Oxidative status and hyperactivation of human spermatozoa: potential biomarkers for predicting assisted reproduction outcomes

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Introduction

Infertility is usually defined as the inability of a couple to conceive after one year of regular unprotected sexual intercourse. Approximately 17% of couples are infertile, and 50% of all cases of infertility are due to male factors ^[1,2]. However, in about 15% of couples no apparent cause can be found, and the cause of infertility remain idiopathic. As a result, more couples undergoing to Assisted Reproduction Technology (ART) procedures to achieve a pregnancy, however, success rates remain low. It is known that Oxidative Status (OS) can impair sperm quality and function. Recently, our lab developed a method to evaluate sperm OS using two fluorescent probes, able to detect hydrogen peroxide and superoxide anion in viable spermatozoa: CRO and DHE ^[3]. This method identifies viable oxidized spermatozoa with better characteristics. Hyperactivated motility, a sperm specific motility pattern acquired in the female genital tract, is another important parameter influencing reproductive outcomes.

Aims

The primary aim of this study was to evaluate the oxidative status and hyperactivated motility of both selected and unselected spermatozoa from male partners of infertile couples undergoing ART procedures, and to assess its correlation with ART outcomes. This study was conducted in collaboration with the Demetra ART Center in Florence (Italy), where we enrolled 179 couples undergoing ART cycles from December 2022 to June 2024. All couples were treated using the ICSI procedure. Fresh embryo transfer was possible in only 26 couples. Indications for deferred embryo transfer included: risk of ovarian hyperstimulation syndrome, elevated progesterone levels (\leq 1.5 ng/ml), and inadequate endometrial thickness on the trigger day. To eliminate potential confounding bias due to the different types of embryo transfer (fresh or frozen), we analyzed the two groups separately and found no statistically significant differences in ART outcomes between them. For this reason, we performed a cumulative statistical analysis including both fresh and deferred transfers.

Results

Preliminary results showed a significant positive correlation between the percentages of positivity for both probes, in both selected and unselected spermatozoa. Furthermore, the correlation between the percentage of spermatozoa positive for both probes, assessed before selection, positively correlated with those after selection. No significant associations were found between sperm oxidative levels and early ART outcomes such as fertilization, cleavage, blastocyst development, or embryo quality. Nevertheless, some intriguing trends were observed. Specifically, higher levels of sperm oxidation following selection were associated with embryo euploidy, biochemical pregnancy and, notably, with pregnancies that did not result in miscarriage. Additionally, although not statistically significant, higher percentages of sperm hyperactivated motility appeared to correlate with achievement of good-quality embryos, euploid embryos, and both biochemical or clinical pregnancies, supporting the idea that hyperactivated spermatozoa exhibit better functions.

Conclusion

Although preliminary, our results suggest that sperm oxidative status, assessed using both probes, is positively linked to pregnancy outcomes and negatively with miscarriage. Additionally, higher sperm hyperactivated motility appears to be associated with improved ART outcomes. These findings encourage to continue the study with the recruitment of a larger number of couples.

Bibliography

1 Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol. 2015;13:37.

2 Pavuluri H, Bakhtiary Z, Panner Selvam MK, Hellstrom WJG. Oxidative stressassociated male infertility: current diagnostic and therapeutic approaches. Medicina (Kaunas). 2024;60(6):1008.

3 Traini G, Tamburrino L, Vignozzi L, et al. Is oxidative stress evaluated in viable human spermatozoa a marker of good semen quality? Front Endocrinol (Lausanne). 2022;13:1012416.