DNA DAMAGE ASSESSMENT IN CUMULUS CELLS BY A NOVEL CHROMATIN DISPERSION ASSAY

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Apoptosis and double strand DNA damage (ds-DNA) in cumulus cells (CC) seem to be related to the developmental competence of the enclosed oocytes, however, conclusive studies are missing, mostly due to the lack of a reliable, cheap, fast and repeatable ds-DNA test. We describe here a new chromatin dispersion test (D3-Max) for ds-DNA analysis in CC and compare it to two dimensional comet assay (TT-comet) in pooled CC from MII containing cumulus-oocyte complexes. This prospective study included CC from 103 oocyte donation cycles.

The D3-max showed a statistically significant correlation with the damage visualized as ds-DNA by TT-comet assay (Spearman Rho 0.624; p=0.003). On the contrary, the correlation was not significant (Spearman Rho -0.141; p=0.554) when the ss-DNA breaks observed with the TT-comet assay was compared to the D3-max.

To prove conclusively that D3-max test was identifying ds-DNA breaks in CC chromatin, we produced controlled ds-DNA breaks by the restriction endonuclease Alu-I. We found a high correlation between TT-comet ds-DNA damage derived from a controlled ds-DNA damage production and that obtained after the D3-max (Spearman Rho 0.720; p=0.019).

In summary, D3-Max test detects specifically ds-DNA break and allows for fast, repeatable and precise evaluation of ds-DNA damage in CC. D3-Max and can be used to investigate the role of CC ds-DNA as marker of oocyte quality and developmental competence.