Impact Of Ovarian Stimulation Protocols On Telomere Biology: investigating Gene Expression in Peripheral Blood Mononuclear and Granulosa Cells

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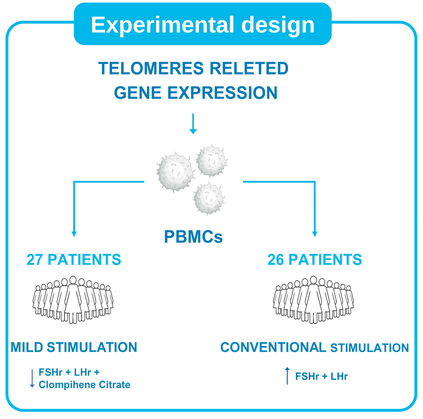
INTRODUCTION

Ovarian stimulation is a fundamental step in assisted reproductive technology (ART), yet its impact on oocyte quality remains a critical area of investigation. While conventional stimulation protocols maximize oocyte yield, concerns have been raised about their potential effects on cellular stress and telomere integrity, factors that influence oocyte competence and embryo developmental potential.[1] Oxidative stress has been identified as a significant side effect of ovarian stimulation impacting biological aging and gamete quality by altering telomere structure and length.[2] Telomeres are specialized nucleoprotein structures located at the ends of linear chromosomes. Telomere integrity is maintained by the Shelterin complex (TRF1, TRF2, TIN2, TPP1, POT1, RAP1) and additional factors like TCAB1 and DKC1, which together ensure proper telomere protection, replication, and telomerase activity. Telomerase is a reverse transcriptase composed of two basic subunits: TERT and telomerase RNA component (TERC) capable of adding de novo repeats to telomeric DNA sequence[3] (Fig1). Mild stimulation protocols have been proposed as an alternative, particularly for patients with diminished ovarian reserve, to mitigate these detrimental effects.[4] However, comparative data on their biological impact at the cellular level are still limited.

OBJECTIVE

This study aims to compare the effects of conventional and mild stimulation protocols, on peripheral blood mononuclear cells (PBMCs), analyzing markers of telomere integrity. To achieve this, PBMCs telomeres related gene expression of two patient groups were analyzed: POSEIDON Group 4 undergoing conventional ovarian stimulation or mild ovarian stimulation.

Experimental design



Telomers biology

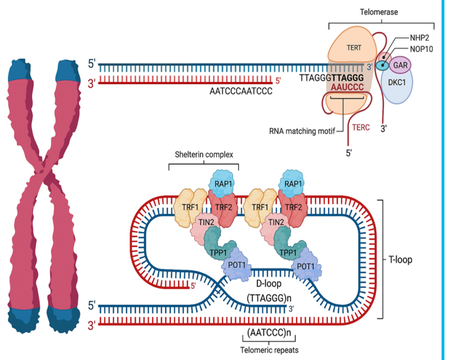


Figure 1: Telomere structure, Shelterin complex and telomerase[6]

METHODS

- Mild stimulation protocol administered on 27 patients (MiniFiv clinic of Madrid) involved:
  Administration of Clomiphene citrate (50 mg every 24h from the first day of the last menstrual period);
  Administration of FSHr + LHr (150 IU every 48h from the sixth day of treatment);
  Trigger induction with rhCG (250 mcg) and/or Triptorelin (0.2 mg);
  +/- antagonist administration (ganirelix 0.25 mg/24 h or cetrorelix 0.25 mg/24 h) on the trigger day;
  Retrieval 34-36 h after the trigger.
- Conventional stimulation protocol administered on 26 patient (IVI Aravaca clinic of Madrid) involved:
  Administration of FSHr/ LHr (minimum dose 225 IU/ 24h from from the first day of the last menstrual period);
  Antagonist administration (ganirelix 0.25 mg/24 h or cetrorelix 0.25 mg/24 h) based on the response;
  Trigger induction with rhCG (250 mcg) and/or Triptorelin (0.2 mg);
  Retrieval 34-36 h after the trigger.
- PBMCs were collected during pickup clinical practice.
- Total RNA isolation was performed using TRIzol Reagent (INVITROGEN).
- 1 mg of RNA was retrotrascripted to cDNA using iScriptcDNA Synthesis Kit (BioRad).
- RT-qPCR was perfomed using SsoAdvanced Universal SYBER Green Supermix (BioRad) in 75000 Fast Real Time PCR System (applied BioSystems).

RESULTS

RAP1, POT1, were found significantly upregulated in patients who underwent mild stimulation compared to those who received conventional stimulation ($p < 0.01$). DKC1, TPP1 exhibited a trend toward differential expression with a higher expression in mild stimulation patient group, although statistical significance was not reached.
These results suggest that patients undergoing mild ovarian stimulation exhibited significantly higher Shelterin genes expression indicating that the telomeric DNA would be better protected under mild ovarian stimulation conditions. Moreover, also telomerase related genes TCAB1 and TERT, were found significantly ($p < 0.001$) upregulated in patients who underwent mild stimulation, suggesting that either telomerase could maintain telomere length, or exert non-canonical functions, such as migrating to the mitochondria to reduce oxidative stress.[5] (Fig.2) Globally, these results suggest that mild stimulation may better preserve telomere integrity and reduce oxidative stress, potentially improving oocyte quality.

Higher SHELTERIN and TELOMERASE's gene expression in PBMCs of women undergoing MILD STIMULATION

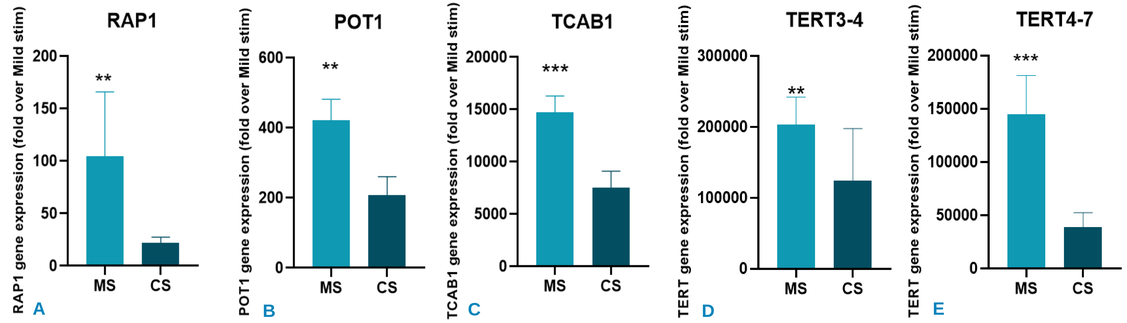


Figure 2: Analysis of the expression of telomere-related genes in PBMCs. A) the graph shows the relative RAP1 expression in samples from women undergoing mild (MS) or conventional (CS) ovarian stimulation protocols. B) The graph shows relative POT1 expression in the samples described in A. C) The graph shows relative TCAB1 gene expression in the samples described in A. D) the graph shows relative expression of the gene TERT3-4 in the samples described in A. E) the graph shows the relative expression of TERT in the samples described in A. n= 27 for conventional ovarian stimulation and 26 for mild ovarian stimulation. Mann-Whitney U test was used to calculate the statistical significance. ** means p< 0.01 and *** means p<0.001).

CONCLUSIONS

Preliminary findings suggest that genes associated with telomere maintenance are lower expressed in the blood cells of patients who underwent conventional ovarian stimulation, indicating potential systemic cellular stress linked to the stimulation protocol. However, due to the small sample size, further studies with a larger cohort are needed to confirm these findings and how mild stimulation affects oocyte quality and reduces oxidative stress to support the identification of more personalized protocols for each patient.

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