FROM FROZEN SEMEN TO THE GROWTH OF BIOTECHNOLOGY IN ANIMAL BREEDING

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About 50 years ago artificial insemination was first adopted in many countries as an important method of animal breeding. This heralded the start of a new era of biotechnology which has now extended into many spheres including embryo transfer, in vitro fertilization and genetic manipulation. All of these have led to major advances in genetic improvement and animal productivity or have a great potential to do so, but artificial insemination still remains the most widely applied technology.

The discovery in 1949 of an effective method for the preservation of spermatozoa by deep freezing led to a revolution in artificial insemination. The first calf from frozen semen was born in 1951 and since then the preservation of bull semen by deep freezing has been very widely applied. The ability to store semen for prolonged periods of time effectively removed the constraints of time and space in animal breeding and led to improved methods of progeny testing and the international exchange of valuable genetic material.

Apart from the practical applications achieved in agriculture the demonstration that spermatozoa could be preserved at very low temperatures by treatment with cryoprotectants such as glycerol and a process of slow cooling led to the opportunity to preserve a wide variety of living cells and tissues of higher animals which otherwise would normally be killed by freezing and thawing.
Important applications in medicine also become possible of which the freezing of red blood cells, bone marrow and cornea are examples.

Early experiments on the freezing of mammalian eggs or embryos were relatively unsuccessful. More dramatic results were achieved, however, in experiments on freezing and thawing of ovarian tissue. Using slices of ovarian tissue treated with media containing glycerol and frozen slowly to low temperatures, orthotopic grafting experiments revealed that fertility could be restored to recipient animals that had previously been sterilized by X-irradiation. This was the first example of the survival of immature oocytes after exposure to very low temperatures.
Nevertheless, it was not until the early 1970's that real progress was made on the deep freezing of mammalian embryos. Survival of mouse embryo was achieved following exposure to a permeating cryoprotective agent and very slow cooling to a temperature as low as —60°C before storage in liquid nitrogen.
An important relationship was also established at this time between the rate of rewarming and the rate of freezing. The first calf born following transplantation of a frozen/thawed embryo was in 1973 and an important step towards achieving this success was the realisation that embryos at the late morula or blastocyst stage of development were more resistant to cooling than were embryos at earlier stages. Today, preservation of embryos by deep freezing is applied quite routinely in cattle embryo transfer and acceptable levels of pregnancy can be achieved.

Over the years a science of cryobiology has emerged and we now know much more about the nature of the damage caused to living cells by freezing and thawing and how it can be avoided. Experiments carried out both on spermatozoa and embryos have played an integral role in the establishment of cryobiological principles. The better understanding of these principles has led to wider opportunities as well as to the development of more recent technologies such as the preservation of cells by vitrification techniques in which the formation of ice crystals is avoided.
Major advances have been made during the last 20 years in techniques for embryo transfer and these are now being applied commercially especially in cattle breeding. Apart from the opportunity for genetic improvement and breed expansion through the greater use of superior female animals, embryo transfer provides a means for distributing the whole animal genome rather than just half the genetic complement as can be achieved with semen. As an advance on frozen semen, therefore, frozen embryos provide the most efficient means of international exchange of animal genetic material. The threat to a reduction in genetic diversity, which in one way is enhanced by modern methods of animal breeding, can at the same time be reduced by the adoption of genetic conversation programmes in which spermatozoa and embryos are preserved in the frozen state.

In contrast to artificial insemination, embryo transfer is still only applied to a very limited extent. New methods are now being developed, however, for the large scale production of embryos by in vitro techniques. These involve the in virto maturation and fertilization of oocytes and the culture of embryos to the blastocyst stage when they can be frozen and distributed for transfer to recipients. Such technologies herald a

major expansion of embryo transfer. The methods for in vitro cattle embryo production are now quite efficient and although early attempts to freeze in vitro produced embryos were not very successful many of these problems have now also been overcome.

The production of embryos by in vitro techniques represents one application of the recent advances that have been made in mammalian embryology. Other opportunities are now also provided such as the multiplication of embryos by nuclear transfer techniques and genetic manipulation by the introduction of foreigh cloned genes.
Many of these new technologies are in their infancy. As they advance it is certain that the ability to freeze and store valuable genetic material in the form of gametes or embryos will play an important role in their future application. It is important, however, that, as with frozen semen, the applications which arise will be applied to enhance the peace and prosperity of mankind.