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THE INFLUENCE OF CRYOPRESERVATION IN SPERM DNA DAMAGE

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Infertility is a worldwide problem and affects 15% of all couples trying to conceive. In general, the cause of infertility involves the male in one-third of cases, the female in another one third, and in the remaining cases, both the male and female, or no cause can be identified. Sperm DNA damage has been related to male infertility and it is associated with reduced fertilization rates, embryo quality and pregnancy rates, higher rates of spontaneous miscarriage and childhood diseases.

Cryopreservation is a common technique used to preserve male sperm. This technique is routinely used in a variety of circumstances including assisted reproduction, pre-radiation or chemotherapy treatment, surgical treatments that may affect fertility and for storage of donor semen. However studies suggest that cryopreservation can cause DNA damage.

In this work it will be compared DNA damage of cryopreserved versus fresh sperm cells.

From December 2015 to February 2016 a comet assay was performed in 11 samples from men aged between 28 and 43 years old, followed in the Hospital Center of Trás-os-Montes and Alto Douro fertility consultation.

Semen was analyzed according to World Health Organization specifications. The comet assay was used to DNA damage analysis where Total Sperm DNA Damage Count (TDC) was assessed and compared in fresh and cryopreserved samples. From each sample, three aliquots were separated: one aliquot was immediately processed to comet assay and the other two aliquots were stored in liquid nitrogen and processed later. DNA damage was quantified by visual classification of nucleoids into five comet classes, according to the tail intensity and length, from 0 (no tail) to 4 (almost all DNA in tail). 50 comets per duplicate gel were analyzed, on a scale of 0-400 arbitrary units (AU).

We observed that all the cryopreserved samples presented higher DNA damage when compared with fresh samples. The average variation between fresh and cryopreserved samples was 190 U. The minimum value obtain was 50 AU and a maximum of 370 AU.

Despite the reduced number of samples, the study suggests that cryopreservation increases DNA damage, thus possibly being a rather counterproductive way to preserve male fertility as DNA damage is associated with reduced fertilization rates.

However more samples are necessary in order to fully comprehend the role of cryopreservation in DNA damage.