (36.6%)	13 (33.3%)
Infertility duration (months)	41.9 (28.7)	42.5 (26.0)
Smoking	4.0 (9.8%)	8.0 (20.5%)
Antral follicle count	5.4 (2.7)	5.3 (2.4)
Basal FSH (IU/l)	9.2 (3.2)	10.1 (4.8)
Basal estradiol (pg/ml)	55.9 (36.1)	70.6 (70.4)
AMH (ng/ml)	0.5 (0.2)	0.5 (0.3)

AMH: anti-Müllerian hormone; duostim: dual ovarian stimulation (in follicular then luteal phase of the same cycle); control: two conventional ovarian stimulation cycles.

Comparing Cycle 1 (follicular phase) with Cycle 2 (luteal phase) in the duostim group (per-protocol analysis N = 28), the number of days of stimulation and the total gonadotrophin dose were not statistically different, and the mean (SD) number of oocytes retrieved was similar in follicular and luteal phases: 2.8 (1.8) versus 2.6 (1.8), respectively ($P=0.66$). The overall oocyte survival rate after vitrification was 81.6%. In the control group (per-protocol analysis N = 17), the duration of stimulation and the total gonadotrophin dose were not statistically different between Cycles 1 and 2. The mean (SD) number of oocytes retrieved in each cycle was similar 2.0 (1.5) and 2.7 (2.2) ($P=0.28$). The mean (SD) time to the second oocyte retrieval was statistically shorter in the duostim group 14.4 (2.3) days versus 82.8 (38.1) in the control group ($P<0.001$).

The implantation rate was similar in both groups. The cumulative clinical pregnancy rate was significantly lower in the duostim group (17.9% versus 39%, $P=0.04$). However, the cumulative live birth rate was not statistically different: 7 live births (17.9%) were obtained in the duostim group and 14 (34.1%) in the control group ($P=0.1$). All the embryos obtained were transferred at the end of the study.

The mean (SD) duration (months) of follow up was similar in the duostim 2.8 (2.3), and the control 2.7 (2.2) groups, $P=0.85$. The mean (SD) time from the start of the study (Day 1 of the first stimulation) to the transfer leading to a clinical pregnancy was not statistically different in the duostim group 2.7 (1.1) compared to the control group 1.6 (1.5), $P=0.11$. The survival curve for the event clinical pregnancy is presented in Fig. 2. The cumulative hazard ratio (95% CI) for clinical pregnancy with the conventional protocol compared to the duostim protocol was 2.24 [0.91–5.5], $P=0.07$. In fact, the hazard ratio in the Cox model was biased (the curves cross each other). The RMST is one of methods suggested in this case (Royston and Parmar, 2013). RMSTs were 147.8 and 128.7 days in the duostim and control groups, respectively. The difference in RMST gives the difference in time to pregnancy in the duostim group +19.1 days [−24.5 to 62.7] compared to the control group ($P=0.39$).

No serious adverse events were reported.

Unfortunately, cost analyses could not be performed (owing to lack of sufficient collected data).

The primary outcome (total number of oocytes) and secondary outcomes (oocyte cryo-survival, fertilization rates and cumulative live birth rate) were reproducible between the four centres.

Discussion

The present study comparing two consecutive ovarian stimulation protocols in patients with POR, one in the same cycle in the follicular then luteal phase (duostim) and the second in two follicular phase cycles, failed to demonstrate a superiority of duostim regarding the cumulative number of oocytes obtained, and mature oocytes. This is the

Table II Ovarian stimulation outcomes per cycle and cumulated cycles comparing the duostim protocol (duostim group) with two conventional antagonist cycles (control group).

Mean (SD) Min-max	Control group	Duostim group	Effect size [95% CI]	*P value*
First ovarian stimulation				
Number of patients	**N = 41**	**N = 39**		
Cancellation rate	2 (4.9%)	3 (7.7%)	1.62 [0.25 12.8]**	*0.60*
Days of stimulation	11.7 (2.7) 9–24	11.7 (2.6) 6–18	0 [−1.2; 1.2]	*0.99*
Dose of gonadotrophins (IU)	3491 (966) 2600–8400	3453 (807) 1800–5400	−37 [−433.3; 358.1]	*0.85*
Number of follicles > 14 mm at HCG	3.5 (2.6) 1–13	3.8 (2.8) 1–13	*0.3 [−0.9; 1.6]*	*0.64*
Oocytes	3.5 (3.2) 0–13	3.3 (2.5) 0–10	−0.3 [−1.6; 1.0]	*0.66*
Metaphase II oocytes	2.5 (2.7) 0–13	2.4 (2.3) 0–8	−0.1 [−1.2; 1.0]	*0.86*
Vitrified oocytes	0	2.1 (2.0) 0–6	2.1 [1.4; 2.8]	*<0.001*
Fertilization rate	77.3%	66.1%*	−11.2 [−29.8; 7.3]	*0.22*
Total embryo	2.2 (2.4) 0–11	0.3 (1.2)* 0–6	−1.9 [−2.7; −0.9]	*<0.001*
Vitrified embryo	0.3 (0.8) 0–3	0.1 (0.8)* 0–4	−0.18 [−0.5; 0.2]	*0.35*
Second ovarian stimulation				
Number of patients	**N = 20**	**N = 32**		
Cancellation (%)	3 (15.0%)	3 (9.4%)	0.58 [0.1; 3.5]**	*0.54*
Time to second oocyte retrieval—months	2.8 (1.3) 1–7	0.3 (0.5) 0–1	−2.5 [−3.2; −1.9]	*<0.001*
Time to second oocyte retrieval—months without Covid interval correction	2.9 (1.4) 1–7	0.3 (0.4) 0–1	−2.6 [−3.3; −1.9]	*<0.001*
Days of stimulation	12.0 (3.3) 7–20	11.2 (2.9) 5–18	−0.8 [−2.7; 1.0]	*0.36*
Dose of gonadotrophins (IU)	3250 (1433) 2640–6000	3325 (882) 1500–5400	74.2 [−673.5; 821.9]	*0.82*
Number of follicles > 14 mm at HCG	3.3 (1.6) 1–7	3.1 (1.7) 1–8	*−0.2 [−1.3; 0.8]*	*0.64*
Oocytes	2.9 (2.1) 0–8	2.6 (1.8) 0–9	−0.3 [−1.5; 1.0]	*0.67*
Metaphase II oocytes	2.4 (2.1) 0–7	2.2 (1.7) 0–8	−0.2 [−1.4; 1.0]	*0.74*
Devitrified oocytes	0.1 (0.5) 0–1	1.9 (1.7) 0–5	1.7 [1.0; 2.4]	*< 0.001*
Fertilization rate	77.3% (34.9)	79.7% (30.8)	2.4 [−19.7; 24.6]	*0.82*
Total embryo	1.9 (1.4) 0–5	2.2 (2.3) 0–11	0.4 [−0.8; 1.5]	*0.56*
Vitrified embryo	0.2 (0.4) 0–1	1.1 (1.4) 0–4	0.9 [0.3; 1.4]	*0.02*

(continued)

Table II Continued

Mean (SD) Min-max	Control group	Duostim group	Effect size [95% CI]	*P value*
Cumulative cycles (intention-to-treat analysis)				
Cancellation in both cycles (%)	2 (4.9%)	3 (7,7%)	1.62 [0.2 12.8]**	*0.60*
Total oocytes (Mean (SD))	4.6 (3.4) 0–13	5.0 (3.4) 0–14	0.4 [−1.1; 1.9]	*0.56*
Total oocytes (Median [IQR])	4[1–7]	5.0 [2.5–7]	1 [−2; 4]***	*0.52*
Total Metaphase II oocytes	3.1 (3.0) 0–13	3.8 (3.2) 0–13	0.7 [−0.6; 2.1]	*0.29*
Total embryos	2.7 (2.6) 0–11	2.0 (2.3) 0–11	−0.7 [−1.8; 0.3]	*0.18*
Embryo transfer (%)	32 (78.0%)	21 (53.8%)	0.3 [0.1; 0.8]**	*0.02*
Total embryo transferred (fresh and frozen)	1.5 (1.1) 0–4	0.9 (1.1) 0–4	−0.6 [−1.1; −0.1]	*0.03*
Positive HCG test (%)	18 (43.9%)	11 (28.2%)	0.50 [0.2; 1.2]**	*0.14*
Implantation rate	33.3%	22.2%	−11.1 [−32.8; 10.6]	*0.32*
Cumulative clinical pregnancy rate—positive heartbeat	16 (39.0%)	7 (17.9%)	0.3 [0.1; 0.9]**	*0.04*
Miscarriage rate	2 (4.8%)	0 (0.0%)	0 [NA; +Inf]**	*0.16*
Cumulative live birth rate	14 (34.1%)	7 (17.9%)	0.47 [0.2; 1.3]**	*0.10*
Multiple pregnancy	1 (7.7%)	0 (0.0%)	0 [NA; +Inf]**	*0.33*
Time to clinical pregnancy—months	1.6 (1.5) 0–5	2.7 (1.1) 1–4	1,1 [−0.1; 2.3]	*0.11*
Time to clinical pregnancy—months without COVID-19 interval correction	1.6 (1.5) 0–5	3 (1.4) 1–5	1.4 [−0.05; 2.8;]	0.06

*Four patients with unexpected, good ovarian response (≥6 oocytes) were given the opportunity to have a fertilization with fresh oocytes and undergo a fresh embryo transfer.
**odds ratio.
***Median difference.
NB: median difference and its CI were estimated by bootstrap with 1000 iteration.
Duostim: dual ovarian stimulation (in follicular then luteal phase of the same cycle).
CODIV-19: coronavirus disease 2019; IQR: interquartile range.

second RCT comparing duostim to two conventional antagonist protocols, with the patients treated with the same fixed dose of gonadotrophins (HMG 300 IU), the same number of days of stimulation and the same ovulation triggering. Consistent with the RCT from Cerrillo *et al.* (2022), a similar number of embryos was obtained with both protocols. However, in the present RCT the need for freeze-all embryos in the duostim protocol tended to reduce the number of useable embryos and the rate of patients with embryo transfer, and enhanced the delay to a transfer leading to a clinical pregnancy with duostim, despite reducing the delay between two oocyte retrievals. The implantation rate was similar in both groups and no serious adverse events were reported. The study was not powered for clinical pregnancy and live birth rates.

This was an open-labelled RCT as the patient and the physicians could not be blinded to the study protocol. To prevent bias regarding the primary outcome (total number of oocytes retrieved), we used a strict registered protocol with a fixed dose of gonadotrophins in both groups, criteria of HCG triggering and applied a standard procedure for the oocyte retrieval with the size of follicles retrieved (>10 mm). Unfortunately, the RCT was impacted, both for recruitment and treatment of patients, by the coronavirus disease 2019 pandemic and a halt in IVF activities for 10 weeks. Delays were recalculated to exclude this period to avoid bias. However, one woman in the duostim group could not have the luteal stimulation. Also, we cannot exclude that some patients' withdrawal or loss of follow up was a result of the impact of the pandemic, in both groups but mostly in the control group. Indeed, patients in the duostim group were kept in the study by the accumulation of their oocytes, that may have participated in the reduction of the drop-out rate and to a higher cumulative pregnancy rate, as shown by Cobo *et al.* (2012). However, the accumulation of oocytes/embryos may also increase the number of unnecessary cycles, as women may have the chance of a pregnancy after the first cycle, as shown in the present RCT. This was a main difference in the present RCT to that of Cerrillo *et al.* (2022), wherein women had oocyte accumulation in both groups, for preimplantation genetic testing for aneuploidy (PGT-A) purposes. Furthermore, reducing the drop-out rate does not appear to be a good patient-centred practice or valid scientific outcome.

We also faced unexpected, good ovarian responses, withdrawals, and pregnancies after the first oocyte retrieval in both groups but with a higher incidence in the control group, specifically owing to pregnancies. This leads to the discontinuation of the study of about 50% of

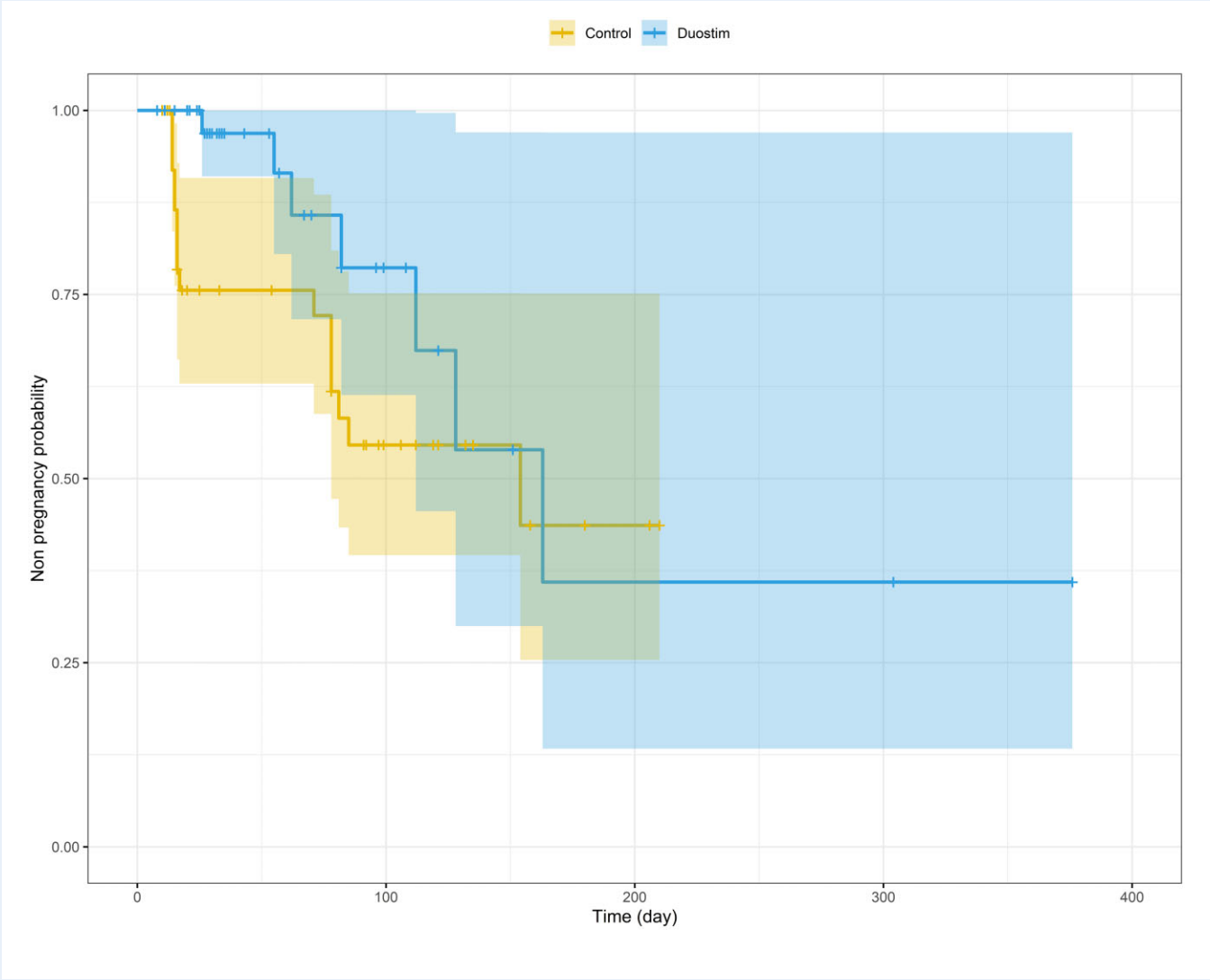

Figure 2. Survival curves for clinical pregnancies in the BISTIM study. BISTIM is a randomized controlled trial comparing dual ovarian stimulation (duostim) with two conventional ovarian stimulations in women with poor ovarian response undergoing IVF. The time to clinical pregnancy is presented in days from Day 1 of the first ovarian stimulation cycle to the embryo transfer leading to a clinical pregnancy (positive foetal heartbeat) for the control (conventional protocol in yellow) and the duostim (in blue) groups.

included patients in the control group, compared to 20% in the duostim group. When designing the study, we decided to allow the possibility of having a fresh transfer for women with at least six or more oocytes; mostly because the patients were primarily selected by their low ovarian reserve markers—for a more homogenous population—and not particularly selected for advanced maternal age (the mean age of this RCT population is 35.6 years) and were thought to have fair chances of having an euploid blastocyst and then a pregnancy, estimated at 30–56% for women with less than nine oocytes (Group 1 and 2 of Poseidon) (Li *et al.*, 2019). Effectively, among women with unexpected, good ovarian response, we reported four live births among nine women in the control group and one live birth among two women with fresh embryo transfer in the duostim group. In our population, primarily defined by POR (mean AFC 5 and mean AMH 0.5 ng/mL), we anticipated a high cancellation rate of 30% for no or insufficient response. Conversely, we were surprised with the incidence of fair ovarian response despite low ovarian reserve markers, consistent with the results of the RCT from Cerrillo *et al.* (2022) with women $\geq$38 years, and moreover with the incidence of pregnancy. This agrees with most of the published studies reporting a predominant effect of age when at least five oocytes are obtained (McLernon *et al.*, 2016). Whatever, regarding the number of patients and the power of this RCT, no conclusion can be reached regarding pregnancy and live birth rates, as the study design also includes fresh oocytes versus oocyte accumulation and fresh versus vitrified-warmed embryo transfer.

Despite the loss of women for the per-protocol analysis (women who effectively underwent two ovarian stimulation cycles), the number of patients calculated for our statistical hypothesis was reached in the duostim group (N = 28). Supported by previous non-randomized, and

mostly retrospectives studies, such as Kuang *et al.* (2014a,b) and Vaiarelli *et al.* (2018, 2020, 2022), we performed a superiority trial based on the hypothesis that more oocytes can be obtained with a luteal phase stimulation after a follicular phase stimulation. In addition, some authors have shown that more oocytes may be retrieved from a luteal phase stimulation, compared to follicular phase stimulation (Li *et al.*, 2016). However, this RCT, like the retrospective study from Li *et al.* (2022) and the RCT from Cerrillo *et al.* (2022), failed to demonstrate any difference in the number of oocytes retrieved from the follicular and subsequent luteal phase, when using the identical dose of gonadotrophins in both ovarian stimulations. Thus, a hypothetic potentializing effect of FSH on the recruitment of early antral follicles (Hsueh *et al.*, 2015) is not demonstrated in this study, at least for a short HMG exposure (mean duration of HMG 12 days). Nevertheless, the present RCT is underpowered to report a lower additional oocytes number in the second stimulation compared to the first stimulation, i.e. +0.4 oocytes with the duostim in the present RCT, not significant.

In the study design, HCG was chosen for all the ovulation triggering, in both groups. This design avoided bias between groups and optimized the chances of pregnancy with fresh embryo transfer, as fresh transfer is not recommended after triggering with GnRH agonist alone (The ESHRE Guideline Group on Ovarian Stimulation *et al.*, 2020) particularly in the absence of luteal support with HCG. However, both Chinese and Italian pilots (Kuang *et al.*, 2014a; Ubaldi *et al.*, 2016) and further studies used GnRH agonist to trigger ovulation. In the study from Kuang *et al.* (2014a), the total dose of gonadotrophins was higher in the luteal phase. Furthermore, in the study from Ubaldi *et al.* (2016) the wash out period after oocyte retrieval was 5 days, i.e. 7 days after GnRH agonist trigger. Without any luteal phase support, GnRH agonist triggering leads to an hormonal environment close to that in early follicular phase (Beckers *et al.*, 2003). Triggering by HCG alone in the present study may have influenced the number of oocytes retrieved compared to GnRH agonist (Haas *et al.*, 2020), but similarly in both groups. To date, there is no published study comparing HCG and GnRH agonist triggering in the duostim protocol, nor comparing types of gonadotrophin or ovulation induction drugs used, nor wash out period between first and second stimulations, to identify a more appropriate protocol.

Consistent with previous studies, the implantation rate in this RCT was similar in both groups (28.9%). Ubaldi *et al.* have largely demonstrated the equal competence (i.e. fertilization, blastocyst and euploidy rates) of oocytes obtained in the luteal phase compared to the follicular phase, with similar clinical outcomes after euploid single-embryo transfer (Ubaldi *et al.*, 2016; Cimadomo *et al.*, 2018), confirmed by the RCT from Cerrillo *et al.* (2022). Ubaldi *et al.* systematically perform PGT-A in association with the duostim protocol for the advanced maternal age/POR population but PGT-A was not used in this RCT, as it is not allowed by law in France. There is no evidence in the literature that selection of an euploid blastocyst enhances the chance of a live birth by started cycle, and some authors do not recommend its systematic use in routine practice (Cornelisse *et al.*, 2020). However, it may help physicians to personalize the duostim protocol for poor prognosis patients, as proposed by Vaiarelli *et al.* (2022). Overall live birth rate (21/80, 26.2%) was close to that reported after two IVF cycles in patients with POR, defined with Poseidon criteria; women with low ovarian reserve markers have estimated chances of a live birth of 24–30% when <35 years (Group 3 of Poseidon) and 9–11% when ≥35 years (Group 4 of Poseidon) (Li *et al.*, 2019). Overall, in this poor prognosis population, using 300 IU HMG with an antagonist protocol or a luteal phase stimulation without antagonist, leads to an accumulation of nearly five oocytes after up to two cycles and fair outcomes (one live birth for four women included). Whereas a small Chinese cohort study reports no live birth defects in 587 infants born after luteal phase stimulation and frozen embryo transfer (Chen *et al.*, 2015), further studies with a larger population and long-term health evaluation are needed.

In the present study, although the total number of oocytes retrieved was similar in both groups, the total number of useable embryos for frozen embryo transfer was reduced in the duostim group. In this group, oocytes were vitrified after the first oocyte retrieval (mandatory by law in France at the time of study design), then thawed and pooled with fresh oocytes for ICSI after the second retrieval. The embryos obtained were then vitrified, and thereafter thawed for transfer. Even if low, the wastage rate of oocytes and embryos at each thaw process may put oocytes/embryos at a higher risk of loss with the duostim protocol, particularly in women with POR. In this RCT, oocyte survival rate was 81.6%. Cobo *et al.* (2018) reported an overall oocyte survival rate of 85.2% (95% CI 83.2–87.2) in non-selected women with elective fertility preservation (mean age 37.2 years, mean number of oocytes retrieved 12.5). The evidence for a lower oocyte survival rate in a poor responder population, as in the present study, is scarce and should be considered when deciding on oocyte versus embryo vitrification. Furthermore, considering the pregnancy and birth outcome risks for fresh and frozen embryo transfers, further studies are needed with a larger population and long-term evaluation.

Finally, no serious adverse events were reported for ovarian stimulations and/or oocyte retrievals in the duostim and the conventional protocols. Physicians reported, off the charts, a more difficult ultrasound monitoring in first week of the luteal phase in the duostim group owing to the presence of one or several corpus luteum, rapidly disappearing thereafter.

In routine practice, the benefit of duostim in patients with POR, selected by low ovarian reserve markers and not specifically by advanced maternal age, is not confirmed in this RCT, moreover when a fresh transfer is feasible. This is because, first, we demonstrate no potentializing effect on the number of oocytes retrieved in the luteal phase after follicular phase stimulation. Second, the freeze-all strategy avoids a pregnancy after the first cycle. However, the duostim appears to be safe for women, and the quality of embryos obtained with duostim seems unimpaired. Caution must be taken on the potential wastage of oocytes/embryos after two vitrification/thaw techniques, particularly in the POR population. This must be considered for future research. The main benefit of duostim is to shorten the time to a second retrieval by 2 weeks, when accumulation of oocytes/embryos is required, as confirmed in a previous RCT.

Further research is needed for evaluation of the live birth rate, and the time and cost effectiveness of duostim compared to two conventional protocols, as well as confirmation of the safety of the double-thaw process in this specific population.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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Authors' roles

All authors actively contribute to this article, including participation to study design (N.M., G.P.-B., N.C., E.D., M.B., H.B.-G.), acquisition of data (N.M., G.P.-B., N.C., E.D., C.P.-V., M.Pi., M,Pa., H.B.-G.), analysis and interpretation of data (N.M., I.A., G.P.-B., N.C., E.D., S.G.), manuscript drafting (N.M., I.A.), critical discussion (N.M., I.A., G.P.-B., N.C., E.D., H.B.-G.), and approval of the final version of the manuscript (all).

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Conflict of interest

N.M. declares grants paid to their institution from MSD (Organon France); consulting fees from MSD (Organon France), Ferring, and Merck KGaA; honoraria from Merck KGaA, General Electrics, Genevrier (IBSA Pharma), and Theramex; support for travel and meetings from Theramex, Merck KGaG, and Gedeon Richter; and equipment paid to their institution from Goodlife Pharma; IA declares honoraria from GISKIT; and support for travel and meetings from GISKIT. G.P.-B. declares consulting fees from Ferring and Merck KGaA; honoraria from Theramex, Gedeon Richter, and Ferring; payment for expert testimony from Ferring, Merck KGaA, and Gedeon Richter; and support for travel and meetings from Ferring, Theramex, and Gedeon Richter. N.C. declares grants from IBSA pharma, Merck KGaA, Ferring, and Gedeon Richter; support for travel and meetings from IBSA pharma, Merck KGaG, MSD (Organon France), Gedeon Richter, and Theramex; and participation on advisory board from Merck KGaA. E.D. declares support for travel and meetings from IBSA pharma, Merck KGaG, MSD (Organon France), Ferring, Gedeon Richter, Theramex, and General Electrics. C.P.-V. declares support for travel and meetings from IBSA Pharma, Merck KGaA, Ferring, Gedeon Richter, and Theramex. M.Pi. declares support for travel and meetings from Ferring, Gedeon Richetr, and Merck KGaA. M.Pa, declares honoraria from Merck KGaA, Theramex, and Gedeon Richter; support for travel and meetings from Merck KGaA, IBSA Pharma, Theramex, Ferring, Gedeon Richter, and MSD (Organon France). H.B.-G. declares honoraria from Merck KGaA and Gedeon Richter; support for travel and meetings from Ferring, Merck KGaA, IBSA Pharma, MSD (Organon France), Theramex, and Gedeon Richter. S.G. and M.B. have nothing to declare.

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