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Assessment of DNA damage in cumulus cells from infertile women using comet assay

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Infertility affects approximately 15% of reproductive-aged couples, which brings an increasing demand to prevent and explore biomarkers for this reproductive and health burden. The comet assay is being used to assess sperm DNA damage, demonstrating that a high level of DNA damage affects reproductive ability. To the best of our knowledge, in women no correlation was obtained mainly due to the scarcity of studies and difficulty in obtaining those tissues. Cumulus cells surround the oocyte, establishing mutual interdependence, fundamental for oocyte development and fertility processes.

This work aims at optimizing and implementing the comet assay protocol to assess DNA damage in cumulus cells (CC) to be applied on samples belonging to infertile women.

The alkaline comet assay was optimized in whole blood and CC samples, using as a positive control cells treated with MMS (0.5 mM). Comet assay was performed in cryopreserved blood and CC of six samples from infertile women, with mean age 33.7 ± 3.4 (range 28-37 years). Data was analyzed using SigmaPlot version 14.0 (Systat Software®Inc.,Chicago, IL, United States). T-test and Mann-Whitney U test were used for continuous comparisons.

The optimization of the assay showed that the concentration of low melting point agarose and the density of cells per gel were critical steps for establishing optimal conditions. In addition, human CC is a very difficult tissue to obtain, conditions cannot be repeated, hence the assay optimization is of paramount importance. Our findings showed that the DNA damage measured by the comet assay was significantly increased in CC compared to the levels found in blood (systemic DNA damage).
These results seem to indicate that the DNA damage found in CC may be related to infertility, as evidenced in male infertility. However, further studies with a higher number of participants are needed to confirm these results.

This study allowed us to successfully implement the comet assay in CC and blood samples from infertile women. Furthermore, positive findings were observed in CC compared to a systemic tissue. Despite the need to increase the number of samples tested, this study highlights the importance of using these two tissues to compare results of DNA damage and supports the use of CC to target the oocyte status as a biomarker with clinical impact in female infertility treatments.

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Comet assay, DNA damage, women infertility, cumulus cells.