

## **Impact of slow freezing on sperm nuclear quality and telomere length**

**Study question:** Does slow freezing affect human sperm telomere length?

**Summary answer:** Slow freezing of human sperm does not alter telomere length despite of increasing DNA oxidation and fragmentation.

**What is known already:** Sperm telomere length (STL) is a potential interesting biological marker to assess male fertility. While its relationship with standard sperm parameters is still controversial, recent studies have shown correlations between STL and DNA alterations, notably DNA fragmentation and oxidation. Likewise, STL is positively related with pregnancy rate. As STL is transmitted to offspring, its alteration could lead to pathologies that may worsen from generation to generation. Human sperm freezing is an indispensable tool for male fertility preservation despite of inducing nuclear spermatid alterations. Nevertheless, nothing is known about its impact on STL.

**Study design, size, duration:** This prospective study was conducted between January and December 2018 and approved by the “Germethèque” Scientific Committee and the Ethical Committee under the French IRB CPP Sud-Est6. Samples were surplus semen obtained from 63 men undergoing routine semen analysis at the Center for Reproductive Medicine. STL measurement was carried out on all the 63 fresh samples, and for 30 of those also after cryopreservation. In addition, nuclear quality was measured before and after freezing-thawing cycle.

**Participants/materials, setting, methods:** The 63 subjects (35 normozoospermic) had a mean age of  $34 \pm 0.6$  years and a BMI of  $27.4 \pm 0.8$ . Spermatozoa were slowly frozen in high security straws using freezing medium and programmable freezer. STL was measured for all fresh and frozen-thawed samples by qPCR. For 10 samples, STL before and after freezing-thawing was additionally assessed by qFISH. For another 10, sperm DNA oxidation (8-OHdG residues immuno-detection) and fragmentation (TUNEL assay) were measured by flow cytometry.

**Main results and the role of chance:** We did not measure any significant impact of freezing on the mean telomere length of sperm samples, whether it was analyzed by qPCR ( $3.25 \pm 0.15$  arbitrary unit (a.u.) *vs.*  $3.46 \pm 0.2$  a.u.,  $n = 30$ ) or by qFISH ( $0.84 \pm 0.1$  a.u. *vs.*  $0.84 \pm 0.04$  a.u.,  $n=10$ ). qFISH method highlighted STL heterogeneity within each sperm sample. The cryopreservation tended to modify STL distribution (segmented by intervals of 0.25 a.u.) even if it was not statistically significant.

In addition, slow freezing-thawing cycle decreased sperm motility and vitality ( $p < 0.05$  for both,  $n=10$ ), and increased DNA oxidation (average oxidation intensity  $839 \pm 210$  a.u. *vs.*  $1445.2 \pm 202$  a.u.,  $p < 0.05$ ,  $n=10$ ) and fragmentation ( $23.1 \pm 5.4$  % *vs.*  $39 \pm 6$  %,  $p < 0.005$ ,  $n=10$ ). Finally, we did not measure any significant correlations between STL and the analyzed spermatid nuclear markers.

**Limitations, reasons for caution:** Standard parameters of analyzed samples were normal or slightly altered because the different measurements required a high number of spermatozoa. Nevertheless, fertility preservation is proposed to men with potentially altered sperm parameters, therefore it would be essential to measure cryopreservation impact on semen of oligo-astheno-teratozoospermic patients.

**Wider implications of the findings:** Although slow freezing increased DNA oxidation and fragmentation, it did not alter STL. To our knowledge, this was the first study to assess the impact of cryopreservation on STL. Compared to qPCR that only gives a mean STL, qFISH provides the distribution of STL within a semen sample.

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Mot clé: Human spermatozoa, telomere, cryopreservation, fragmentation, oxidation.

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