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## Abstract title:

High levels of Follicular fluid oxidative stress are present in younger patients and fertile donors

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### Study question:

Is the information provided by the oxidative status of the follicular fluid related with fertility and maternal age?

### Summary answer:

A correlation was found among the follicular fluid oxidative status, its fertility status (donor/patient), woman age and number of retrieved oocytes.

### What is known already:

Oxidative stress has been reported in literature as a useful biomarker in male and female infertility. A novel technique detecting the oxidative status of different biological fluids, the Thermochemiluminescence (TCL) Analyzer<sup>TM</sup> (Carmel Diagnostics, Israel), has proved to be effective in different studies regarding seminal plasma, amniotic fluid and the spent embryo culture media. In this last one, we found a close relationship between the oxidative status of media and the subsequent clinical outcomes. Our present purpose is to examine the relationship between follicular fluid's oxidative status and relevant characteristics of an *in vitro* fertilization (IVF) treatment.

### Study design, size, duration:

This prospective observational study included 40 patients (from 31 to 45 years) undergoing an IVF cycle and 17 donors (from 20 to 34 years) enrolled in an egg donation program during the period from March 2018 to October 2018. The oxidative analysis was performed with the TCL Analyzer<sup>TM</sup> and embryos were cultured and evaluated in a time-lapse technology system.

### Participants/materials, setting, methods:

A total of 57 follicular fluid samples were collected during oocyte retrieval. 50 µl samples were assessed by the TCL Analyzer<sup>TM</sup>, based on a heat-induced oxidation of biological fluids leading to the production of light energy, counted as photons emitted per second (cps), recorded after 55 seconds (H1), 155 seconds (H2) and 255 seconds (H3). A smoothing algorithm (sm) was used to normalize data. Data was statistically analyzed with Pearson chi-square test and one-way ANOVA.

### Main results and the role of chance:

Donor follicular fluid showed significantly higher values ( $p < .05$ ) for the oxidative parameters than patients ones: **H1sm** =  $290.53 \pm 85.59$  cps for patients vs.  $361.88 \pm 124.46$  cps for donors, **H2sm** =  $412.09 \pm 116.92$  cps for patients vs.  $514.71 \pm 133.20$  cps for donors and **H3sm** =  $628.37 \pm 213.23$  cps for patients vs.  $783 \pm 173.62$  cps for donors. Regardless the follicular fluid origin (donor or patient), a significantly negative correlation was found between woman age and the level of oxidative stress in the sample ( $p < .05$ ). The mean and standard deviation of the TCL parameters were as follows (cps):  $555.39 \pm 130.84$  for  $\leq 26$  years,  $506.11 \pm 125.29$  from 27 to 35 years,  $429.10 \pm 166.81$  from 36 to 40 years and  $424.37 \pm 135.08$  for  $\geq 41$  years. Congruent with the above results, significantly higher TCL parameters were found as number of retrieved oocytes increased ( $p < .05$ ). For less than 20 oocytes retrieved: **H1sm** =  $297.89$  cps, **H2sm** =  $418.53$  cps and **H3sm** =  $634.46$  cps and for more than 20 oocytes retrieved: **H1sm** =  $367.49$  cps, **H2sm** =  $534.69$  cps and **H3sm** =  $823.49$  cps. However, no differences in TCL results were found regarding Body Mass Index (BMI).

### Limitations, reasons for caution:

The study of the follicular fluid can only give us information about the full oocyte and embryo cohort development, but never individual information of each embryo. This is a pilot study with a small sample size which must be increased in order to verify our conclusions.

**Wider implications of the findings:**

An increase of the sample size would allow us to assess the impact of follicular fluid oxidative status over the embryo development and clinical outcomes. The measurement of follicular fluid oxidative status might help us designing improved strategies to better and more personalized IVF treatments.

**Trial registration number:**

not applicable

**Keywords:**

Follicular fluid  
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oocytes retrieved