IJAE Vol. 115, n. 1/2 (Supplement), 2010

Cryogenic temperature protects biological material from gamma ray induced effects

Giulia Cugia[1,2], Filippo Centis[3], Maria Dattena[4], Genny Del Zotto[1], Alessandra Lucarini[1,5], Piero Bonelli[6], Daniela Sanna[4], Elisabetta Argazzi[7], Monica Bono[7], Giampaolo Zini[7], Massimo Valentini[3], Francesco Picardi[3], Werther Cesarini[2,7], Loris Zamai[1,2]

[1] Department of Human, Environmental and Natural Sciences, University of Urbino "Carlo Bo", Italy
[2] INFN Gran Sasso National Laboratories, Assergi (AQ), Italy
[3] Laboratory and Image Diagnostic Department, San Salvatore Hospital, Pesaro, Italy
[4] Agris-Sardegna, DIRPA (Agricultural Research Agency of Sardinia, Department of Animal Science) Reproduction Division, Sassari, Italy
[5] ASLEM (Associazione Sammarinese Leucemie ed Emopatie Maligne), San Marino
[6] Experimental Zooprophylactic Institute of Sardinia, Sassari, Italy
[7] Health Physics Department, San Salvatore Hospital, Pesaro, Italy

Cryopreservation of cells, tissues and organism in liquid nitrogen (LN) offers the most secure form of conservation, nevertheless frozen biological materials are exposed to natural background of ionising radiation (IR). It is known that IR can induce cell death and tumors in living cells, furthermore radiation can cause abortion and teratogenic effects in embryos, but on the response of cryopreserved cells and embryos only few information are available. The aim of this study is to evaluate the effects of IR on frozen and unfrozen peripheral blood mononuclear cells (PBMCs) and sheep embryos irradiated in LN with different doses of γ-rays. PBMCs were directly irradiated at room temperature, then immediately frozen, or frozen and then irradiated in LN with different (0, 0.1, 0.3, 0.9, 3.0, 18,6 Gy) doses of IR. After thawing, cells were incubated and percentages of cell death were evaluated by flow cytometry at different time points, using both hypodiploid peak detection and supravital propidium iodide staining. On the other hand, zygotes from fertilized oocytes with fresh ram semen were cultured for 6-7 days in 20 µl droplets of synthetic oviduct fluid. Embryos were vitrified and exposed to different radiation (0, 0.3, 2.4, 19.2 Gy) doses in LN. After thawing, embryos were all transferred in pairs into synchronized ewes. Pregnancy was confirmed by ultrasonography.

Interestingly, PBMC cell death gradually increased both with dose radiation and incubation time and was relevantly higher in PBMCs irradiated at room temperature than in those frozen. Moreover, lambing rates were 28% (5/18), 21% (5/24), 0% (0/10), 50% (4/8) for 0.3, 2.4, 19.2 Gy and control group respectively. In conclusion, these results suggest that cryogenic temperature protects biological material from gamma ray induced effects.

Key words
Gamma radiation, cryopreservation, cell death, embryo, abortion