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Origins of techniques in human and animal embryology

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Abstract

Increasingly innovative and imaginative techniques are being developed to investigate the development of animal and human embryos. Among the types of techniques that have been developed are ones that deal with oocyte maturation and culture, the isolation and utilization of stem cells, cryopreservation of reproductive cells and tissues, and various procedures to manipulate early embryos. To appreciate the derivation of these sophisticated techniques, it seems appropriate to consider the very early origins of these current techniques.
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1. Introduction

As one contemplates the disciplines of human and animal embryology in 2006, it is difficult not to be astounded with the remarkable progress in basic understanding and practical applications that have been achieved over the past three or four decades. This progress depended to a considerable extent upon the derivation of increasingly sophisticated techniques by numerous investigators. Chief among these techniques was the derivation of various methods to transfer embryos, as exemplified by the founding of a scientific organization called the International Embryo Transfer Society.

During the 1960s, emphasis was placed on various methods to superovulate females so as to increase the number of oocytes released and to synchronize their recovery [1], on methods to fertilize oocytes in vitro [2], and on techniques to culture resultant embryos [3]. Coincident with studies of these procedures, other investigations were made to derive procedures to transfer embryos into recipients to study pre- and post-implantation development of embryos and of fetuses, as well as to carry fetuses to term [4]. Yet other experiments were performed to monitor early development of embryos and to manipulate them in various ways. In the 1970s, embryo transfer, especially of domestic animal embryos, became widespread and was rapidly adopted by the cattle industry throughout the world [5,6]. This has resulted in the production of tens of millions of cattle. It was also at this time that the first baby conceived by in vitro fertilization was born, an event that presaged a veritable explosion of various methods to assist human reproduction, so-called assisted reproductive techniques [7]. Since then, literally millions of children have been born as a result of ART. It was also during the 1970s that various methods to manipulate embryos were derived, resulting in the birth of genetically identical individuals of several species as well as of hexaparental animals [8]. Procedures were also developed to cryopreserve embryos [9]. In the 1980s, efficient methods to produce

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embryos and offspring from abattoir-derived oocytes were derived [10]. In addition, it was at this time that practical techniques were invented by which the sex both of spermatozoa [11] and of embryos [12] could be determined, permitting the sex of offspring to be controlled. And it was in the 1990s that nuclear transfer of adult somatic cells was reported [13], an observation that mesmerized the entire world and provoked intense investigations to apply this powerful technique to study basic aspects of early embryological development as well as to attempt to produce multiple genetically identical offspring [14].

The last 10 years have witnessed numerous applications of that technique of nuclear transfer to produce even unusual results. Among the techniques that have contributed to these results are those that form the agenda of the current symposium. In contemplating these various achievements of the past few decades, and in attempting to visualize their future applications, it may help to put them into perspective by briefly examining some of the initial preliminary observations that led to the present results.

2. A very brief synopsis of embryological techniques

One place to begin such an examination is what Lillie [15] referred to as ''the fertilization problem''. In 1897, Walter Heape summarized the origin of attempts to perform artificial insemination (AI) of animals and fertilization of oocytes [16]. Heape ascribed the first successful AI to the Italian abbe, Lazzaro Spallanzani in 1770. Heape then traced the evolution of techniques to perform AI on a variety of mammalian species, including horse, dogs, and humans. It must be noted that it was also Walter Heape who was the first person to describe the successful transfer of mammalian embryos.

In Lillie's description of the history of development of techniques to perform fertilization of oocytes [15], he stated that there was no progress made in understanding the phenomenon of fertilization between 1770 and 1824. Then, Prevost and Dumas conducted a long series of experiments on frogs. They concluded that the sperm penetrated the egg and stated that ''the prolific principle resides in the spermatic animacules''. It was not until 1841 that Lallemand wrote that ''Fertilization is the union of two living parts which mutually complete each other and develop in common''. In the latter half of the nineteenth century, independent studies by O. Hertwig and H. Fol both showed definitive evidence that ''the most important process involved (in fertilization) is the fusion of the two nuclei''. Lillie continues his description of fertilization by noting that for fertilization to occur, both germ cells must undergo maturation, and that there is a specificity between the egg and the sperm. Remarkably, writing only 90 years ago, Lillie concludes by stating that ''It remains for the biology of the future to elucidate the chemical foundations of chromosome behavior and to identify the Mendelian factors in these chemical foundations''. Although the importance of oocyte maturation was recognized long ago, it remains the subject of considerable interest [17].

Another technique that has become increasingly important in studies of human and animal embryology is that of cryobiology, especially the preservation of reproductive cells and tissues. As a curious coincidence, the first formal description of attempts to preserve mammalian cells was also those of Lazzaro Spallanzani in the late eighteenth century. Using a rudimentary microscope, he observed that motile spermatozoa of the horse stopped moving when cooled in snow but resumed their motion when warmed to body temperature. Several years later, having cooled semen to −17 °C, an Italian physician, Mantegazza, speculated that it might be possible to preserve the fertility of soldiers killed on the battlefield by cooling their sperm to very low temperatures.

Although it is difficult to ascribe exact credit for each step in the early derivation of cryopreservation procedures, considerable credit must be given to Hahn, Parkes, Chang, and Shettles, each of whom made important observations of the freezing of spermatozoa in the 1930s to early 1940s. However, the observation most often cited as the foundation of modern cryobiology is that of Polge et al. [18]. Attempting to freeze fowl spermatozoa, these investigators accidentally discovered that glycerol plus albumin protected the sperm against damage when the sperm were cooled to −79 °C. They soon found that glycerol would also protect bovine and human spermatozoa against freezing damage, and that such cryopreserved sperm could be used successfully to inseminate females. The significance of these early experiments on low temperature biology cannot be exaggerated. Millions of cattle and other domestic animals as well as many tens of thousands of women have been successfully impregnated with cryopreserved spermatozoa. These results, in turn, have led to derivation of procedures to cryopreserve gametes, embryos and reproductive tissues of numerous species. Very recent studies have been concerned with cryopreservation of human and animal oocytes and ovarian tissue, as well as investigation of basic aspects of cryobiology [19–22].

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