# RESEARCH PROTOCOL

## Prospective Randomized Single-Blind Study of the Effectiveness of the Superovulation Stimulation Protocol Using hCG Compared to Standard Protocols with Different Gonadotropin Doses in In Vitro Fertilization Programs

Protocol Version: 1.2

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## 1. RELEVANCE of the Problem

Ovarian stimulation is a key stage of assisted reproductive technology (ART) programs, since the number of oocytes retrieved, their quality, and the subsequent embryological outcome depend directly on its effectiveness. Despite significant progress in reproductive medicine, the optimization of controlled ovarian stimulation protocols remains a relevant issue.

In current clinical practice, protocols using gonadotropins as well as ovulation triggers, in particular human chorionic gonadotropin (hCG), are widely used. At the same time, interest is growing in modified approaches aimed at increasing effectiveness and reducing risks, in particular ovarian hyperstimulation syndrome. An additional economic issue for Ukraine is the high cost of standard controlled superovulation protocols, driven mainly by the price of recombinant gonadotropins, which creates a barrier to the accessibility of in vitro fertilization (IVF) for infertile couples in Ukraine.

A separate scientific problem is the lack of a unified position on the equivalence of alternative stimulation protocols to standard regimens. This creates the need for comparative studies with clearly defined embryological endpoints.

Human chorionic gonadotropin (hCG) is a well-studied and widely available drug traditionally used for trigger administration for the purpose of final oocyte maturation. Due to its structural similarity to luteinizing hormone (LH), hCG is able to bind to LH/hCG receptors in the theca and granulosa cells of the ovary, supporting steroidogenesis and folliculogenesis.

A number of studies indicate the possibility of using hCG in sub-trigger doses as a component of the stimulation protocol instead of or together with standard gonadotropins. Such an approach may substantially reduce the cost of an IVF cycle while maintaining comparable clinical outcomes.

Given that the ultimate goal of ART programs is to obtain high-quality mature oocytes and viable embryos, these parameters are key for evaluating the effectiveness of any protocol.

The lack of a sufficient number of randomized studies demonstrating the equivalence of alternative protocols using hCG to standard regimens determines the need for this study.

In modern evidence-based medicine, non-inferiority studies are attracting increasing attention, the purpose of which is to demonstrate that a new method is not inferior to the standard method in terms of key clinical outcomes.

In reproductive medicine this is particularly relevant, since even minor changes in the protocol can affect the embryological outcome and clinical pregnancy.

Thus, the evaluation of the superovulation stimulation protocol using hCG as a potentially equivalent alternative to standard regimens is relevant from both a scientific and a clinical point of view.

## 2. STUDY OBJECTIVE

To evaluate the effectiveness of the superovulation stimulation protocol using human chorionic gonadotropin (hCG) by demonstrating its equivalence to standard controlled ovarian stimulation protocols in terms of the number of oocytes retrieved, their morphological quality, blastulation, and perinatal outcomes, while maintaining an economic advantage.

## 3. STUDY OBJECTIVES

3.1. To compare the number of oocytes retrieved in patients after superovulation stimulation using hCG versus patients on standard controlled ovarian stimulation protocols.

3.2. To evaluate oocyte quality (morphological parameters) in each group and the proportion of degenerative forms in both groups.

3.3. To determine the rate of mature oocytes (MII stage) obtained in the study protocols and perform a comparative analysis thereof.

3.4. To analyze embryological outcomes, in particular the fertilization rate and early embryo development, in the two groups.

3.5. To evaluate the safety of the protocols used, including the incidence of ovarian hyperstimulation syndrome and other possible complications.

3.6. To perform a comparative economic analysis of the cost of the stimulation protocols.

3.7. To establish equivalence or differences between the protocols in terms of key indicators of superovulation stimulation effectiveness.

3.8*. To evaluate the equivalence of clinical pregnancy and live birth rates between the groups.

## 4. STUDY DESIGN

Study type: prospective, randomized, single-blind (single-blind outcome assessment) (non-inferiority / equivalence)

Number of groups: 2

**Object of study:** women of reproductive age undergoing ART programs and subject to controlled ovarian stimulation.

*Inclusion criteria:* women aged 18-40 years with all types of ovarian reserve, presence of indications for superovulation induction, consent to participate in the study, BMI up to 35 kg/m$^2$.

*Exclusion criteria:* women with ovarian endometriomas, prior severe OHSS, severe endocrine disorders, oncological diseases, contraindications to hormonal stimulation, male factor (cryptozoospermia).

### Group A — "hCG protocol" (main group)

- Protocol: stimulation using hCG instead of, or replacing a significant part of, standard gonadotropins once the main follicular cohort reaches 11-12mm
- Medications: hCG (Choriogonadotropin, etc.) in sub-trigger doses of 200 IU without FSH
- Specificity: hCG is used as a substitute for LH activity and to support folliculogenesis.
- Suppression protocol: fixed GnRH antagonist protocol from stimulation day 6.
- Trigger: Dual (dual trigger)

**Group B — "standard protocol"**

- Protocol: standard controlled ovarian stimulation.
- Gonadotropin dose: 100–225 IU/day
- Medications: FSH or FSH/LH preparations
- Suppression protocol: fixed GnRH antagonist protocol from stimulation day 6.
- Trigger: Dual (dual trigger)

## 5. PRIMARY ENDPOINTS

- Number of oocytes retrieved (oocyte yield).
- Proportion of mature oocytes MII (%).
- Fertilization rate (%).
- Embryo quality on day 3 and day 5 (% of good-quality blastocysts).
- Total dose of gonadotropins/hCG (IU).

Oocyte and embryo quality is assessed in accordance with the standardized ESHRE/ALPHA consensus criteria (Istanbul Consensus, 2025 update): Coticchio G. et al. "*Istanbul consensus update: a revised ESHRE/ALPHA consensus for the assessment of oocytes, zygotes and embryos*" // Human Reproduction. — 2025. — Vol. 40, No. 6. — P. 989–1023. DOI: https://doi.org/10.1093/humrep/deaf025

## 6. SECONDARY ENDPOINTS

- Clinical pregnancy rate (%).
- Live birth rate (%).
- Incidence of OHSS (moderate and severe).
- Mean duration of stimulation (days).
- Endometrial thickness on trigger day (mm).
- Cost of the protocol per cycle (UAH and USD).

## 7. SAMPLE SIZE CALCULATION

Study design — non-inferiority. The non-inferiority margin (δ) is set at 1.5 oocytes for the primary endpoint "number of oocytes" and 10% for the endpoint "clinical pregnancy rate."

- Significance level: $\alpha = 0.05$ (one-sided test).
- Study power: 80% ($1 - \beta = 0.80$).
- Size of each group: 180 patients.
- Number of groups: 2.
- Total sample: ~360 patients (2 groups × 180).

The sample size calculation accounts for an expected drop-out rate of up to 10%.

## 8. STATISTICAL ANALYSIS

- Descriptive statistics: mean ± SD or median (IQR) depending on the distribution of the data.
- Shapiro–Wilk test to assess normality of distribution.
- One-way analysis of variance (ANOVA) or Kruskal–Wallis test for comparing three groups.
- Pairwise comparison: Tukey's post-hoc test or Dunn's test with Bonferroni correction.
- Categorical variables: $\chi^2$ (chi-square) test or Fisher's exact test.
- For non-inferiority analysis: calculation of the difference between groups and the 95% confidence interval (CI).
- Software: SPSS v.25 or R (version 4.x).
- Level of statistical significance: $p < 0.05$.

### – 8.1. Randomization and Allocation Concealment

- Randomization of patients will be performed using the block randomization method with stratification by institution. Block size — 6 (three allocation pairs). The randomization sequence will be generated using computer software (R package “blockrand” or equivalent) by an independent person who is not involved in patient recruitment or outcome assessment.
- Allocation concealment (allocation concealment) will be ensured using centralized, sequentially numbered, opaque sealed envelopes (Sequentially Numbered Opaque Sealed Envelopes — SNOSE). The envelopes will be kept by the study's responsible administrator, whom the investigator will contact only after confirming the patient's compliance with the inclusion criteria and signing of informed consent. The envelope cannot be opened until the patient enrollment procedure is complete — this prevents selective enrollment based on the anticipated treatment group.
- The study is open-label (open-label) given the differences in medication administration regimens between groups. To minimize bias associated with the open-label design, blinding is provided for the assessment of primary endpoints: the embryologist evaluating oocyte and embryo quality will have no information about the patient's treatment group. Assessment of pregnancy and live birth rates will likewise be performed without knowledge of group assignment (single-blind outcome assessment).

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- 8.2. Assessment of Protocol Adherence
- Protocol adherence / compliance will be assessed based on the following parameters:
- –Complete dose administration of the prescribed medications: recorded in the patient's outpatient medical record at each monitoring visit; any deviation from the prescribed protocol is regarded as a violation.
- Compliance with the monitoring visit schedule: recorded in the patient's outpatient medical record.
- Patients with a documented protocol violation will be included in the analysis on an intention-to-treat (ITT) basis, but will be excluded from the Per-Protocol analysis (see Section 8.3).

- **8.3. ITT and Per-Protocol Analysis**
- In accordance with the recommendations of ICH E9 and the requirements for equivalence/non-inferiority studies, two parallel analyses will be performed:
- – Intention-to-Treat (Intention-to-Treat (ITT)) analysis: includes all randomized patients regardless of treatment actually received, protocol adherence, or premature withdrawal from the study. Missing values for primary endpoints will be imputed using multiple imputation (multiple imputation) accounting for covariates (age, AMH, group). The ITT analysis is the primary analysis for evaluating effectiveness.
- – Analysis based on the Per-Protocol (PP) principle: includes only patients who fully completed the prescribed treatment protocol without significant deviations (adherence ≥ 90%, no unauthorized medications, availability of data for all primary endpoints). The PP analysis is confirmatory and particularly important for confirming the conclusions of non-inferiority.
- The conclusion of non-inferiority will be made only if it is confirmed simultaneously in both analyses (ITT and PP). If the results of the analyses diverge, this is recorded as a sensitivity analysis and discussed in the “Discussion” section. Inclusion/exclusion criteria for the PP population are defined prior to unblinding of the randomization codes (a priori).

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- 8.4. Multivariable Regression Analysis
- In order to adjust for the potential influence of confounding factors on the primary and secondary endpoints, a multivariable regression analysis will be performed.
- For continuous endpoints (number of oocytes, proportion of MII, duration of stimulation), multiple linear regression or Poisson regression (for count data) will be used. For binary endpoints (clinical pregnancy, live birth, OHSS) — logistic regression.
- The following covariates will be included in the regression models:
- Patient age (years, continuous variable)
- Body mass index (BMI, kg/m², continuous variable)
- Ovarian reserve (AMH ng/mL and AFC, continuous variables)
- Primary cause of infertility (categorical variable: tubal-peritoneal factor, male factor, endocrine factor, combined factor, idiopathic infertility)
- The results of the regression analysis will be presented as adjusted relative risks (aRR) or odds ratios (aOR) with 95% confidence intervals and p-values. Multicollinearity will be assessed using the VIF (variance inflation factor); a VIF value > 10 will be considered unacceptable.

## 8.5. Economic Evaluation of the Stimulation Protocol

The economic evaluation of the stimulation protocol's effectiveness is performed by calculating the total cost of medications used over the entire course of stimulation (superovulation stimulation protocol) for each patient.

The calculation is performed according to the following methodology:

- Total cost of medications used = sum of the products of the number of doses administered of each medication and its current retail price as of the date of protocol administration (UAH).
- For Group A (hCG protocol): the total number of IU of hCG (Choriogonadotropin, etc.) and GnRH antagonists administered during the protocol is taken into account.
- For Group B (standard protocol): the total number of IU of gonadotropins (FSH or FSH/LH) and GnRH antagonists administered during the protocol is taken into account.
- The trigger medication (hCG or GnRH agonist) is included in the total cost.
- The cost is recorded in Ukrainian hryvnia (UAH) and converted to US dollars (USD) at the official NBU exchange rate on the date of administration.

The results of the economic analysis will be presented as the mean cost of the protocol per cycle (M ± SD) for each group, with calculation of the absolute difference and percentage difference between groups.

## 9. ETHICAL ASPECTS

- Obtaining approval from the institution's local ethics committee prior to the start of patient enrollment.
- Registration of the study on ClinicalTrials.gov or in the Register of Clinical Trials of the Ministry of Health of Ukraine.
- Signing of informed consent by each patient after receiving complete information about the study.
- Compliance with the principles of the Declaration of Helsinki of the World Medical Association (2013 revision).
- Ensuring the confidentiality of personal data in accordance with the GDPR Regulation and the Law of Ukraine "On Personal Data Protection."

## – 10. MONITORING OF ADVERSE EVENTS AND ADVERSE REACTIONS

### – 10.1. Definitions and Classification

- The study will apply a standardized safety monitoring system in accordance with the requirements of ICH E6 (R2) Good Clinical Practice and the Law of Ukraine "On Medicinal Products."
- The following definitions will apply:
- Adverse Event (AE / Adverse Event, AE): any untoward medical occurrence in a patient receiving investigational treatment, regardless of causal relationship to the treatment.
- Serious Adverse Event (SAE / Serious Adverse Event, SAE): any AE resulting in death, is life-threatening, requires or prolongs hospitalization, results in persistent disability, or a congenital anomaly.
- Unexpected Adverse Drug Reaction (UADR): an AE whose nature or severity is not consistent with the available information about the medication.
- The severity of AEs will be classified according to the CTCAE (Common Terminology Criteria for Adverse Events, v5.0):
- Grade 1 — mild: asymptomatic or with minimal symptoms, no intervention required
- Grade 2 — moderate: requires minimal/local intervention, limits instrumental activities of daily living

- Grade 3 — severe: requires hospitalization or significant medical intervention, limits self-care
- Grade 4 — life-threatening: requires urgent intervention
- Grade 5 — death

- **10.2. Collection and Reporting Procedure**
- All AEs will be recorded in the patient's outpatient medical record at each monitoring visit. Active monitoring will be conducted at each ultrasound monitoring session and 24–48 hours after oocyte retrieval.
- Reporting timelines:
- Grade 1–2 AEs: recorded in the patient's outpatient medical record within 24 hours of detection; reported to the sponsor/study coordinator at the next visit.
- Grade 3 or higher AEs / SAEs: immediate notification of the responsible investigator (within 24 hours); written report to the Biomedical Ethics Committee within 7 calendar days; notification to the Ministry of Health of Ukraine within the timelines established by law.
- Death or life-threatening SAE: immediate notification (within 24 hours) to the Ethics Committee, the Ministry of Health, and ClinicalTrials.gov.

- **10.3. Specific Monitoring of OHSS**
- Ovarian hyperstimulation syndrome (OHSS) is a key expected adverse reaction subject to enhanced monitoring, especially in Group A (hCG protocol) due to the longer half-life of hCG.
- The following RCOG/ESHRE OHSS classification will be applied:
- Mild OHSS: abdominal discomfort, ovaries up to 8 cm — outpatient observation.
- Moderate OHSS: nausea/vomiting, ovaries 8–12 cm, ascites on ultrasound — outpatient treatment with daily monitoring.
- Severe OHSS: pronounced ascites, respiratory failure, hypovolemia, thrombosis — hospitalization, recorded as an SAE.
- If signs of moderate or severe OHSS are detected in Group A patients, the prescribed trigger (hCG) is replaced with a GnRH agonist, and a freeze-all strategy is implemented (cryopreservation of all embryos without fresh transfer). This event is recorded in the patient's outpatient medical record and taken into account in the Per-Protocol analysis.

## 11. EXPECTED RESULTS AND SCIENTIFIC NOVELTY

### Scientific Novelty of the Study

For the first time, a comparative analysis will be conducted of the effectiveness of the superovulation stimulation protocol using human chorionic gonadotropin versus standard controlled ovarian stimulation protocols, within a non-inferiority (equivalence) study design.

A new approach will be developed for evaluating the effectiveness of stimulation protocols, not only using traditional quantitative indicators (number of oocytes retrieved), but also with an emphasis on comprehensive embryological criteria, in particular the proportion of mature oocytes (MII) and their morphological quality.

The results obtained will provide scientific grounds for the use of the alternative stimulation protocol as equivalent to standard regimens, expanding existing understanding of the variability and flexibility of approaches to controlled ovarian stimulation.

### Practical Significance of the Study

The results of the study will have direct clinical significance for the practice of reproductive medicine, as they will allow assessment of the feasibility of implementing the alternative superovulation stimulation protocol using human chorionic gonadotropin.

If the equivalence of the proposed approach to standard protocols is demonstrated, clinicians will gain an additional tool for individualizing patient treatment in ART programs. Criteria will be developed for selecting patients suitable for the hCG protocol; justification will be provided for the clinical use of hCG as a full-fledged component of the stimulation protocol.

This will increase the flexibility of stimulation protocol selection depending on the clinical situation; it will contribute to a potential reduction in medication burden; optimization of embryological outcomes without loss of effectiveness;

improved treatment safety, including a reduced risk of ovarian hyperstimulation syndrome in certain patient groups.

Economic justification for the widespread implementation of the hCG protocol will contribute to a potential reduction in the cost of the stimulation cycle by 30–60%.

Thus, this work has significant applied value for improving the effectiveness and personalization of ART programs.