

STUDY PROTOCOL

Official Title:

A Within-Patient Comparison of CAPA-IVM (Biphasic Capacitation IVM) With the Contralateral Ovary Undergoing Minimal Controlled Ovarian Stimulation (OS-IVF)

Short title: Intra-ovarian CAPA-IVM vs Standard IVM (IO-CAPA Study)

Protocol Number: IVM-001

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Sponsor: New Hope Fertility Center

1. Principal investigator / study sites

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2. Background / Rationale (brief)

In vitro maturation (IVM) of oocytes represents a promising approach to reduce or eliminate the need for ovarian stimulation, thereby minimizing patient burden and risk while maintaining good embryological outcomes. Recent advances in biphasic IVM systems (CAPA-IVM), which include a pre-maturation phase prior to final maturation, have improved developmental competence compared with traditional single-step IVM. CAPA-IVM adds a pre-maturation “capacitation” step (often using C-type natriuretic peptide, CNP, or a similar meiotic-arresting environment) for approximately 24 hours before maturation culture. Published data demonstrate enhanced nuclear and cytoplasmic maturation and higher embryologic outcomes in multiple series. Additionally, this approach has been used clinically in pilot and comparative trials as well as in registered clinical studies, showing particular benefit for oocytes from small antral follicles and in patients with PCOS. Safety data to date have not indicated increased obstetric or neonatal risks in small follow-up cohorts, although larger surveillance is recommended.

Conversely, conventional controlled ovarian stimulation (OS) cycles, which use standard gonadotropin dosing, remains an established approach for IVF, providing in vivo-matured oocytes for fertilization and embryo development.

This study employs a prospective, within-patient, same-menstrual-cycle design. At the Day 3 baseline visit, the right ovary is assigned to CAPA-IVM and the left ovary is assigned to minimal stimulation OS-IVF. Immature oocytes are first retrieved from the right ovary for CAPA-IVM before ovarian stimulation begins. Minimal stimulation of the left ovary then begins on Day 5 of the same menstrual cycle.

This design allows a direct within-patient comparison of the number and quality of oocytes and embryos produced by the two protocols, including oocyte competence, fertilization, and early embryonic development, while providing an internal clinical and safety reference.

3. Objectives

Primary Objective

- Assess and compare the developmental competence, number, and quality of oocytes and embryos derived from CAPA-IVM versus those obtained from the contralateral ovary after minimal controlled ovarian stimulation (OS-IVF), with the primary endpoint being the maturation rate to metaphase II (MII) for CAPA-IVM oocytes.

Secondary Objectives

- Compare fertilization rates (2PN) after ICSI between CAPA-IVM and OS-IVF oocytes.
- Compare cleavage rates, blastocyst formation (day 5/6), high-quality blastocyst rates, and embryo euploidy (if PGT-A is performed) between embryos derived from each arm.
- Compare implantation, clinical pregnancy, ongoing pregnancy, and live birth rates from embryos originating from each ovary.
- Evaluate perinatal outcomes, including birth weight, gestational age, and short-term neonatal outcomes.
- Assess safety outcomes, including rates of ovarian hyperstimulation syndrome (OHSS), procedure-related complications, and ovarian reserve markers (AMH / AFC changes at 3–6 months).
- Laboratory endpoints: cumulus expansion score, meiotic spindle integrity (if available), and selected molecular markers (optional sub-study).

4. Study design

Study Design

- Prospective, **within-patient comparative experimental design**.
- **Population:** Women undergoing fertility treatment who meet inclusion/exclusion criteria (see below).
- **Ovary assignment for each subject:**
 - **Left ovary: Minimal controlled ovarian stimulation (OS-IVF), followed by routine oocyte retrieval and standard IVF/ICSI procedures.**
 - **Right ovary: CAPA-IVM, in which immature oocytes are retrieved on or after menstrual cycle Day 3, before initiation of stimulation of the left ovary, and matured using the biphasic CAPA-IVM protocol.**

Rationale for design:

- This within-patient approach reduces inter-patient variability and allows direct comparison of oocyte competence, fertilization, and embryo development between CAPA-IVM and standard minimal OS-IVF within the same physiological environment.
- Each patient serves as her own internal control, with the minimal OS-IVF ovary providing reference outcomes for clinical and laboratory endpoints.

Note:

- No randomization is performed at the oocyte or follicle level. Assignment is fixed at the Day 3 baseline visit: right ovary to CAPA-IVM and left ovary to OS-IVF. Both approaches occur within the same menstrual cycle, with CAPA-IVM oocyte retrieval performed first.
- All immature oocytes from the CAPA-IVM ovary are processed using the biphasic IVM protocol.

- After completion of right-ovary CAPA-IVM aspiration, minimal stimulation of the left ovary begins on Day 5. The left ovary then follows minimal controlled ovarian stimulation and routine IVF/ICSI procedures.

5. Study population — inclusion / exclusion

Inclusion Criteria

- Female, age 18–38 years (adjustable per center).
- Indication for ART (infertility).
- Both ovaries present and anatomically normal, with no prior oophorectomy.
- Adequate ovarian reserve for study participation (e.g., AMH >0.5 ng/mL; AFC >6/Ovary).
- Willing and able to provide written informed consent and comply with study procedures, including embryo storage and transfer options.
- Access to partner or donor sperm for fertilization/ICSI per clinic protocol.
- Both ovaries deemed suitable for their assigned protocol: one ovary for CAPA-IVM (immature oocyte retrieval) and the contralateral ovary for standard OS-IVF.

Exclusion Criteria

- Severe endometriosis (stage III–IV) or known diminished ovarian reserve (AMH <0.2 ng/mL), unless the study is specifically designed for DOR patients.
- Active pelvic infection or untreated hydrosalpinx.
- Known chromosomal abnormalities in either partner (unless testing is planned).
- Contraindication to oocyte retrieval or sedation.
- Any medical or gynecological condition that, in the investigator's judgment, would interfere with study participation, oocyte retrieval, or safety.

6. Interventions — Clinical and Laboratory Procedures

Overview of Cycle Management

- **Minimal Stimulation (OS-IVF) Ovary — Left Ovary:**
 - Minimal controlled ovarian stimulation begins on menstrual cycle Day 5, after aspiration of the right CAPA-IVM ovary. The regimen uses clomiphene citrate (Clomid) for approximately 9 days and follitropin beta (Follistim) injections for approximately 3 days, with ultrasound and/or hormonal monitoring. Final oocyte maturation is initiated with leuprolide acetate (Lupron), a GnRH-agonist trigger.
 - Oocytes retrieved from this ovary are mostly mature (MII) and processed for IVF/ICSI and embryo culture per routine. These outcomes provide a safety and clinical reference for each patient.
 - Only one OS-IVF attempt involving the left ovary is permitted per participant under this protocol. There is no protocol-defined minimum or maximum number of oocytes required for collection. If premature ovulation occurs before the scheduled egg retrieval, the study cycle will be cancelled.
 - Although the right ovary is aspirated before stimulation begins, any residual follicles in either ovary may respond to the subsequent medications. Follicular response in both ovaries will be documented separately. The right ovary will not undergo a second study retrieval during that cycle; the protocol-defined OS-IVF retrieval is from the left ovary.

- **CAPA-IVM Ovary — Right Ovary:**
 - The right ovary is not stimulated and does not receive an ovulation trigger before CAPA-IVM oocyte collection. Its follicles are aspirated first, beginning on or after menstrual cycle Day 3 and before Day 5 initiation of left-ovary OS-IVF stimulation.
 - At the first oocyte pick-up (OPU), follicles from the right CAPA-IVM ovary are punctured and cumulus-oocyte complexes (COCs) are collected separately and transported to the laboratory in pre-warmed medium. A later retrieval is performed for the stimulated left ovary after the Lupron trigger.
 - Only immature oocytes (GV or MI) are processed using the CAPA-IVM biphasic protocol. Any in vivo-matured oocytes (rare) are recorded but not included in CAPA-IVM.

Laboratory Handling & Culture Protocols

Common Initial Steps

- Identify and record number of follicles punctured, COC grading (cumulus expansion, compactness), and GV/MII status.
- Avoid full denudation of immature COCs if cumulus is needed for CAPA-IVM; denude only if clinically required.

CAPA-IVM (Biphasic) — Suggested Parameters

- **Pre-maturation (capacitation) step:** Culture intact COCs in a meiotic-arresting capacitation medium for approximately 24 hours at room temperature. This maintains nuclear arrest while supporting cytoplasmic maturation and cumulus-oocyte communication.
- **Maturation step:** Transfer COCs to standard IVM maturation medium (FSH/LH/EGF environment) for ~24–30 hours, then assess for MII.
- **Total culture time:** ~48–54 hours (24 h pre-maturation + 24–30 h maturation).
- Document exact media composition and batch number in the protocol appendix.

Minimal Stimulation OS-IVF Ovary — Standard IVF/ICSI in NHFC

- MII oocytes retrieved after minimal stimulation undergo IVF/ICSI per NHFC clinical protocol. Oocyte assessment and selection for ICSI, and the ICSI procedure itself, are established clinical treatments and are part of standard of care rather than research-specific procedures.
- Embryo culture to cleavage/blastocyst stage follows standard procedures.

Fertilization & Embryo Culture

- Matured MII oocytes from CAPA-IVM undergo ICSI according to NHFC clinical practice. Oocyte assessment and selection for ICSI, and the ICSI procedure itself, are standard-of-care clinical procedures; the research component is the CAPA-IVM maturation method and the comparison of outcomes by ovary of origin.
- Embryos from both ovaries are cultured per standard protocols.
- Traceability of embryo origin is maintained through unique labeling. Embryo transfer policies should be pre-specified (e.g., prioritize euploid or best morphology, irrespective of origin).

Laboratory Blinding and Traceability

- Laboratory personnel performing assessments should ideally be **blinded to ovary of origin** (e.g., culture dishes coded).
- If blinding is not feasible, the embryologist performing morphological scoring should be different from the one managing ovary assignment and culture protocol.

- All oocytes and embryos are tracked using unique identifiers to ensure traceability between ovary of origin, culture protocol, fertilization, and embryo development outcomes.

7. Outcomes / definitions

Primary endpoint

- Maturation rate = number of oocytes reaching MII / number of immature oocytes assigned to the CAPA-IVM arm, assessed at prespecified time points (e.g., 24 h/30 h/48 h).

Secondary endpoints

- Fertilization rate (2PN / injected MII)
- Cleavage rate (number of embryos day 2–3 / 2PN)
- Blastocyst rate (blastocysts / injected MII)
- High-quality blastocyst rate (per center's grading schema)
- Implantation rate, clinical pregnancy (heartbeat at ~7–8 weeks), ongoing pregnancy >12 weeks, live birth
- Adverse events: OHSS (graded), procedure complications, ovarian reserve changes (AMH, AFC at 3–6 months)
- Neonatal outcomes: gestational age, birth weight, congenital anomalies (report per standard)
- Laboratory measurements: cumulus expansion score, time to polar body extrusion, spindle assessment (optional).

8. Safety monitoring & adverse events

- **Definitions and Reporting:**
 - **Adverse Event (AE):** Any untoward medical occurrence in a study participant, whether or not causally related to the intervention.
 - **Serious Adverse Event (SAE):** Any AE that results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent or significant disability, or is otherwise judged medically significant.
 - **Reporting timelines:** All SAEs must be reported to the IRB and/or relevant regulatory authority within **24–48 hours** per local regulations. AEs should be documented in the study CRFs and monitored continuously.
- **Expected Risks:**
 - Oocyte retrieval complications (e.g., bleeding, infection, pain, injury to surrounding tissues)
 - Sedation/anesthesia-related risks
 - Ovarian hyperstimulation syndrome (OHSS), primarily associated with the minimal stimulation OS-IVF ovary
 - Procedure-related discomfort or other minor complications
- **Monitoring:**
 - Only the left ovary is intentionally stimulated. The participant will be monitored for ovarian hyperstimulation symptoms and OHSS using standard

clinical assessments. If clinically significant ovarian hyperstimulation symptoms or OHSS develop before retrieval, stimulation and the study cycle will be cancelled. If symptoms arise after retrieval, further study-related procedures will stop and the participant will receive appropriate clinical management and follow-up.

- Safety monitoring should include post-retrieval assessment, laboratory evaluation, and follow-up visits as clinically indicated.
- **Independent Oversight:**
 - A **Data Safety Monitoring Board (DSMB)** or independent safety monitor should review interim safety data, e.g., after the first 10–20 patients, to ensure participant safety and trial integrity.
- **Stopping Rules:**
 - Participant-level stopping rules are: (1) cancellation if premature ovulation occurs before egg retrieval; (2) cancellation if clinically significant ovarian hyperstimulation symptoms or OHSS develop before retrieval; and (3) discontinuation of further study-related procedures for any SAE or other condition that, in the investigator's judgment, makes continued participation unsafe. Only one left-ovary OS-IVF attempt is permitted per participant, and there is no minimum or maximum oocyte-yield threshold for proceeding with collection.
 - Any signals of adverse embryologic, clinical, or neonatal outcomes associated with either arm should trigger review by the DSMB and consultation with the IRB before continuation.
 - At the study level, any unexpected SAE judged related or possibly related to a study procedure, or any pattern of clinically significant OHSS or procedure-related complications, will prompt immediate review by the Principal Investigator and independent safety monitor and may result in study suspension pending IRB review.

9. Consent / ethical considerations

- Full informed consent must describe:
 - Experimental nature of culture comparisons (CAPA-IVM is increasingly used but may be considered investigational depending on jurisdiction).
 - That oocytes from rival arms will be used for fertilization, embryo creation and potential transfer — clarify embryo selection/transfer policy.
 - Potential risks/benefits and alternative options (standard OS for whole cycle, or not to participate).
 - The fixed ovary assignment and timing: right-ovary CAPA-IVM aspiration beginning on or after Day 3, followed by initiation of left-ovary OS-IVF stimulation on Day 5 within the same menstrual cycle.
 - The medications used for the OS-IVF comparator arm: clomiphene citrate (Clomid), follitropin beta (Follistim), and leuprolide acetate (Lupron) as the GnRH-agonist trigger, including their reasonably foreseeable risks and discomforts as listed:
 - **Lupron (leuprolide):** Headache, hot flashes, mild fatigue, mood changes, and injection-site soreness.
 - **FSH (Follistim/Gonal-f):** Bloating, pelvic fullness or discomfort, headache, breast tenderness, fatigue, and injection-site soreness.
 - **Clomid (clomiphene citrate):** Hot flashes, mood changes, headache, bloating, breast tenderness, nausea, and occasional blurred vision.

- That oocyte assessment/selection for ICSI and ICSI itself are established standard-of-care clinical procedures; the research-specific procedures are CAPA-IVM and the within-patient comparison.
- Data and sample storage (biobanking of cumulus cells, molecular tests) — optional consent sections for research substudies.
- Privacy and data handling.
- Provide patient with contact details for study team and IRB.

10. Data collection and CRFs

- Baseline: demographics, BMI, infertility diagnosis, AMH, AFC, prior IVF history.
- Cycle data: menstrual cycle day and fixed ovary assignment, timing of right-ovary CAPA-IVM aspiration and Day 5 left-ovary stimulation start, stimulation medications/doses, Lupron trigger timing, follicular response in each ovary, number of aspirated follicles per ovary, COC counts/grades, timing of culture steps (exact hours), maturation times, premature ovulation/cancellation, and outcomes.
- Embryology: fertilization, cleavage, embryo grades, PGT-A results if any.
- Clinical outcomes: transfer details, pregnancy tests, ultrasound confirmations, pregnancy follow-up to delivery.
- Adverse events log.
- Facility to link oocyte→embryo→transfer→pregnancy by unique IDs while preserving privacy.

11. Statistical analysis plan

Unit of Analysis

- **Primary analysis: oocyte-level within-patient comparison, with each oocyte classified by fixed ovary of origin as either CAPA-IVM (right ovary) or minimal stimulation OS-IVF (left ovary).**
- Because oocytes are clustered by patient, analyses must account for **intra-patient correlation**, using **mixed-effects logistic regression** or **generalized estimating equations (GEE)** with patient as the clustering variable.

Primary Endpoint Testing

- **Outcome:** maturation rate to MII.
- Compare the proportion of MII oocytes between CAPA-IVM and minimal stimulation OS-IVF using **GEE (binomial, logit link) with robust standard errors** or **mixed-effects logistic regression** with a random intercept for patient.

Secondary Endpoints

- **Fertilization and blastocyst formation:** analyze using GEE or mixed-effects models at the oocyte or embryo level.

- **Pregnancy and live birth outcomes:** analyze at the patient or embryo transfer level using appropriate models (e.g., per embryo transfer logistic regression; per-patient outcomes compared descriptively and with paired tests if appropriate).

Sample Size — Worked Example

- Because definitive estimates are limited, a **pilot study** is recommended. Example calculation (paired design):
 - Assume mean maturation proportion under **minimal stimulation OS-IVF** = **0.80**, anticipated CAPA-IVM maturation = 0.70 ($\Delta = -0.10$ if comparing directly, or adjust depending on hypothesis).
 - Suppose the standard deviation of paired differences ≈ 0.25 .
 - Using paired t-test approximation:

$$n = \left(\frac{(Z_{\alpha/2} + Z_{\beta}) \times SD}{\Delta} \right)^2$$

- $Z_{\alpha/2} = 1.96$ ($\alpha = 0.05$ two-sided), $Z_{\beta} = 0.84$ (power = 80%)
- $n = ((1.96 + 0.84) \times 0.25 / 0.10)^2 \approx 49$ patients
- Therefore, a pilot of **20–30 patients** is reasonable to assess feasibility, safety, and variability, with a larger sample needed for definitive trials powered to detect differences in maturation or secondary outcomes.
- For a definitive trial, sample size should be calculated using observed variance from the pilot and hierarchical clustering factors.

Missing Data & Multiplicity

- Pre-specify handling of **missing oocyte outcomes** (e.g., lost samples or failed assessments).
- Address **multiplicity adjustments** for multiple secondary outcomes, distinguishing exploratory vs confirmatory analyses.
- Optional: provide a **sample size spreadsheet** to estimate required patient numbers, considering expected oocyte yield per patient and the primary endpoint definition (oocyte-level vs patient-level).

12. Data management & confidentiality

- Secure electronic data capture (REDCap or similar).
- De-identify data; link key stored separately.
- Access limited to study team.
- Retention per institutional policy.

13. Ethical & regulatory considerations

- Justification for within-patient design: minimizes inter-patient confounders and directly compares the number and quality of oocytes and embryos under both protocols in the same participant. The CAPA-IVM right ovary is aspirated before stimulation begins, and the left ovary then undergoes one minimal-stimulation OS-IVF attempt. Participants are informed that assigning one ovary to CAPA-IVM may reduce the number of in vivo-matured oocytes otherwise available from a bilateral conventional retrieval.
- Ensure compliance with local laws on embryo research and use.
- If genetic testing (PGT-A) or molecular assays on leftover cells are planned, include separate consent and IRB language.
- CAPA-IVM is not standard of care, label it as investigational and get appropriate approvals (e.g., trial registration).

14. Timeline (example)

- Start-up/IRB approvals: 2–3 weeks
- Recruitment/pilot accrual (20–30 patients): 6–12 months (depends on clinic volume)
- Lab analyses and pregnancy follow-up to 12 weeks: +6 months
- Data analysis and manuscript: +3 months

15. Budget & resources (high-level)

- Additional lab reagents (CNP, special media), extra culture dishes / personnel time for longer CAPA culture.
- Randomization and data management infrastructure.
- Optional costs: PGT-A, molecular assays, long-term follow up.

16. Appendices

📄 **Informed Consent Form** – detailing study procedures, experimental nature, risks, embryo handling, optional biobanking, and privacy considerations.

📄 **SOPs** – for:

- OPU separation by ovary
- Culture media for CAPA-IVM and minimal stimulation OS-IVF

📄 **Example Lab Worksheets**

📄 **Sample Size Calculation Spreadsheet & Statistical Analysis Plan (SAP)** – for primary and secondary endpoints, including intra-patient clustering and pilot study calculations.

📄 **Key References** – supporting CAPA-IVM safety, efficacy, and published clinical experience.

17. Key references

1. Sanchez F. Biphasic in vitro maturation (CAPA-IVM) — conceptual descriptions and early clinical data. *Hum Reprod* / review.
2. ASRM Committee Opinion: In-vitro maturation — clinical guidance. 2021.
3. Gong X. CAPA-IVM improves oocyte cytoplasmic quality and embryological outcomes (2023).
4. ClinicalTrials.gov entries of CAPA vs standard IVM pilot trials (examples).
5. Reviews of modern IVM techniques and biphasic strategies.