Official Title: Exosome-Enriched Culture Media to Enhance In Vitro Maturation and Embryo Development in Poor Ovarian Responders Undergoing IVF

NCT Number:
Document Type: Study Protocol

Document Date: July 2025

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1. Background and Rationale Poor ovarian responders often yield a limited number of mature oocytes following ovarian stimulation, resulting in reduced chances for successful IVF. Preliminary evidence suggests that exosomes, extracellular vesicles rich in bioactive molecules, may support oocyte maturation by enhancing intercellular communication in the follicular environment. This study aims to investigate whether incorporating exosomes into IVF culture media improves the short-term in vitro maturation of MI oocytes in poor responder patients.

2. Objectives

- **Primary Objective:** To determine if exosome-enriched culture media increases the proportion of MI oocytes maturing to MII within 4 hours compared to standard IVF media.

- **Secondary Objectives:** To assess subsequent fertilization rates, embryo quality, blastocyst development, implantation, and clinical pregnancy rates.

3. Study Design A prospective, single-center, randomized controlled trial. Patients will undergo standard ovarian stimulation protocols. Retrieved MI oocytes will be allocated within each patient to two different culture conditions: exosome-enriched media vs. standard media.

4. Study Population

- **Inclusion Criteria:**
  - Women aged 35–42 years
  - Undergoing IVF with antagonist protocols
  - Meet at least two Bologna criteria for poor ovarian response
  - Able to provide written informed consent
- **Exclusion Criteria:**
  - Severe male factor infertility requiring surgical sperm retrieval
  - Known untreated hydrosalpinx
  - Uterine anomalies impairing implantation
  - Known chromosomal abnormalities
  - Participation in another investigational study within the last 3 months

5. Interventions

- **Control:** MI oocytes cultured in pre-equilibrated standard IVF media.
- **Intervention:** MI oocytes cultured in the same media supplemented with 10 μg/well of GMP-certified exosomes. Maturation status will be assessed at 2, 3, and 4 hours.

6. Outcome Measures

- **Primary Outcome:** Proportion of MI oocytes achieving MII maturation within 4 hours.
- **Secondary Outcomes:** Fertilization rates, day 3 embryo quality, day 5 blastocyst quality, implantation rate, and clinical pregnancy rate.

7. Statistical Considerations Categorical outcomes will be compared using chi-square or Fisher's exact tests. Logistic regression will adjust for

patient age, AMH, and gonadotropin dose. A p-value <0.05 will be considered statistically significant.

8. Ethical Considerations The study will adhere to the Declaration of Helsinki and Good Clinical Practice guidelines. Ethical approval will be obtained prior to enrollment. All participants will provide written informed consent. Confidentiality will be maintained, with data anonymized using unique study codes.

9. Data Handling and Confidentiality Data will be stored in secure, password-protected systems. No personally identifiable information will be shared or published.

10. References

- Melo P, Navarro C, Jones C, Coward K, Coleman L. J Assist Reprod Genet. 2020;37(4):855–863.
- Zhang H, Luo W, Zhu X, et al. Reprod Sci. 2024; Epub ahead of print.
- Additional relevant literature on exosomes and oocyte maturation.

End of Protocol Document.

Official Title: Evaluating the Efficacy of Exosome-Enriched Culture Media to Enhance In Vitro Maturation and Embryo Development in Poor Ovarian Responders Undergoing IVF: A Randomized Controlled Trial

NCT Number: J-298384255-2

Document Type: Statistical Analysis Plan (SAP)

Document Date: July 2025

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1. Introduction This Statistical Analysis Plan (SAP) details the planned analyses for the study evaluating whether exosome-enriched culture media enhances the in vitro maturation of MI oocytes and subsequent embryo development in poor responder patients undergoing IVF.

2. Study Objectives and Endpoints

- **Primary Endpoint:**
  - Proportion of MI oocytes maturing to MII within 4 hours of culture.
- **Secondary Endpoints:**
  - Fertilization rate (percentage of matured MII oocytes achieving normal 2PN post-ICSI)
  - Day 3 and blastocyst embryo quality
  - Implantation rate (number of gestational sacs per embryo transferred)
  - Clinical pregnancy rate (confirmed fetal heartbeat at 8–10 weeks)

3. Sample Size and Power A total of 60 patients will be enrolled, based on preliminary estimates indicating that this sample size provides >80% power to detect a 25% difference in maturation rates at a two-sided alpha of 0.05, assuming maturation rates improve from ~40% (control) to ~65% (exosome group).

4. Analysis Populations

- **Full Analysis Set (FAS):** All retrieved MI oocytes allocated to either exosome or standard culture conditions.
- **Per Protocol Set (PPS):** Oocytes that completed the planned culture period without protocol deviations.

5. Statistical Methods

- **Primary Analysis:**
  - Compare the proportion of MI oocytes maturing to MII between exosome and standard media using chi-square or Fisher's exact tests.
- **Secondary Analyses:**
  - Compare fertilization rates and embryo quality using similar tests.
  - Implantation and clinical pregnancy rates summarized descriptively and analyzed if numbers permit.
- **Adjustment:** Logistic regression models may adjust for patient age, baseline AMH, and total gonadotropin dose.
- **Significance:** Two-sided tests, $p<0.05$ considered statistically significant.

6. Handling of Missing Data Missing maturation outcomes will be treated conservatively as non-matured. Sensitivity analyses may exclude such oocytes. No imputation planned for secondary reproductive outcomes.

7. Interim Analyses and Data Monitoring No formal interim analyses are planned. Data will be reviewed periodically for safety and protocol compliance by the study team.

8. Reporting Conventions Descriptive statistics will include means, medians, standard deviations, and ranges. Tables and figures will illustrate maturation rates and embryo development outcomes by intervention.

End of Statistical Analysis Plan.

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NCT Number: J-298384255-2

Document Type: Informed Consent Form

Document Date: July 2025

Informed Consent Form (ICF)

Introduction: You are being invited to participate in a research study conducted at [Clinic Name]. This study is investigating whether adding exosomes to the culture media used in IVF can improve the maturation of certain types of eggs (oocytes) retrieved during your IVF treatment.

Purpose of the Study: The goal is to determine if supplementing the standard IVF media with exosomes can help more of your retrieved eggs mature, potentially increasing the number available for fertilization and embryo development.

Procedures:

- You will undergo your planned ovarian stimulation and egg retrieval as part of standard IVF care.
- After retrieval, some of your immature eggs (MI oocytes) will be cultured in either the usual IVF media or IVF media supplemented with exosomes.
- This does not change your medical treatment, medications, or procedures.

Risks and Discomforts:

- There are no known additional risks to you since the study procedures are performed entirely on eggs after retrieval.
- Standard risks of IVF treatment still apply.

Benefits:

- There may be no direct benefit to you, but information gained may help improve future IVF treatments.

Confidentiality:

- Your data will be assigned a study ID number. No personal identifiers will be used in publications or shared outside the study team.

Voluntary Participation:

- Your participation is voluntary. Choosing not to participate or to withdraw at any time will not affect your medical care.

Questions: If you have questions about the study, please contact [Study Coordinator Name and Contact Info].

Consent Statement: By signing below, you confirm that the study has been explained to you, your questions have been answered, and you agree to participate.

Participant Name: _____

Participant Signature: _____ Date: _____

Investigator Signature: _____ Date: _____

End of Informed Consent Form.