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Risk of progesterone elevation in low ovarian reserve patients undergoing long-acting plus low-dose r-FSH versus daily high-dose r-FSH ovarian stimulation protocols for IVF: a randomized controlled trial

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Abstract

Research question: Does the long-acting stimulation protocol with corifollitropin alfa (CFA) followed by daily low-dose recombinant FSH (r-FSH) reduce the risk of premature progesterone elevation (PPE) in women with low ovarian reserve compared to high-dose daily r-FSH?

Design: This randomized controlled trial, conducted from February 2022 to May 2024, enrolled 110 patients who met Bologna criteria for expected poor ovarian response. Participants were randomized into two groups: the intervention group received long-acting CFA followed by 150 IU r-FSH from Day 8; the control group received daily 300 IU r-FSH. The primary outcome was the proportion of patients with progesterone levels ≥

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1.1 ng/mL on hCG trigger day. Secondary outcomes included number of retrieved oocytes, fertilization rates, fresh embryo transfers, and pregnancy rates.

Results: A significantly lower rate of PPE was observed in the CFA group: 2/56 (3.6%, 95% CI 0.9–12.1) vs. 12/54 (22.2%, 95% CI 13.2–34.9) in the control group. CFA stimulation was associated with an 89% reduction in odds of PPE (OR 0.11, 95% CI 0.02–0.55; $p < 0.01$). Fresh embryo transfer was achieved in 34/52 (65%, 95% CI 51.8–76.8) in the CFA group vs. 21/48 (44%, 95% CI 30.7–57.7; $p = 0.04$) in controls.

Conclusions: Ovarian stimulation with long-acting CFA plus daily 150 IU of r-FSH significantly reduced the risk of PPE and increased the feasibility of fresh embryo transfer in patients with low ovarian reserve. This strategy may offer a better approach for managing PPE and potentially improve overall IVF success for this specific patient population.

Key words: poor ovarian responders / progesterone elevation / fresh embryo transfer / long-acting stimulation / progesterone at trigger / freeze-all

ClinicalTrials.gov ID: NCT04695483 - https://clinicaltrials.gov/study/NCT04695483

Trial registration date: 01 December 2020

Introduction

The treatment of patients with poor ovarian response (POR) is still under debate in Assisted Reproduction Technologies (ART). POR is characterized by a reduced number of ovarian follicles, fewer retrieved oocytes, and often poorer oocyte quality, which can significantly affect the chances of a successful ART cycle.

Recent evidence from the U.S. SART registry (Acharya et al., 2018) suggests that for patients with low oocyte recovery (< 5 oocytes) in IVF treatment, the best approach is to perform fresh embryo transfer, which appears to be associated with more favourable clinical pregnancy rates (CPR) and live birth rates (LBR) compared to freeze-all and subsequent frozen-thawed embryo transfer cycles.

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One of the main indications for freeze-all as a rescue strategy in IVF cycles is premature progesterone elevation (PPE) (Kaponis et al., 2018), reported to affect up to 25% of patients with POR (Mitra et al., 2021).

Previous evidence has suggested that high serum progesterone levels on the day of ovulation triggering can negatively impact the pregnancy outcomes in IVF cycles (Bosch et al., 2010). Indeed, elevated progesterone levels can jeopardize endometrial receptivity (Hussein et al., 2019), and this has been associated with reduced implantation rates in fresh IVF cycles.

The cut-off values of progesterone on the trigger day that can significantly impact the success rate of a fresh IVF cycle have been studied extensively, and the latest evidence suggests that the value of 1.14 ng/mL is the most appropriate to use in clinical practice, because the LBR after freeze-all and subsequent thawed embryo transfer significantly surpasses that from fresh (Vuong et al., 2019).

PPE in assisted reproduction is associated with various factors, including age, ethnicity (Hill et al., 2017), body mass index (BMI) (Shen et al., 2023), the type of controlled ovarian stimulation (COS) protocol, baseline progesterone levels at the beginning of COS (Papaleo et al., 2014), daily follicle-stimulating hormone (FSH) dosage, total gonadotrophins dosage, duration of the COS cycle, the number of retrieved oocytes, and peak oestradiol levels. These factors can impact the risk of PPE and should be considered in ART treatment planning to optimize fertility outcomes.

In current clinical practice, COS in POR patients is carried out by daily administration of daily high-dose gonadotropins up to 300 IU, with the aim of increasing number of oocytes retrieved at ovum pick up (OPU) and minimising the risk of cycle cancellation due to non-response (Ngwenya et al., 2024).

However, this treatment regimen has not shown significant effects on clinical outcomes in ART and was most associated with risk of PPE (Lawrenz et al., 2018), that would significantly impact the percentage of IVF cycles in which a rescue freeze-all strategy should be applied, ultimately decreasing the possibility of fresh transfer and thus also the total IVF pregnancies in POR patients.

Especially in patients with a poor ovarian response, the choice of treatment protocol for ovarian stimulation must take into account the fact that one of the aims during treatment must be the feasibility of achieving a fresh embryo transfer.

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In the field of ovarian stimulation drugs, a promising molecule in this respect has proved to be corifollitropin alfa (CFA) (Drakopoulos et al., 2017; Kolibianakis et al., 2015), which, with a pharmacokinetic profile that mimics a step-up/step-down protocol (Zandvliet et al., 2016), seems to have a key role in managing intra-follicular progesterone production. In fact, a post hoc analysis of data from two multicentre, randomized, blinded, non-inferiority trials, ENGAGE (Devroey et al., 2009) and PURSUE (Boostanfar et al., 2015), has shown a PPE incidence of 5.4% among patients treated with CFA-only (Lawrenz et al., 2016).

Our study aims to investigate prospectively whether ovarian stimulation with CFA followed by daily low dose of recombinant FSH (r-FSH) can reduce the incidence of PPE in the expected POR patient population, compared to conventional stimulation with high-doses daily r-FSH.

Materials and methods

This was a, monocentric, unblinded, superiority, explanatory randomized-controlled trial, aiming to investigate whether a long-acting stimulation protocol with CFA followed by 150 UI of daily r-FSH could reduce the proportion of patients with progesterone ≥ 1.1 ng/ml on the day of hCG trigger in poor ovarian responders, compared to a stimulation protocol with daily high dose r- FSH (300 UI/die).

Participants in the study were individuals seeking for fertility care at San Raffaele Hospital in Milan, Italy. Enrolment of patients started in February 2022 concluded in January 2024. The follow-up concluded in May 2024. There were no significant changes to the methods or outcome assessment throughout the study period. The trial was concluded upon completion of planned enrollment and follow-up.

Inclusion criteria

Women undergoing IVF cycles, whether they were undergoing intracytoplasmic sperm injection (ICSI) or not, and meeting the criteria for predicted poor ovarian response, were being considered for enrolment in the study.

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The inclusion criteria for individuals predicted to have a poor ovarian response were defined, according to Bologna criteria, by meeting one or more of the following conditions: 1) an antral follicle count (AFC) of less than 7, 2) an anti-Müllerian hormone (AMH) level of less than 1.1 ng/ml, 3) a history of retrieving ≤ 3 oocytes in a previous ART cycle.

To be eligible for recruitment, patients had to meet all the following criteria at the time of randomization: age between 25 and 42 years, normal BMI (between 18.5 and 24.9 kg/m2), having regular menstrual cycles.

Exclusion criteria

Patients were considered ineligible for the trial if they met any of the following exclusion criteria: other indications for elective freeze-all, diagnosis of polycystic ovarian syndrome (PCOS), personal history of untreated autoimmune diseases and/or endocrine or metabolic disorders, chronic kidney diseases, previous ovarian surgery (i.e. cystectomy or oophorectomy), current ovarian endometriosis, diagnosis of malignant gynaecological cancer, basal FSH ≥ 20 IU/L, hypersensitivity to the investigated drugs. Patients were also excluded from the trial if they were concurrently using hormonal therapies that are not conventional for treating infertility, such as transdermal testosterone or specific hormonal priming regimens prior to ovarian stimulation, which are currently considered experimental or used in specific research protocols.

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Patient and public involvement

The development of the research questions, selection of outcome measures, study design, and study conduct did not involve the participation or input of the patients or the general public.

Intervention

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The hypothesis of the study was to test whether a long-acting stimulation protocol with CFA might reduce the risk of progesterone ≥ 1.1 ng/ml on the day of hCG trigger in poor ovarian responders, compared to a daily stimulation protocol with r-FSH. The randomization for the study protocol was performed at the time of scheduling the patient for IVF treatment.

Treatment plans

The treatment plan for both COS protocols began on day 2-3 of the menstrual cycle. Starting from day 6, patients initiated a daily subcutaneous injection of 0.25 mg of the GnRH antagonist (Ganirelix), which continued until the day of trigger. In the investigation group a single subcutaneous injection of 150 µg of CFA on day 2 or 3 of the menstrual cycle was administered and COS continued with a daily dose of 150 IU of r-FSH from stimulation Day 8 if criteria for ovulation trigger were not met. In the control group patients receive a daily subcutaneous injection of 300 IU of recombinant r-FSH until criteria for triggering were obtained. When there was an absence of adequate follicular growth, it was defined as no response, and the ovarian stimulation cycle was cancelled.

Ovulation triggering was performed if at least one leading follicle reaches a diameter of ≥ 17 mm and was provided by the administration of 10,000 IU of urinary hCG.

OPU took place 36 hours after the administration of hCG. The retrieved MII oocytes were fertilized with the partner's semen on the same day. Embryo transfers were carried out according to our IVF standard procedure, either three or five days after fertilization. In case of supernumerary embryos, freezing at blastocyst stage was performed. In cases where progesterone levels on the day of trigger exceeded 1.1 ng/mL, embryo culture was extended to the blastocyst stage, and a freeze-all strategy was mandatorily applied, with deferred embryo transfer in a subsequent cycle.

Fourteen days following the embryo transfer pregnancy test was done. All participants with a positive pregnancy test result underwent an ultrasound scan at both six and twelve weeks of gestation to confirm clinical pregnancy. Follow-up continued until the 20th week of gestation, at which time point it was considered an ongoing pregnancy.

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Primary outcome

The primary outcome under investigation in this study was the proportion of subjects with progesterone levels of ≥1.1 ng/mL on the day of ovulation trigger, with an expected rate of 5% in the investigation group, compared with an expected rate of 25% in the control group. Progesterone levels were determined through blood sample testing using the Tosoh AIA fluorometric system with ST-AIA-PACK immunoassay (Tosoh Corporation). The assay demonstrated a sensitivity of 0.1 ng/ml, and both intra-assay and inter-assay variation coefficients were reported at 11% and 13%, respectively.

Secondary outcomes

Number of collected oocytes; fertilization rate; number of obtained embryos; the percentage of freeze-all cycles due to PPE; biochemical pregnancy rate, defined as a positive pregnancy test; ongoing pregnancy rate, defined as the presence of a viable foetus at 20 weeks of gestation.

Sample size

The sample size for this study was determined based on the following assumptions: 1) an estimated 10% cancellation rate due to COS failure; 2) a desired statistical power of 80% for the study, 3) a type I error rate (α) of 0.05 for a two-sided test. We assumed that the incidence of elevated progesterone in the CFA + 150 IU/day r-FSH group would be similar to that reported in the CFA-only arm of the study by Lawrence et al. (2016), while the incidence in the control group was derived from existing literature on conventional stimulation protocols. Using a continuity-corrected formula (Bell et al., 2014) and the pre-specified parameters, we have determined that a sample size of 55 women in each study arm, totalling 110 patients, was appropriate. This sample size was designed to detect a reduction in the risk of PPE from 25% in the control group to 5% in the intervention group.

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Recruitment and randomization

All women scheduled for IVF treatment at the study centre were assessed for eligibility. Those who agreed to participate and provided written informed consent were enrolled in the study. Study participants were allocated to the two intervention arms based on the allocation sequence in a 1:1 ratio, generated using permuted block randomization with random block sizes. The randomization was unpaired. The allocation sequence was generated before the start of the study using the R package randomizeR (Uschner et al., 2018). The allocation sequence was generated and maintained solely by the study statistician, who assigned treatment groups without disclosing the sequence to investigators or the principal investigator, ensuring strict allocation concealment.

Statistical analysis

Descriptive analysis of the study population was performed to evaluate differences between the two study groups. Continuous variables were analyzed using the Mann-Whitney test and are presented as median and interquartile range (Q1–Q3).

The primary study outcome—the proportion of patients with progesterone levels ≥1.1 ng/ml at induction—was initially evaluated in the Intention-to-Treat (ITT) population, followed by analysis in the Per-Protocol (PP) population. The ITT population comprised all randomized participants, whereas the PP set included only patients who completed the study without major protocol deviations and met criteria for ovulation triggering in the ovarian stimulation protocol.

The primary outcome in both ITT and PP datasets was assessed using logistic regression models adjusted for potential confounding factors, including body mass index (BMI, kg/m^2), maternal age (years), ovarian reserve (AMH, ng/mL) and basal progesterone (ng/mL).

Progesterone values of the patients with cycle cancellation prior to trigger were addressed in the ITT population through multiple imputation (MI) using predictive mean matching (PMM). Maternal body mass index (BMI), maternal age, and treatment group (intervention or control), all with no missing data, were

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included as predictor variables in the imputation model (Austin et al., 2021). The MI was performed using the mice package in R (van Buuren & Groothuis-Oudshoorn, 2011).

Secondary outcomes were evaluated to identify potential differences between the intervention and control group in the PP population. Continuous secondary outcomes are reported as median and interquartile range (Q1–Q3), analyzed using the Mann-Whitney test, with 95% confidence intervals calculated using bootstrap method. Categorical secondary outcomes are presented as frequencies and percentages and were analyzed using the Chi-square test. Confidence intervals for proportions were calculated using the Wilson score interval method.

All analyses adhered to a pre-specified significance level (α) of 0.05.

R studio version 2022.12.0.353 was used to perform the statistical analysis.

Ethics approval and trial registration

The study proposal was approved by the Ethics committee of IRCCS San Raffaele Institute. The trial was registered on EudraCT.ema.europa.eu under the number 2020-004329-21 and in ClinicalTrials.gov with the number NCT04695483.

Results

Patient selection and baseline characteristics

Figure 1 depicts the study inclusion process. A total of 147 patients were considered eligible and of these 110 were randomised, 56 in the intervention group and 54 in the control group. Among the total number, 100 participants completed the study: 52 in the intervention group (withdrawn consent n=1, treatment discontinued for concurrent medical condition n=1, treatment discontinued for no response n=2), and 48 in the control group (spontaneous pregnancy before COS n=1, withdrawn consent n=2, treatment discontinued for concurrent medical condition n=1, treatment discontinued for no response n=2).

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Table 1 presents the baseline characteristics of the study population, including age, ovarian reserve parameters (AMH, AFC), BMI, basal FSH levels and total motile sperm count of the partner (TMSC).

Primary outclome

Table 2 presents the number of patients with progesterone levels >1.1 ng/mL on the day of ovulation trigger for both the per-protocol and ITT populations. In the ITT population, 2 patients (3.6%) in the intervention group and 12 patients (22.2%) in the control group (OR 0.11, CI 0.02–0.55, $p < 0.01$) exceeded this threshold. The PP set had similar results, with 2 patients (3.8%) in the intervention group and 10 patients (20.8%) in the control group exceeding the threshold (OR 0.13, CI 0.02–0.68, p=0.02).

IVF Laboratory outcomes

Table 3 shows the IVF cycle outcomes per group and the comparison across study arms. The duration of the stimulation was a median 10 (9-12) days in both intervention and control group (p=0.29). Peak oestradiol levels on the day of ovulation trigger were 901 (673-1382) pg/mL in the intervention group and 973 (600-1301) pg/mL in the control group (p=0.92). Progesterone level at triggering was 0.47 (0.28-0.72) ng/mL in the intervention group and 0.64 (0.45-0.98) ng/mL in the control group.

The mean number of oocytes retrieved was 3 (2-6) in the intervention group and 3 (1-4) in the control group (p=0.06). The number of mature oocytes (MII) was 3 (1-5) and 2 (1-3) and in the intervention and control groups, respectively (p=0.10). Fertilization rate was 79% (30-100) in the intervention group and 78% (0-100) in the control group, p=0.80). The total number of embryos in day 3 obtained in the two groups were 1.5 (0-3) in the control group and 2 (0.8-4) in the intervention group (p=0.06).

A total of 34 (65%,95%CI 51.8–76.8) fresh transfers were performed in the intervention group and 21 (44%, 95% CI 30.7–57.7) in the control group (p = 0.04).

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In the intervention group, 23 (44.2%) fresh embryo transfers were performed at the cleavage stage (day 3), 10 (19%) at the blastocyst stage (day 5), and 19 (36.5%) patients did not undergo a fresh embryo transfer. In the control group, 19 (40%) fresh embryo transfers were performed at the cleavage stage, 2 (4%) at the blastocyst stage, and 27 (56%) patients did not undergo a fresh embryo transfer.

In the intervention group, six patients received only CFA injections, and the trigger was administered without any additional gonadotropin dose. A total of 36 patients (69%) in the intervention group and 29 patients (60%) in the control group had at least one cleavage-stage embryo available in the cycle. Among them, 2 out of 36 patients (5.5%) in the intervention group and 6 out of 29 patients (20.7%) in the control group had at least one cleavage-stage embryo available in the laboratory but underwent a freeze-all strategy due to progesterone elevation at the time of trigger (p=0.13). The mean number of cryopreserved blastocysts was 0.79 ± 1.07 in the intervention group and 0.5 ± 0.85 in the control group. In the intervention group, 25 patients (48%) had at least one blastocyst cryopreserved, of which 9 (17%) had two or more blastocysts cryopreserved. In the control group, 15 patients (31%) had at least one blastocyst cryopreserved, and 7 patients (15%) had two or more blastocysts cryopreserved.

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Pregnancy outcomes

Table 4 depicts the pregnancy outcomes in patients that completed the study. In the entire population, 21 patients had a biochemical pregnancy after a fresh embryo transfer, 10 (20.8%) in the control group and 11 (21.2%) in the intervention group (p=1.00). Six (12.5%) patients in the control group and five (9.6%) patients in the intervention group had an ongoing pregnancy beyond 20 weeks of gestation (p = 0.65). Analysing by IVF cycle, considering also all the frozen embryo-transfers, 17 (32.7%) patients in the intervention group and 15 (31.2%) patients in the control group had at least one positive pregnancy test (p=0.99), of whom 15 (28.8%) in the intervention group and 10 (20.8%) in the control group had at least an ongoing pregnancy (p=0.49). At the time of data collection, 5 (9.6%) patients in the intervention group and 1 (2.1%) in the control group had not yet achieved a clinical pregnancy but still had at least one cryopreserved embryo available for

transfer ($p = 0.2$). All of these patients had already undergone at least one embryo transfer during the study period.

Discussion

This study demonstrated that, in patients with low ovarian reserve, the combination of CFA followed by a lower daily dose of r-FSH effectively reduced the risk of premature progesterone elevation (PPE) compared to standard high-dose daily r-FSH. As a result, a significantly higher number of fresh embryo transfers were performed in the intervention group, reducing the need for a rescue freeze-all strategy. Given that fresh embryo transfer is particularly beneficial in poor ovarian responders, this approach may improve treatment outcomes in this challenging population.

Elevated serum progesterone at the time of trigger is well-documented to negatively impact pregnancy rates after fresh embryo transfer, as it disrupts endometrial receptivity and shifts the implantation window. While a rescue freeze-all strategy can mitigate this issue by enabling embryo transfer in a more optimal endometrial environment (Racca et al., 2021; Wong et al., 2021; Celada et al., 2020), this approach is less favorable for patients with low ovarian reserve. In this population, delaying embryo transfer may result in lower overall pregnancy rates, as suggested by SART registry data (Acharaya et al., 2018). More recently, evidence has emerged indicating that freeze-all strategies should even be considered contraindicated in poor prognosis patients, as they may further compromise their chances of success while increasing the financial burden of treatment (Gleicher et al., 2023).

The threshold for defining PPE remains debated, with initial studies suggesting 1.5 ng/mL as a critical cut-off. However, more recent findings indicate that levels above 0.8–1.0 ng/mL (Venetis et al., 2013; Wu et al., 2019) may impair implantation, particularly in poor ovarian responders. Importantly, a threshold of 1.1 ng/mL has been identified as the point at which freeze-all becomes preferable to fresh embryo transfer (Vuong et al., 2019), forming the rationale for its adoption in our study.

Various strategies have been explored to minimize PPE while maintaining effective follicular stimulation (Lawrenz et al., 2018), including step-down protocols (Lawrenz et al., 2021) and the use of hMG instead of r-

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FSH (Bosch et al., 2024). The pooled analysis of the ENGAGE and PURSUE trials (Lawrenz et al, 2016), provided valuable insights into the role of CFA in modulating progesterone levels, particularly when lower r-FSH doses are introduced from day 8 of stimulation. Our findings align with this evidence, reinforcing the efficacy of this approach in preventing PPE and thus preserving the opportunity for fresh embryo transfer.

By combining CFA with a lower r-FSH dosage, our study aimed to achieve two key objectives: on one hand, ensuring effective follicular recruitment, and on the other, preventing excessive progesterone production. This strategy is particularly relevant as it mimics the physiological FSH decline observed in natural ovulatory cycles, where FSH levels naturally decrease as dominant follicles mature (Ecochard et al., 2014), promoting optimal follicular development while maintaining endocrine balance. By applying this principle to controlled ovarian stimulation, the protocol may support a more physiologic hormonal environment, reducing the risk of excessive progesterone elevation while maintaining follicular growth.

Our results confirm that this strategy significantly reduces the risk of PPE and increases the likelihood of fresh embryo transfer when compared to the most used high-dose r-FSH protocols. This is a clinically meaningful outcome for predicted poor ovarian responders, who may benefit the most from tailored stimulation strategies that also optimize hormonal dynamics.

Currently, no single ovarian stimulation protocol has been definitively proven superior in terms of clinical pregnancy and live birth rates across different responder populations, as highlighted in the latest ESHRE guidelines (The ESHRE Guideline Group on Ovarian Stimulation et al., 2020). However, refining protocols to better accommodate the unique challenges of low ovarian reserve patients remains a critical area of research.

In conclusion, our findings suggest that the combination of CFA followed by a low daily dose of r-FSH offers an advantage over standard high-dose daily r-FSH by reducing PPE and, consequently, lowering the need for a rescue freeze-all strategy. This may enhance the likelihood of fresh embryo transfer and, on a broader scale, improve ART success rates in poor ovarian responders. However, given that our study was not powered to detect significant differences in live birth rates, further research is needed to validate these findings and assess their impact on clinical important outcomes.

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Limitations

One limitation of this study is the choice of the progesterone cut-off value, as there is no established consensus on the optimal threshold. Using different cut-offs could potentially impact the final results. Beyond the primary comparison of CFA 150 mcg versus rFSH 300 IU during the first seven days of stimulation, from day 8 onward the two groups followed different fixed protocols. In the CFA group, patients received a lower fixed daily dose of rFSH, while in the control group, the initial 300 IU/day dose was maintained until the end of stimulation. This resulted in a higher total gonadotropin exposure in the control group. Therefore, the observed difference in progesterone elevation cannot be attributed solely to the use of CFA, as it may also be influenced by the higher FSH dosage administered in the control group. This protocol reflects common clinical practice in low responders, where dose reductions are rarely applied due to the minimal risk of OHSS; however, it represents a limitation of the study and should be taken into account when interpreting the results.

The trial was designed as a superiority study for the primary outcome of progesterone elevation at trigger, with the primary objective of assessing whether the intervention reduced the proportion of patients experiencing PPE compared to the control group. This study design led to a sample size which was not powered to formally assess the non-inferiority of the two treatments in terms of clinical outcomes. Therefore, while clinical outcomes were analysed, the sample size does not allow for definitive conclusions regarding non-inferiority between the two protocols.

Finally, the study's monocentric design, while ensuring consistency in protocols and patient management, may limit the generalisability of the findings to other settings with different patient populations, clinical practices, or laboratory conditions.

Conclusions

The results suggest that the long-acting ovarian stimulation protocol with corifollitropin alfa followed by a low-dose daily r-FSH dose could be advantageous over the conventional stimulation protocol with high-dose

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r-FSH, particularly in reducing the PPE risk among expected poor ovarian responders. This could potentially lower the rates of cancelled fresh cycles and increase chances of IVF success in this particular population. The data show that patients in the intervention group had lower progesterone levels on the day of ovulation trigger and a lower proportion of subjects with PPE. Further analyses are advisable to confirm these preliminary findings and to better understand their potential clinical implications. This study was conducted in a population of low responder patients identified based on established classification criteria. To broaden the applicability and relevance of these results, it will be important for future studies to validate the findings in comparable populations also classified using different criteria.

Data availability

The data that support the findings of this study are available on request from the first author, E.P.

Author's role

E.P., A.Q. and M.Z. conceived the original idea and the overall design of the study. D.M. performed the statistical analysis. V.S.V., E.D. and G.B. substantially contributed to data acquisition, interpretation, and critical review. A.Q. wrote the first draft of the article. E.P. and M.C. critically revised the initial and all subsequent drafts and the final manuscript. All authors have read and approved the final manuscript.

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Conflict of interest

E.P. reported grants and personal fees from MSD; grants from Ferring, IBSA, Organon, TEVA, and Gedeon Richter; and grants and personal fees from Merck. V.S.V. reported consultancies from IBSA and Merck Serono; personal fees from Gedeon Richter. The other authors declare no confict of interest.

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During the preparation of this work, the authors used ChatGPT-4o to assist with language editing and improving readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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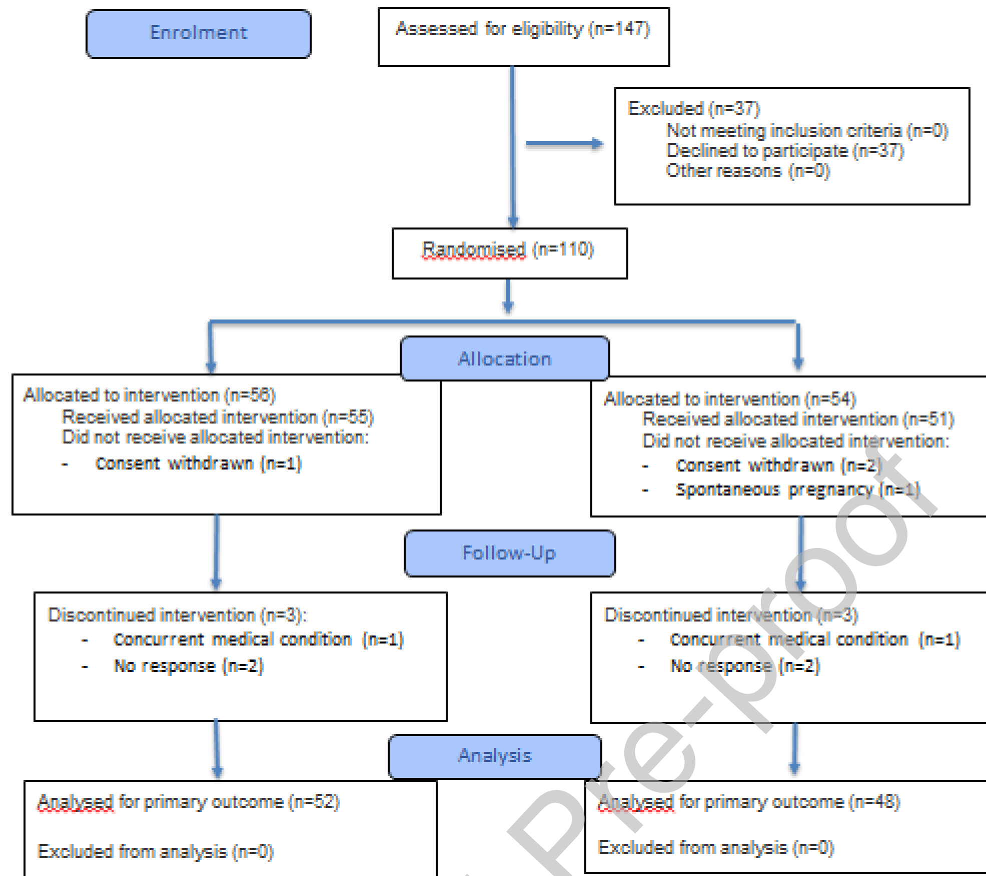
Figure 1. CONSORT flow diagram of patient enrollment, randomization, and follow-up in the study.



Flow diagram presenting the progression of participants through the randomized controlled trial, including reasons for exclusion prior to randomization, the number of patients who did not receive the allocated intervention, discontinuation of the intervention, and the number of participants included in the primary outcome analysis. The diagram is structured according to the CONSORT 2025 guidelines.

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Table 1

	Intervention Group (n=56)	**Control Group (n=54)**	**p-value**
Age (years) *Median (Q1-Q3)*	39.0 (37.0-40.0)	39.0 (38.0-41.0)	0.09
BMI (kg/m^2) *Median (Q1-Q3)*	21.4 (19.9-23.4)	21.0 (19.5-22.9)	0.39
AMH (ng/mL) *Median (Q1-Q3)*	0.67 (0.34-0.86)	0.58 (0.35-0.8)	0.37
FSH (mUI/mL) *Median (Q1-Q3)*	9.3 (7.1-13)	9 (7-11.7)	0.63
AFC (n) *Median (Q1-Q3)*	6 (4-7)	5.5 (4-7)	0.99
Basal progesterone (ng/mL) *Median (Q1-Q3)*	0.37 (0.22-0.57)	0.41 (0.22-0.48)	0.09
TMSC (million) *Median (Q1-Q3)*	22.5 (7.3-58.4)	21.6 (5.9-54.0)	0.97

Table 1: Baseline characteristics of the ITT study population: intervention group (n=56) and control group (n=54).

BMI, body mass index; AMH, anti-Mullerian hormone; FSH, follicle-stimulating hormone; AFC, antral follicle count; TMSC, total motile sperm count; Q1–Q3, quartile 1–quartile 3

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Table 2

	Intervention Group	**Control Group**	**OR [95% CI]**	**p-value**
ITT population	**N=56**	**N=54**		
Progesterone ≥ 1.1 ng/mL *n (%)*	2 (3.6%)	12 (22.2%)	0.11 [0.02-0.55]	<0.01
PP population	**N=52**	**N=48**		
Progesterone ≥ 1.1 ng/mL *n (%)*	2 (3.8%)	10 (20.8%)	0.13 [0.02-0.68]	0.02

Table 2: No. of patients with progesterone levels at trigger ≥ 1.1 ng/mL in the ITT and PP study population.

Progesterone levels at trigger were analyzed as a dichotomous variable using a cut-off of 1.1 ng/mL.

Analyses were performed using logistic regression adjusted for confounders (BMI, maternal age, AMH, basal progesterone). Control group was used as reference level.

Table 3

	Intervention Group (n=52)	**Control Group (n=48)**	**Median Difference [95% CI]**	**p-value**
Duration of COS (days) *Median (Q1-Q3)*	10.0 (9.0 – 12.0)	10.0 (9.0 – 12.0)	0.00 [-0.01;2.00]	0.29
Peak oestradiol levels (pg/mL) *Median (Q1-Q3)*	901.0 (673.2–1382.2)	973.0 (599.5–1301.0)	-72.0 [-266.0;219.5]	0.80
Progesterone levels (ng/mL) *Median (Q1-Q3)*	0.47 (0.28-0.72)	0.64 (0.45-0.98)	-0.17 [-0.30;-0.01]	<0.01
Oocytes retrieved (n) *Median (Q1-Q3)*	3.0 (2.0-6.0)	3.0 (1.0-4.0)	0.00 [-1.00;2.50]	0.06
MII oocytes (n) *Median (Q1-Q3)*	3.0 (1.0-5.0)	2.0 (1.0-3.0)	1.00 [-0.50;2.00]	0.10
Fertilization rate *Median (Q1-Q3)*	0.79 (0.3-1.0)	0.78 (0.0-1.0)	0.01 [-0.31;0.30]	0.80
Day 3 embryos (n) *Median (Q1-Q3)*	2.0 (0.8-4.0)	1.5 (0.0-3.0)	0.50 [-0.50;2.00]	0.06
			OR [95% CI]	**p-value**
Fresh embryo transfer performed *n (%)*	34 (65%)	21 (44%)	2.41 [1.01 - 5.90]	0.04
Cycles with at least one day3 embryo	**N=36**	**N=29**		
Freeze-all cycles due to PPE/patients with day 3 embryos[1] *n (%)*	2 (5%)	6 (20.7%)	0.23 [0.02-1.44]	0.13

Table 3: Per-Protocol analysis results: IVF cycle outcomes in patients who completed the study. The median difference (calculated as the difference between the medians of the two groups, intervention group-control group) was assessed, and 95% confidence intervals (95% CI) were estimated using the bootstrap method. Q1–Q3 indicates quartile 1–quartile 3

[1] Percentages of Freeze-all cycles due to PPE were calculated for cycles with at least one embryo obtained on day 3, with group sizes of n=36 for the intervention group and n=29 for the control group.

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Table 4

	Intervention Group (n=52)	Control Group (n=48)	OR [95% CI]	p-value
Positive pregnancy test *n (%)*	11 (21.2%)	10 (20.8%)	1.02 [0.35-3.02]	1.00
Clinical pregnancy *n (%)*	10 (19.2%)	8(16.7%)	1.19 [0.38-3.85]	0.80
Ongoing pregnancy *n (%)*	5 (9.6%)	6 (12.5%)	0.74 [0.21-2.62]	0.65
Cumulative positive pregnancy test *n (%)*	17 (32.7%)	15 (31.2%)	1.07 [0.42-2.71]	0.99
Cumulative ongoing pregnancy per patients *n (%)*	15 (28.8%)	10 (20.8%)	1.53 [0.56-4.35]	0.49
Patients with cryopreserved embryos and no clinical pregnancy *n (%)*	5 (9.6%)	1 (2.1%)	5 [0.56-44.5]	0.2

Table 4: Per-protocol analysis results: Pregnancy outcomes in patients who completed the study. Positive pregnancy tests, clinical pregnancies, and ongoing pregnancies refer to fresh embryo transfers. Cumulative pregnancies refer to the number of oocyte retrievals that resulted in at least one pregnancy, considering outcomes from both fresh transfers and subsequent thawed embryo transfers.

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Biography

Enrico Papaleo graduated in medicine and surgery from the University of Milan, Italy in 1999 and completed his residency in obstetrics and gynaecology at the University Vita-Salute San Raffaele, Milan, Italy in 2005. He is currently head of the Reproductive Unit at San Raffaele Hospital, a member of ESHRE and the author of more than 50 peer-review publications.

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Key Message

In women with low ovarian reserve, long-acting corifollitropin alfa followed by low-dose r-FSH significantly reduces premature progesterone elevation and increases fresh embryo transfer rates, offering a promising strategy to improve IVF outcomes in this high-risk group.