Solidification of water normally occurs by the way of crystallization. It implies rearrangement of water molecules to form an ice crystal. Cells could survive if the temperature is lowered gradually permitting the very slow growth of water crystals in the extracellular space. The growth of ice induces cell dehydration and increase the intracellular concentration of cryoprotectants. A frozen straw looks milky and opaque.

But solidification of water may also occur by vitrification. Cell could survive only if intra-and extracellular ice crystals are absent, provided the cooling is very rapid. In this case, the straw containing the cells / embryos is immersed in LN2, resulting in a brutal solidification in which crystallization does not have time to occur. It implies an infinite increase of viscosity: the straw is solidified in its liquid conformation, and appears transparent.
Embryo cryopreservation by slow rate freezing method (SF) has been described already in 1972 by Whittingham et al and by Wilmut. Till now it is the most commonly used method for cell and embryo freezing. During a SF, cooling begins after a complete equilibration of cells in the cryoprotective solution and at room temperature. After this step, embryos are packed in straws and these are placed in a programmable freezer to lower the temperature gradually (0.3°C/min) and in a perfectly controlled manner: this requires the use of programmable freezers. The apparition of the first ice crystal is manually seeded at -7°C. Lowering the temperature gradually permits the very slow growth of water crystals in the extracellular space. The growth of ice crystal induces cell dehydration and increase the intracellular concentration of cryoprotectants (CPs). After the slow cooling step until about -50°C, the intracellular concentration of CPs reaches the threshold allowing the intracellular vitrification. This explains why the cells survive when the straw containing the embryos is plunged into LN2.
This graph represents phase diagrams. The liquid phase is above the freezing curve, the solid phase (crystalline) under. Liquid freezing medium start to freeze at -7°C. An ice crystal is only composed with water molecules, so its growth implies an increase of the concentration of solutes in the remaining liquid phase. As a consequence, crystal growth needs the temperature to decrease again.

In the presence of cells, we have to consider two phase curves corresponding to the intracellular (blue) and the extracellular (pink) compartments. Because of the higher concentration of solute in the intracellular compartment, the corresponding freezing curve is always lower than its extracellular counterpart. As a consequence, when tissue is slowly cooled, ice first forms between cells. The growing ice crystals increase the concentration of solutes in the remaining liquid around them, causing osmotic dehydration of cells. If cryoprotectants are present, the freezing point of the unfrozen solution drops sooner and faster, limiting the total amount of ice that forms. As the temperature drops below -50°C, the cryoprotectant concentration becomes so high in the remaining unfrozen solution that ice stops growing. Cells survive suspended in the residual unfrozen liquid between ice crystals. As the temperature drops below about -130°C, this unfrozen solution containing the cells becomes a glassy solid. Although the concentration of cryoprotectants seems to be dangerously high at the final phases (-50°C), this happens at low temperatures, where the real toxic effect is minimal.
Slow freezing protocols need to lower the temperature very gradually (0.3°C/min) and in a perfectly controlled manner: this requires the use of programmable freezers.

After full equilibration of embryos (20 to 30 minutes at room T°) in the freezing medium, eggs are loaded in straws. These are placed in a freezing machine already pre-cooled at 0°C. Temperature is decreased rapidly (2°C/min) until -7°C. At this temperature, the freezing medium is still liquid, and crystallization is manually seeded to avoid surfusion. Once crystallization has progressed all along the straw, the temperature is slowly decreased (at -0.3°C/min) until -50°C to dehydrate progressively the cells when the crystal size increases. Cell dehydration arises intracellular CP concentration, and at -50°C conditions of intracellular vitrification are reached, so the straw may be plunge directly in LN2.
Vitrification implies the exclusion of any intra-and extracellular ice crystal. It involves first partial equilibration of embryos in the cryoprotective solution before to dehydrate the cell by submitting it to another hyperosmotic mixture of non-penetrating CPs. In a few minutes at room temperature, both intra- as well as extracellular conditions of vitrification are satisfied provided the cooling is very fast (~1000°C/min). The straw containing the cells / embryos is immediately immersed in LN2, resulting in a brutal solidification in which crystallization does not have time to occur: it implies an infinite increase of viscosity: intra- and extracellular compartments are solidified in their liquid conformation. This technique requires more skill because the manipulation of embryos is performed in viscous media (it involves working with CPs concentrations greater than for a SF method). After equilibration, which is only partial in the vitrification procedure, the intracellular concentration of cryoprotectants is lower than in SF method. Vitrification method does not need any specific cooler.
This table summarizes the differences about cell survival conditions between vitrification and slow freezing methods.

Cell survival in LN2 is only possible in the case of intracellular vitreous solidification. This could be obtained either by the SF procedure or by ultra-rapid cooling (vitrification procedure). Vitrification excluded the presence of ice crystals in both intra- and extracellular compartments. On the opposite, SF implies the apparition and the growth of ice crystals in the extracellular compartment, which results in the dehydration of the cell. Growth of ice crystals must be perfectly controlled to avoid cell mechanical damages. Solution effect resulting from ice growth dehydrates the cells and concentrates CPs. At the end of the slow cooling process, intracellular concentration of CPs could reach 10M ! Despite a very long exposition to CPs, the toxic effect is lowered due to a very low T° exposition. In the case of vitrification, cells / embryos are exposed to CPs during a very short duration allowing cell survival. Exposition to CPs induces a rapid osmotic shock when exposed to vitrification solution 1 (VS1). During the cooling process of the SF procedure, the osmotic shock is more progressive but more important.
In the practice, vitrification in contrast to SF, does not need any specific equipment. On the other hand, it requires more practical skill because it implies to manipulate embryos very rapidly and precisely in viscous media. That’s why training is more important. In human practice, because of the long duration of the SF process, embryos from several patients need frequently to be frozen together, increasing the mismatch risk. In the mouse species, vitrification limits the number of embryos to be cryopreserved in the same session. This is why SF is still preferred when more than hundred embryos have to be cryopreserved in the same session.