

The significance of Sperm DNA oxidation in embryo development and reproductive outcome in an oocyte donation program; A new model to study a male infertility prognostic factor.

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Background: Free radical damage is an important factor in sperm function. Despite extensive research efforts in male fertility biomarkers in recent years, there is still a great need for additional research on the applicability of free radical damage analysis in humans. One product resulting from free radical damage is the DNA-hydroxylation also known as DNA oxidation. Oocyte donation is a powerful tool to study sperm quality, since oocyte and endometrial characteristics can be controlled and are comparable among the recipients. To gain understanding about the effects of sperm DNA oxidation on embryo quality and reproductive outcome, we used pairs of oocyte donation cycles i.e. the same oocyte donors were employed and the only difference between the two treatments was the use of different sperm source..

Materials and Methods: Semen samples were obtained from the infertile partners of couples undergoing oocyte donation cycles (n = 38). We obtained 76 semen aliquots before and after semen processing by swim up. A total of 19 oocyte donors were employed and they donated for two different recipients.. Our model includes two original design features difficult to find in the previous literature in order to overcome female factor heterogeneity: 1) sperm OXI DNA was determined by analyzing aliquots of the same ejaculated sample that was used for ART and 2) ovum donation offers a powerful tool in the study of sperm quality in assisted reproduction treatments, since it reduces the variability within oocyte quality that female infertility may introduce. We used the OXI DNA assay kit based on the direct binding of a fluorescent probe to the DNA adduct 8-oxoguanine. The FITC-conjugate binds to 8-oxoguanine in cells with damaged DNA and fluorescence was quantified using flow cytometry (excitation 495 nm, emission 515 nm).

We analyzed all the data obtained and correlated sperm OXI DNA features with embryo quality parameters, and pregnancy outcome. Furthermore, we grouped pairs of sperm samples with same oocyte donor, and calculated the difference in OXI DNA to correlate this indicator with the variation observed in the numerical parameters of the embryos obtained from them. Correlation between fertilization and implantation rates, triploid rate and embryo quality parameters (number of blastomeres, percentage of embryo fragmentation, total number of frozen embryos on day 2 and 3) with OXI DNA results was done.

Results: No significant differences were seen in sperm concentration, motility and morphology in couples achieving pregnancy to those that did not.. A positive correlation was seen between embryo fragmentation and DNA oxidation of capacitated samples at 48 and 72 h after fertilization (r = 0.318 and r = 0.353 respectively). A negative correlation was seen between the percentage of DNA oxidized sperm cells and the total number of cryopreserved embryos which reflects the average embryo quality of our cohort. Similarly, a positive correlation was seen between this percentage and the triploid rate formation.

However when we analyzed the differences in the fertilization rates and IVF outcome parameters of the couples who shared the oocyte cohort (same donor) with the differences in the OXI DNA values, we observed further relationships that are represented in the following table.

IVF parameter	Fertilization rate	Implantation Rate	Number of blastomeres (48 h)	Embryo Fragmentation (72 h)	Number of triploid embryos	Number of frozen embryos
Fresh	-0.083	-0.131	0.317*	0.479*	0.288*	0.115
Swim-up	-0.406*	-0.220	-0.024	0.534*	0.206	-0.333*

* Denote a significant linear relationship, p<0.05.

Conclusions: The association observed between early embryo quality features (48 and 72 h) and sperm DNA oxidation supports the relevance of the paternal contribution of the sperm in the early stages of embryo development (before the whole embryo translational machinery is activated). It highlights the importance of DNA oxidation defining sperm quality, and could be considered as a biochemical marker. The lack of differences in DNA oxidation among those couples who achieved a pregnancy to those who did not may probably be due to embryo selection before transfer, we did not select poor prognosis embryos based on their morphological features.