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*Implications of storage and handling conditions
on glass transition and potential devitrification
of oocytes and embryos*

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5 **Implications of storage and handling conditions on glass**
6 **transition and potential devitrification of oocytes and**
7 **embryos**

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26 **ABSTRACT**

27 Devitrification, the process of crystallization of a formerly crystal-free,
28 amorphous glass state, can lead to damage during the warming of cells. The
29 objective of this study was to determine the glass transition temperature of a
30 cryopreservation solution typically used in the vitrification, storage and
31 warming of mammalian oocytes and embryos using Differential Scanning
32 Calorimetry. A numerical model of the heat transfer process to analyze
33 warming and devitrification thresholds for a common vitrification carrier (open-
34 pulled straw, OPS) was conducted. The implications on specimen handling
35 and storage inside the dewar in contact with nitrogen vapor phase at different
36 temperatures were determined. The time required for initiation of
37 devitrification of a vitrified sample was determined by mathematical modeling
38 and compared with measured temperatures in the vapor phase of liquid
39 nitrogen cryogenic dewars. Results indicated that the glass transition ranged
40 from -126 to -121°C and devitrification was initiated at -109°C. Interestingly,
41 samples entered rubbery state at -121°C and therefore could potentially
42 initiate devitrification above this value, with the consequent damaging effects
43 to cell survival. Devitrification times were calculated considering an initial
44 temperature of material immersed in liquid nitrogen (-196°C) and two
45 temperatures of liquid nitrogen vapors within the dewar (-50 and -70°C) to
46 which the sample could be exposed for a period of time, either during storage
47 or upon its removal. The mathematical model indicated samples could reach
48 glass transition temperatures and undergo devitrification in 30 seconds.
49 Results of the present study indicate storage of vitrified oocytes and embryos
50 in the liquid nitrogen vapor phase (as opposed to completely immersed in
51 liquid nitrogen) poses the potential risk of devitrification. Due to the reduced
52 time-handling period before samples reach critical rubbery and devitrification
53 values, caution should be exercised when handling samples in vapor phase.

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55 Keywords: Vitrification, embryo, glass transition, devitrification, liquid nitrogen

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