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Commentary

Fresh Versus Frozen Testicular Sperm in Non-obstructive Azoospermia: Laboratory Dynamics and Clinical Relevance

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INTRODUCTION

The use of testicular sperm extraction (TESE) combined with intracytoplasmic sperm injection (ICSI) has substantially expanded reproductive options for men with nonobstructive azoospermia (NOA). Despite its widespread adoption, important clinical and laboratory questions remain unresolved, particularly regarding the optimal timing of sperm retrieval and the use of cryopreserved testicular sperm. Clinicians are frequently faced with the dilemma of synchronizing micro-TESE with oocyte retrieval or planning these procedures independently by relying on cryopreserved gametes. This decision has implications not only for laboratory outcomes, but also for treatment logistics, patient burden, and counselling.

The existing literature comparing fresh and frozen-thawed testicular sperm is heterogeneous. Many studies are limited by inter-patient variability, differences in surgical and laboratory techniques, relatively small sample sizes, and retrospective designs. Moreover, most reports focus on conventional endpoints such as fertilization, pregnancy, or live birth rates, without exploring the dynamic aspects of early embryo development. As a result, the biological mechanisms underlying potential differences between fresh and frozen testicular sperm remain incompletely understood.

Ferracuti et al. address several of these gaps through an observational retrospective cohort study uniquely designed to compare fresh and frozen-thawed testicular sperm obtained from the same NOA patients across different treatment cycles. By eliminating interindividual variability, this intra-patient approach allows a more refined assessment of the true impact of cryopreservation on post-insemination events. The cohort included 980 oocytes retrieved across 72 cycles from 30 couples, with embryos cultured in a time-lapse incubator and evaluated using both conventional laboratory outcomes and detailed morphokinetic parameters. In addition, blastocysts underwent trophectoderm biopsy for pre-implantation genetic testing for aneuploidy (PGT-A), enabling assessment of chromosomal competence alongside developmental dynamics.

From a laboratory perspective, the study demonstrated that frozen-thawed testicular sperm was associated with significantly lower fertilization rates leading to a reduced proportion of usable blastocysts per inseminated metaphase II oocyte when compared with fresh testicular sperm. Importantly, once normal fertilization was achieved, blastulation rates per fertilized oocyte were comparable between groups. Furthermore, after adjustment for female age, no significant differences were observed in euploidy rates per biopsied blastocyst. These findings suggest that while cryopreservation may impair the efficiency of early post-insemination events, it does not appear to compromise the chromosomal competence of embryos that successfully reach the blastocyst stage. However, fertilization remains a critical determinant of overall cycle success, particularly in cycles involving testicular sperm, where the available gametic material is limited and therefore especially precious.

A key strength and innovative aspect of this work lies in the integration of time-lapse morphokinetic analysis. Embryos derived from frozen-thawed testicular sperm exhibited subtle but consistent alterations in early developmental timing, particularly during the pronuclear phase. Frozen-derived gamete dyads were associated with an earlier appearance of pronuclei and a prolonged two-pronuclear (2PN) phase, resulting in a longer 2PN lag time. Similar alterations were observed in embryos derived from vitrified oocytes, suggesting that cryopreservation-related stress may influence early nuclear dynamics in both gametes.

The biological relevance of a prolonged 2PN phase remains an area of ongoing investigation. Pronuclear formation and dissolution reflect the coordination of parental genome replication, epigenetic reprogramming, and preparation for the first mitotic division. The observed delays may indicate compromised sperm functionality following thawing, potentially related to cryodamage affecting plasma membrane integrity, centrosomal function, or paternal DNA integrity. Alternatively, they may reflect an increased requirement for DNA repair mechanisms after cryopreservation. Although these morphokinetic differences did not translate into altered euploidy rates in this study, they provide biologically meaningful insights that cannot be captured by static morphological assessment alone.

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From a clinical standpoint, the findings contribute directly to the ongoing debate regarding synchronization of micro-TESE with oocyte retrieval. The data support the preferential use of fresh testicular sperm when logistically feasible, as this approach appears to maximize fertilization efficiency and blastocyst yield per oocyte. At the same time, the comparable euploidy rates observed between fresh and frozen-TESE cycles offer reassurance that cryopreserved testicular sperm remains a valid and clinically effective option, particularly when fresh sperm retrieval is not possible or fails on the day of oocyte retrieval.

The study also provides valuable evidence supporting oocyte vitrification as a reliable backup strategy. The use of vitrified oocytes combined with fresh testicular sperm resulted in fertilization, blastulation, and euploidy rates comparable to those achieved with fresh oocytes. This observation suggests that oocyte vitrification may better preserve gamete competence than testicular sperm freezing in the context of NOA, an important consideration for treatment planning and patient counselling.

As highlighted by the reviewers of the original manuscript, caution is warranted when interpreting laboratory outcomes in the absence of embryo transfer data. Implantation, pregnancy, and live birth remain the gold standard for validating embryo competence. Morphokinetic parameters and laboratory endpoints should therefore be viewed as intermediate markers that inform, but do not replace, clinical outcomes. Nevertheless, in clinical scenarios where transfer has not yet occurred such as freeze-all strategies or delayed transfers these data provide meaningful guidance for embryologists and clinicians.

In conclusion, this work provides a refined understanding of how cryopreservation of testicular sperm influences early post-insemination dynamics in non-obstructive azoospermia. By leveraging an intra-patient design and integrating time-lapse morphokinetics with chromosomal assessment, the study moves beyond traditional static laboratory endpoints and highlights subtle developmental signatures associated with frozen-derived gametes. Clinically, the findings support the preferential use of fresh testicular sperm when feasible, while reinforcing the role of cryopreserved sperm and vitrified oocytes as effective and clinically meaningful alternatives. Importantly, the absence of differences in euploidy among embryos reaching the blastocyst stage suggests that cryopreservation primarily affects early efficiency rather than intrinsic chromosomal competence. As the field of andrology continues to emphasize individualized treatment strategies, prospective studies linking morphokinetic signatures to implantation and live birth outcomes will be essential to translate laboratory dynamics into improved reproductive care.