P-457- Cohesins SA1/SA2 expression profiling and DNA telomere sizing of cumulus cells as potential indicator of oocyte quality and embryo competence

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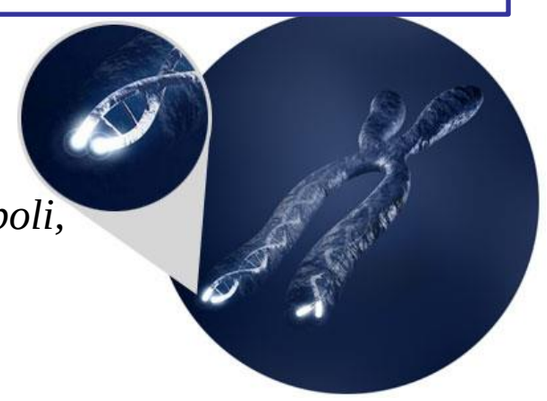

Study question: Are the relative telomere length and cohesins SA1/SA2 mRNA quantitative evaluation of cumulus cells predictive biomarkers of oocyte quality and embryo development potential?

Summary answer: The relative telomere length and expression profiling of cohesins SA1/SA2 of cumulus cells are eligible biomarkers of oocyte competence in ART scenario.

What is known already: Cohesin-SA1 and SA2 form a protein complex that mediates sister chromatid cohesion at telomere termini (SA1) and alongside chromatid arm (SA2), allows chromatin accessibility to regulate gene transcription and triggers DNA repair in G2. Inadequate expression of cohesion SA1by ageing or cytoplasmic immaturity may led to telomere shortening (defective replication) and affect oocyte quality and embryo competence.

Study Design, Size, Duration: Collectively 280 cumulus/oocyte complex samples were recovered from a total of 51 patients undergoing different stimulations protocols (FSH, FSH+LH, FSH+lH like).

Partecipants, Materials, Setting, Methods: Cumulus cells were separated from the oocyte-cumulus complex under a microscope. DNA and total mRNA were extracted from cumulus cells and assayed for telomere sizing and SA1/SA2 expression profiling by qPCR and qRT-PCR. The quantification of telomere DNA was accomplished relative to a single copy housekeeping gene (albumin) to generate a T/S ratio (Telomere/single copy gene). Expression levels of SA1/SA2 cohesins and telomere length measurements of patients/samples were ranked in relation to the clinical setting parameters (BMI, age at oocyte recovery, hormonal protocol stimulation) and oocyte development indicators (embryo formation and positive reproductive outcome).

Main results and the role of chance: Cohesins SA1 and SA2 mRNAs were both expressed in all cumulus cells analyzed in this study, even if their expression level resulted significantly different. Both SA1 and SA2 resulted more expressed in young (< 34 years old) in comparison with old patients (>38 years old) (Fig 1). When the expression level was evaluated according to oocyte number retrieved at pick up, we observed an increase of both SA1 and SA2 levels in cumulus cells. A significant increase in cohesins expression was detected in high responder women (producing more than 6 oocytes at the day of pick up) compared to poor responders (less than 4 oocytes) (Fig.2).

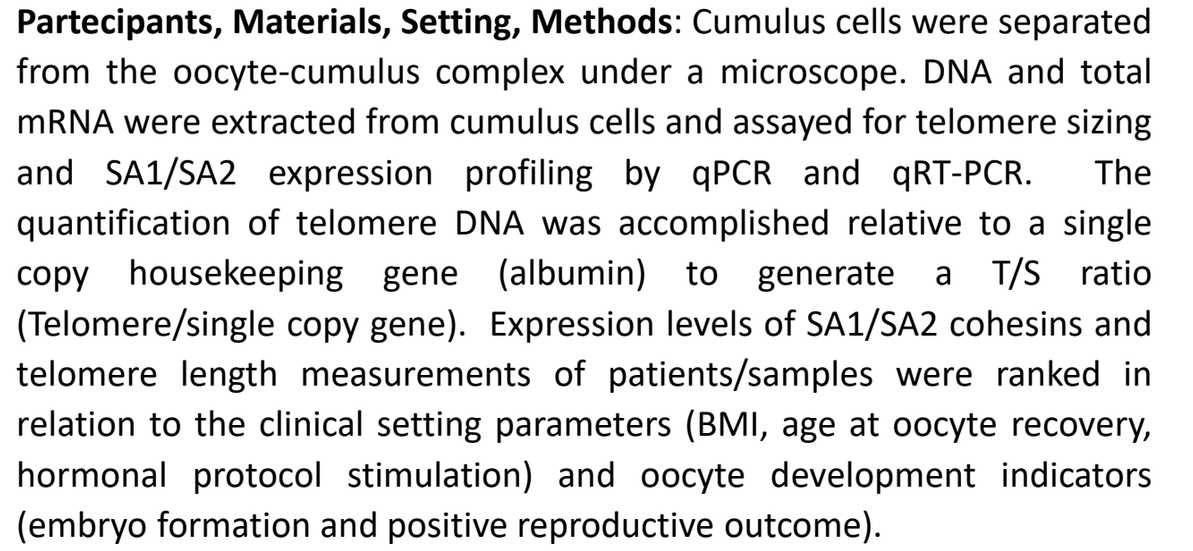

Figure 1: *SA1(panel A) and SA2 (panel B) mRNA expression in cumulus cells prepared from young (< 34 years old) in comparison with old patients (>38 years old). Fold change (y axis) represents SA1or SA2 expression normalized to HPRT1, relative to young women, considered to be equal to 1. All values are presented as means ± SD. *P<0.05.*

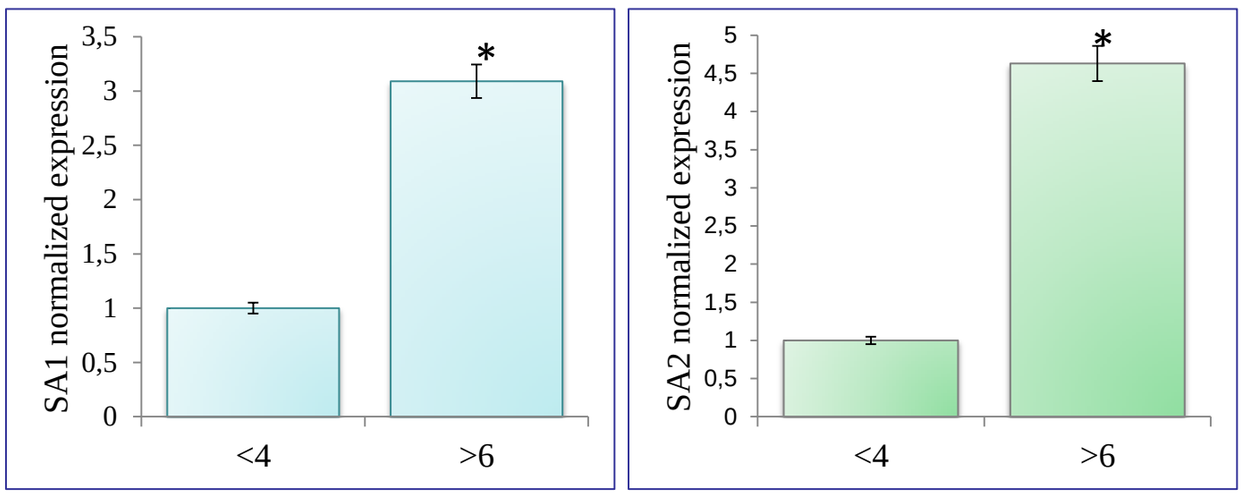

Figure 2: *SA1 and SA2 normalized expression in cumulus from women*

We also analyzed the relation between cohesins mRNA levels and telomere length in the same samples, highlightening a significant raise of SA1 expression level in cumulus cells with longer telomere (R=0.42). The same analysis for SA2 mRNA expression level showed a less significant correlation (R= 0.36) (Fig.3). A significant correlation between SA1 and SA2 expression level has been established (R=0.8) (Fig .4).

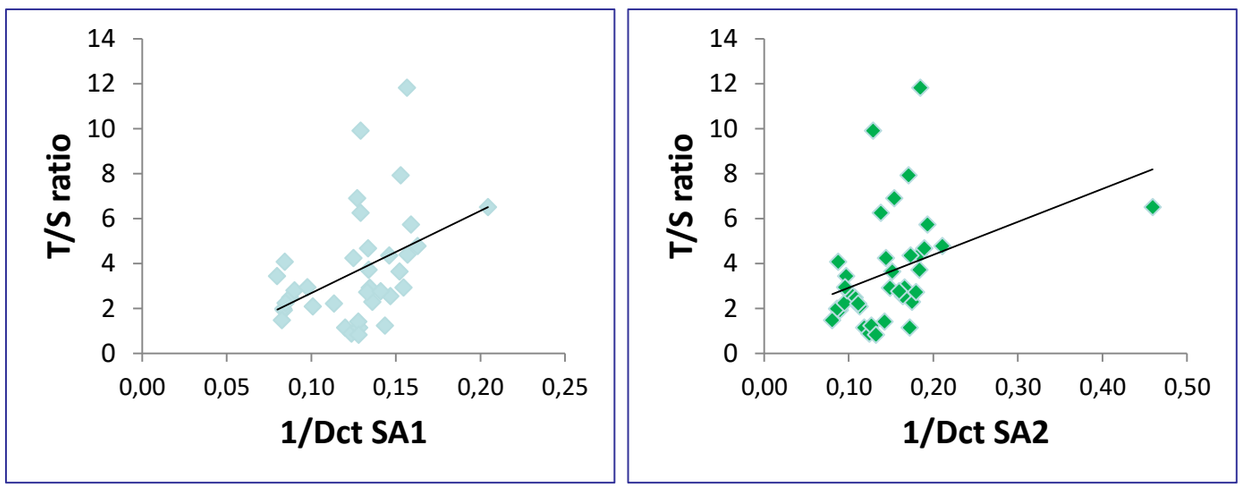

Figure 3*: Correlation between cohesins mRNA levels and telomere length.*

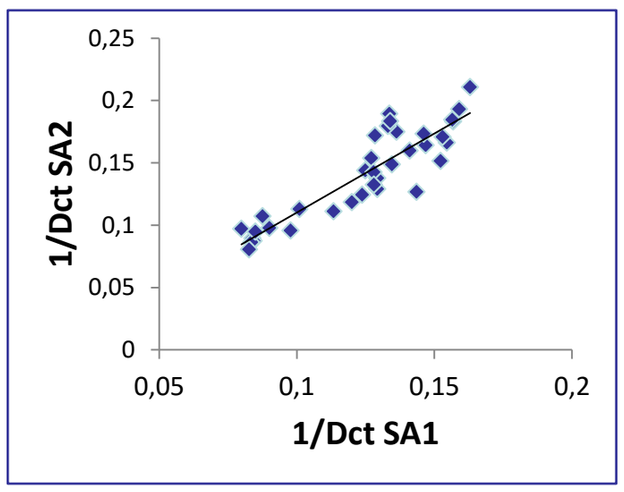

Figure 3*: Correlation between SA1 and SA2 mRNA.*

Wider implications of the findings. To our knowledge this is the first study addressing the issue of Cohesins SA1 and Sa2 expression in cumulus cells together with the telomere relative length sizing as biomarkers of oocyte competence. The reported observations may stimulate further research to uncover the role of cohesins in oocytes aneuploidies.

In conclusion we report, for the first time, novel and unique data concerning the involvement of cohesin SA1/SA2 in telomere homeostasis of cumulus cells suggesting their eligibility as biomarkers of oocyte ontogeny.

Acknowledgment: We tank Merk for finalcial support .