# Modified natural cycle is a reasonable alternative in women over 40 years old: a retrospective analysis on 26,039 single embryo transfers

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## Introduction

The natural cycle has been associated with better obstetric and neonatal outcomes compared to AC-ET, reducing the risks of preeclampsia, preterm birth, macrosomia, low birthweight and early pregnancy loss. However, the natural cycle is typically reserved for younger women with normal ovulatory cycles, as women over 40 years old are often considered unsuitable due to presumed ovarian dysfunction. Limited data exist on the efficacy of NC-ET in older women, despite their potential benefit demonstrated in younger women. This study investigated whether mNC-ET may be an effective alternative for women over 40 years old.

#### **Objective**

Aim of this study was to investigate whether Modified-Natural Cycle Embryo Transfer (mNC-ET) achieves a comparable Live Birth Rate (LBR) to Artificial Cycle Embryo Transfer (AC-ET) in women over 40 years old.

## Material and methods

A retrospective multicentric cohort study analyzed 26,039 Single Embryo Transfers (SET) performed between January 2019 and December 2023 across 21 IVI clinics in Spain and Italy. In mNC-ET, hCG was administered at dominant follicle 13-22 mm, followed by low-dose progesterone supplementation (400 mg/day). In the AC-ET group, oral estradiol valerate (6 mg/day) was administered, followed by vaginal progesterone (800 mg/day). The primary outcome was LBR. Secondary outcomes were clinical pregnancy, ongoing pregnancy, miscarriage rates. Women aged 40–49 undergoing SET were included. Only euploid frozen ETs were considered for autologous cycles, while both fresh and frozen ETs were included for egg donor cycles. Inclusion criteria were regular menstrual cycles (21–35 days), endometrial thickness <sup>3</sup>7 mm and blastocyst-stage embryos. Exclusion criteria included double embryo transfer, severe uterine pathologies and hydrosalpinx. Statistical analyses included chi-square and Student's t-tests for group comparisons. Logistic regression adjusting for confounders was used for LBR.

## **Results**

The study analyzed 26,039 SETs (2,694 mNC-ETs vs. 23,345 AC-ETs) in 16,579 patients (43.1±2.4 y.o.). No significant differences were found in BMI, endometrial thickness, sperm source, or serum E2 and P4 levels on the day of endometrial thickness assessment. The AC-ET group included older patients (43.2±2.4 vs 42.2±2; p<0.001), a higher proportion of egg donation cycles (84.7%, 95% CI: 84.2–85.1 vs 54.9%, 95% CI: 53–56.8; p<0.001), lower proportion of PGT-A cycles (21.1% vs 51.7%; p<0.001) and younger oocyte age at retrieval (27.5±6.9 vs 32±8.5 y.o.). No statistical differences were found in LBR between mNC and AC-ET (40.2%, 95% CI: 38.3–42.1 vs. 41%, 95% CI: 40.4–41.7, p=0.44), nor in ongoing pregnancy rates (44%, 95% CI: 42.1–45.9 vs 44.3%, 95% CI: 43.7–44.9; p=0.78). The mNC-ET group had lower clinical pregnancy rate (50.1%, 95% CI: 48.2–52 vs 53.9%, 95% CI: 53.25–54.53); p<0.001), but also lower miscarriage rate (11.8%, 95% CI: 10.2–13.7 vs. 17.4%, 95% CI: 16.8–18.1; p<0.001). Multivariate analysis adjusted for confounders (patients and oocyte age, BMI, oocyte and sperm source, endometrial thickness, serum E2 levels, PGT-A, blastocyst stage/grade) confirmed no significant LBR advantage for AC- ET over mNC-ET (OR=0.93, 95% CI 0.84-1.04; p=0.21).

#### Conclusions

mNC-ET demonstrated non-inferior LBR compared to AC-ET in women over 40, supporting its feasibility as an endometrial preparation method in women of advanced maternal age and suggesting a shift toward individualized endometrial preparation. Further research should confirm the impact on reproductive outcomes and evaluate potential obstetric and perinatal benefits in this population.