CASE STUDIES Background to Controlled rate Freezing

Cryopreservation of tissue in recent times started with the freezing of fowl sperm, which in 1949 was cryopreserved for the first time by a team of scientists in the UK led by Dr Christopher Polge. The process moved into the human world in the 1950s with pregnancies obtained after insemination of frozen sperm. However the rapid immersion of the samples in liquid nitrogen did not, for certain of these samples – such as types of embryos, bone marrow and stem cells – produce the necessary viability to make them usable on thawing. Increased understanding of the mechanism of freezing injury to cells emphasised the importance of controlled or slow cooling to obtain maximum survival on thawing of the living cells. A controlled rate cooling process, allowing biological samples to equilibrate to optimal physical parameters osmotically in a cryoprotectant (a form of anti-freeze) before cooling in a predetermined, controlled way proved necessary.

The ability of cryoprotectants, in the early cases glycerol, to protect cells from freezing injury was discovered accidentally. Freezing injury has two aspects – direct damage from the ice crystals and secondary damage caused by the increase in concentration of solutes as progressively more ice is formed. In 1963 Peter Mazur, at Oak Ridge National Laboratory in the USA, showed that lethal intracellular freezing could be avoided if cooling was slow enough to permit sufficient water to leave the cell during progressive freezing of the extracellular fluid. That rate differs between cells of differing size and water permeability: a typical cooling rate around 1°C/minute is appropriate for many mammalian cells after treatment with cryoprotectants such as glycerol or dimethyl sulphoxide, but the rate is not a universal optimum. Controlled-rate cooling machines evolved to help with the process, and there are now tens of thousands of these machines used in laboratories, clinics, and cell and tissue banks around the world to provide the range of cooling rates needed in practice.

An early gas phase cooling machine, built by Pegg, Hayes and Kingston in England in the early 1970s, formed the basis for the first commercial controlled rate freezing machines (Planer Products Ltd, Union Carbide Inc. and Matburn Ltd), and work on human bone marrow cryopreservation was carried out by Dr (now Professor) David Pegg. There are early references to autologous bone marrow transplants in the early 1960s. In the 1970s and 1980s progress was made worldwide in the successful cryopreservation of many types of biological matter that could previously not be frozen, and manufacturers, lead by Planer, supplied various types of controlled-rate freezers to assist. The first calf derived from the transfer of frozen bovine embryos was reported by Dr (now Professor) Ian Wilmut in 1973, and the first pregnancy derived from a frozen human embryo was reported by Allan Trounson & Linda Mohr in 1983 - although the pregnancy aborted spontaneously at about 20 weeks of gestation. A pregnancy derived from a frozen human embryo were reported by Zeilmaker et al. and Zoe Leyland, born from a stored frozen embryo helped by Dr Alan Trounson and Dr Carl Wood and a Planer freezer, was born in Melbourne, Australia on 28 March 1984,. Early haematopoietic stem cell transplants were undertaken in the 1970s, with some bone marrow work being done using a phase cooling machine between 1968 and 1971. In 1985 the first IVF twins were born from frozen embryos in Australia.

World usage data is hard to come by but it was reported (Andersen et al 2005) in a study of Fertility in 23 countries that almost 42,000 frozen human embryo transfers were performed during 2001 in Europe. Estimates give between 300,000 and 500,000

successful births world-wide between the mid-1970s and 2008 from ‘slow frozen’ embryos cryogenically preserved in liquid nitrogen. The rate of usage is rising as Single Embryo Transfer becomes a preferred procedure – and a recent analysis showing that frozen embryos are as likely to be healthy as fresh. The first human egg, as opposed to fertilised embryo, was successfully frozen in a Planer controlled rate machine by Dr Christopher Chen in 1986. Subsequently few births from frozen eggs were reported, although in recent years there has been increased application of this technology using freezing solutions with increased sucrose, based on the work of Raffaella Fabbri (2001).

Other relevant fertility areas include ovarian tissue freezing. In 2010 a former cancer patient gave birth to a second daughter making medical history. Before starting chemotherapy part of her right ovary was removed and frozen. Strips of frozen ovarian tissue were thawed and transplanted and she gave birth in February 2007. The following year she conceived naturally leading Prof CY Andersen, of Copenhagen, to state “As long as the tissue remains properly stored in liquid nitrogen, it could remain functional for 40 years.” The first successful ovarian tissue transplant was made in 2003 by Pro Donnez of Belgium, also using Planer freezers

Away from the fertility side tissue, such as umbilical cord blood is becoming widely stored by controlled rate freezing. Seen as a valuable source of stem cells, the blood contains haematopoietic stem/progenitor cells that are proving useful in clinical applications to reconstitute the system in children and some adults. Reports show that cord blood, containing mesenchymal stem/progenitor cells, have additional uses on their own or in conjunction with the haematopoietic counterparts. To effectively utilise cord blood clinically it must be frozen and banked and the protocols used for this have largely been adapted from those originally designed for the bone marrow haematopoietic stem/progenitor cells. There is no consensus yet on optimal procedures for these cord blood cells, although many cryopreservation strategies suggest using dimethyl sulfoxide (DMSO), slow cooling and rapid thawing.

Hematopoietic stem cell transplantation (HSCT) was pioneered using bone-marrow-derived stem cells by a team at the Fred Hutchinson Cancer Research Center from the 1950s through the 1970s led by E. Donnall Thomas, who showed that bone marrow cells infused intravenously could repopulate the bone marrow and produce new blood cells. Autologous HSCT involved extraction of haematopoietic stem cells from the patient and their storage in a freezer prior to treatment whereafter cells are returned to the body. They should then replace destroyed tissue and resume the patient's normal blood cell production. Autologous HSCT as one of the standard second-line treatments for such diseases such as lymphoma. Allogeneic treatment may be preferred for other conditions and with the availability of stem cell growth factors hematopoietic stem cell transplantation procedures are now often performed using stem cells collected from the peripheral blood, rather than from the bone marrow.

Scientific researchers’ attention is now turning towards the preservation of other, often larger, samples which are currently impossible to successfully freeze and thaw - such as cartilage. Researchers are experimenting with new approaches involving dynamic changes in the rates of cryoprotectant concentration with the lowering of temperature in specially constructed freezing machines. If successful one day even whole organs might be preserved for later transplantation.