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Recipient of the 2005 Pioneer Award: Steen M Willadsen, DVM, PhD

Dr Steen Willadsen, born in Copenhagen, Denmark, attended the Royal Veterinary College of Copenhagen. A few years after graduating, he returned again to the Royal Veterinary College to obtain a degree in reproductive physiology. Ever since, Dr Willadsen has made a significant contribution to our science on numerous occasions and in several areas.

His scientific work has been mainly in experimental embryology and embryo transplantation, with an emphasis on innovation and application. The work was originally aimed at developing new effective ways of breeding livestock and this is still one of its main practical objectives. However, since 1992, he has also given to developing techniques and insights of relevance in human assisted reproduction.

Most of the problems he has worked on over the years, and hence the results of the work, have been of wider biological interest than selective breeding of livestock and alleviation of infertility in humans. Over the years he has experimented with oocytes and embryos of a fairly large number of species, including domestic cow, zebu, domestic sheep, domestic goat, horse, donkey, pig, cat, mouse, hamster, rabbit and human.

During the period 1971–1973, he worked primarily on *in vitro* maturation of follicular oocytes in cattle. During the period 1974–1977, at the British Agricultural Research Council's Unit on Reproductive Physiology and Biochemistry, he successfully developed a process for efficiently freezing, storing and then thawing livestock embryos.

The first lambs resulting from transfer of frozen–thawed sheep embryos were produced by him in 1974, and almost immediately afterwards he showed that the method was equally effective for freezing certain stages of cattle embryos. Because one calf had already been produced from a frozen–thawed embryo in 1972, his were not the first. However, the procedure he developed was more effective, and the first frozen livestock embryos to be shipped internationally (from the UK to New Zealand, 1977) in compliance with all rules and regulations were frozen, thawed and transferred by Dr Willadsen. This allowed embryo freezing to be integrated into elite cattle breeding during the late 1970s and early 1980s. Perhaps more importantly, his work uncovered the general principles that govern the survival of mammalian embryos during freezing and thawing, and subsequently, former students and collaborators of his were the first to successfully freeze human embryos, using methods virtually identical to those described by him.

The basic approach currently used in virtually all mammalian embryo freezing work owes much to this work. He has maintained an active interest in gamete and embryo freezing with special reference to human assisted reproduction. He was a co-inventor of a patented procedure for the freezing of single sperm (to be used in extreme cases of oligospermia) and a co-inventor of a patented freezing medium (to be used primarily for oocytes and embryos).

During the period 1978–1983, he worked on various cellular manipulations of embryos, again primarily sheep and cattle embryos. He was the first to produce monozygotic sheep, cattle and horse twins of pre-selected parentage (in 1978, 1979 and 1980, respectively) by blastomere separation ('embryo splitting'). Willadsen attempted to divide the single-celled embryo of a sheep, mimicking Hans Spemann's work with frogs in 1902. He could divide the embryos, but when the individual cells were placed in sheep oviducts to grow, the original embryo division caused the cells to die. Willadsen discovered that a substance called agar could protect the embryos, and succeeded in completing the experiment. Willadsen's discovery of the agar coating technique allowed scientists to perform new types of experiments, because embryos could now be manipulated in drastic ways without the worry of damaging the embryo. Both this technique and his embryo freezing research proved to be instrumental in Willadsen's later successful cloning experiments.

A natural extension of that work dealt with the development of embryos produced by aggregation of cells from two or more parent embryos, so-called chimaeras. Until then, such work could only be done with mouse embryos, as no other species of mammalian embryo could be successfully cultured in the laboratory. Among the most extreme results of his work with chimaeric embryos were animals that were sheep–cow, and sheep–sheep × goat chimaeras. Effective methods for the artificial production of monozygotic twins and chimaeras of pre-selected parentage in a wide range of mammals resulted from these experiments. This work, which also included the development of an extremely effective procedure for culture of mammalian embryos, opened the way for even more radical micromanipulations of embryos in a wide range of mammals. Willadsen wanted his work to be used to increase the populations of endangered species by having closely related common animals serve as surrogate mothers in the births of endangered organisms.

During the period 1984–1990 he worked primarily on the cloning of sheep and cattle embryos by nuclear transplantation. He was the first to undisputedly clone mammals by nuclear transplantation (sheep, 1984; cattle, 1985–1986). Following this success, Willadsen joined Grenada genetics in 1985, and used his technique to clone cattle embryos. At Grenada genetics Willadsen conducted an unpublished

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experiment in which he cloned a cow from a differentiated 1-week-old embryo cell. The donor nuclei were taken from early embryos up to and including blastocysts. He was also able to demonstrate that cloned embryos and frozen embryos could be used as nucleus donors, and he was probably also the first to transfer nuclei between species in a cloning context. The results of the cattle embryo cloning work were immediately implemented in practice with relative success. He later joined Canadian breeding company Alta Genetics who implemented his work.

The work done by Dr Willadsen established the basic method of mammalian embryo cloning by nuclear transplantation, the central elements of which are being used in virtually all mammalian cloning work.

During the period 1991 to the present, he has been working primarily on reconstitution of oocytes, zygotes, gynogenetic and androgenetic embryos in cattle and more recently in mice and humans. Among the objectives of this work is the inducement of sexual reproduction in embryos and asexual reproduction in gametes. However, the field that has been opened is much wider and has enormous scientific and biotechnical potential. New concepts he has developed over the years are relevant to the field of reproductive physiology and more specifically of embryology. Dr Steen M. Willadsen is a worthy recipient of the 2005 IETS Pioneer Award.

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