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Editorial

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Assessment of Ageing-Dependent Effects on Sperm Functions following Semen Cryopreservation

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There has been an increased improvement in the cryopreservation protocol of semen, which contributes to the preservation of genetic resources.[1,2] Cryo-induced damage to spermatozoa is attributed to membrane deterioration caused mainly by the formation of intracellular ice and increased oxidative stress, resulting in a loss of the sperm fertilizing ability.[3] It is noteworthy that ageing-dependent processes in frozen-thawed semen occur in different sperm compartments, and are associated with membrane modifications of specific lipid-protein interactions, activation of an apoptosis-like mechanism and reduced genomic integrity, which consequently compromise sperm cryo-survival.[4,5] Moreover, ageing is a natural process occurring in sperm cells during semen preservation, and there is no single sperm test that can effectively predict the fertilizing capacity of semen following the different reproductive technologies.[6]

Cryo-induced ageing-dependent processes in spermatozoa have marked effects on different sperm attributes, either directly or indirectly, resulting in impaired fertilizing ability of the post-thaw semen.[1] A plethora of sperm markers have provided evidence of the ageing-dependent effects on sperm cryo-survival.[5] Accordingly, molecular markers, used to detect the sperm ageing-dependent processes associated with the freezing-thawing procedure, have made possible a more widespread analysis of different sperm attributes that are considered as a prerequisite for successful fertilization.[6] Cryo-induced sperm ageing processes have been manifested in compromised sperm motility, mitochondrial membrane potential (MMP), plasma membrane integrity (PMI) and acrosome membrane integrity (AMI), which are the key determinants of semen quality.[1] Even though motility characteristics, analyzed by the computer-assisted semen analysis (CASA) system, reflect several essential aspects of the sperm metabolic activity, it is still unclear whether these variables are reliable to predict fertilization.[4] Moreover, the sperm membranes are an integral part of the acrosome reaction and are involved in the fertilization-related events, so deterioration in the structural and functional integrity of spermatozoa is an obvious sign of the ageing processes, as is evident after cryopreservation.[5] Assessment of the cryo-induced sperm ageing processes, using a combination of different fluorescent probes (lipophilic cationic JC-1 in combination with propidium iodide – PI, JC-1/PI; rhodamine 123, R123/PI; MitoTracker Green, MTG; SYBR-14/PI; carboxyfluorescein diacetate, CFDA/PI; Hoechst 33258, H258; fluorescein isothiocyanate (FITC)-labeled peanut agglutinin, FITC-PNA/PI; FITC-labeled *Pisum sativum* agglutinin, FITC-PSA/PI), has provided more detailed information on several sperm attributes that are required to identify individuals with poor and good semen freezability.[1,5] Furthermore, the ageing-associated decrease in post-thaw sperm viability is concomitant with an increase in lipid peroxidation measured by malondialdehyde (MDA) production, capacitation-like destabilization of sperm membranes (antibiotic chlortetracycline, CTC; Merocyanine 540, M540), apoptotic sperm cells (Annexin-V/PI; Yo-Pro-1/PI; caspase activation) or sperm DNA fragmentation (Comet assay, sperm chromatin structure assay, SCSA; terminal deoxynucleotidyl transferase-mediated dUDP nick end labelling assay, TUNEL; sperm chromatin dispersion assay), which significantly compromises the fertilizing ability of frozen-thawed spermatozoa.[4,5] Despite the effectiveness of these sperm assays, the underlying mechanisms involved in the cryo-induced ageing-dependent changes, within different compartments of the spermatozoon, are still poorly understood.

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Sperm attributes react differently to the cryopreservation conditions among individuals.[6] This has contributed to renewed interest in developing new sperm markers that can be used during routine semen analysis to identify individuals differing in semen freezability.[7] It is envisaged that transcriptome studies on RNA-sequence data will identify markers directly associated with the cryo-induced ageing processes in spermatozoa.[8] Analysis of the ageing processes in gene expression profiles of frozen-thawed spermatozoa will provide new opportunities to develop freezability markers for the improvement in the technology of semen cryopreservation. Supported by a project from the National Science Centre, Poland (2015/19/B/NZ9/01333).

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