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Factors influencing the success of embryo transfer in cattle

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Introduction

Embryo transfer is one step in the process of removing one or more embryos from the reproductive tract of a donor female and transferring them to one or more recipient females. Embryos also can be produced in the laboratory via techniques such as in vitro fertilization (IVF) or somatic cell cloning. But the actual transfer of an embryo is only one step in a series of processes that may include some or all of the following: superovulation and insemination of donors, collection of embryos, isolation, evaluation and short-term storage of embryos, micromanipulation and genetic testing of embryos, freezing of embryos and embryo transfer.

Embryo transfer, first successfully accomplished by Walter Heape in 1890, started as a research tool and became a commercial enterprise in cattle in the early 1970s. The development of embryo transfer technology recently was reviewed historically very comprehensively by Betteridge (1). Commercial embryo transfer is now a large, international business.

Superovulation

There have been few improvements in the superovulation of cattle over the last 25 years. Illustrating this, it was recently shown that the average number of embryos recovered from superovulated cattle at Em Tran, Inc. was 4.6 from 248 donors in 1979 and 4.8 from 1485 donors 20 years later in 1999 (7). These data included all donors superovulated, whether or not they came into estrus or were rejected due to no palpable ovarian response at the time of embryo collection. A serious problem is that approximately 20% of donors produce no usable embryos.

In spite of the fact that embryo production per donor has not improved, there have been increases in embryo production per donor on a per unit time basis. This has been made possible largely through the use of intravaginal or subcutaneous progesterone-releasing devices. Superovulation can now be initiated following insertion of a progesterone-releasing device at any time of the estrous cycle. In addition, it has been clearly shown that donors do not benefit from having two estrous cycles between superovulations as was widely formerly believed. Donors are now repeatedly superovulated for a period of 1 to 2 years, every 40 days or less with very satisfactory results (7).

Dominant follicle removal has been shown to increase the number of embryos produced by superovulation in some studies but not others. Increased understanding of the processes of oocyte growth and maturation is essential to improving the efficiency of superovulation (8).

Embryo Recovery

Following the widespread adoption of non-surgical recovery, often referred to as flushing, of embryos in the mid 1980s, the procedures for recovering embryos have received little attention. Virtually all practitioners utilize Foley-type catheters with an inflatable cuff. Most practitioners opt for using a large volume (one to two liters) of flush fluid that is introduced by gravity flow, although opinions seem to be equally divided on uterine body versus horn flushing. For body flushing, the cuff is inflated just anterior to the cervix, allowing the uterine body and both horns to be flushed simultaneously. For each horn to be flushed separately, the catheter is inserted part way up one horn and then the other. Efficacy of embryo recovery appears similar for both approaches. In contrast, some practitioners achieve fine results by introducing a very small volume of medium with a syringe.

Traditionally, Foley catheters were composed of rubber or latex. Recently, several manufacturers have produced silicone catheters specifically designed for recovering embryos from cattle. These catheters have several advantages including the ability to withstand autoclave sterilization, cuffs that maintain a concentric conformation and multiple drainage ports.

Embryo Evaluation and Handling

Initially, the commercial embryo transfer industry primarily utilized simple media such as phosphate buffered saline supplemented with serum for flushing and storage of embryos. A number of companies now offer more complex media specifically designed for embryo transfer, although it remains to be proven that this has led to an improvement in success rates.

Evaluation of embryos is now relatively well standardized for both stage of development and quality based on definitions developed by the International Embryo Transfer Society. Several procedures for predicting the viability of embryos based on metabolism have been described. Currently, however, only microscopic morphology is used for evaluating embryos. Although morphology does not offer predictability on any given embryo, average pregnancy rates relative to embryo quality are highly predictive (6).

Embryo Transfer

Success rates with embryo transfer in many commercial situations are consistently high, often exceeding 70% pregnancy rates. In fact, when high quality fresh embryos are transferred into suitable recipients, pregnancy rates can average nearly 80% (6). Assuming technical competence on the part of the practitioner, the major factors influencing pregnancy rate are probably embryo quality and recipient suitability. Consequently, future increases in pregnancy rates beyond what is currently technically possible will probably be very incremental. For example, any change in technique involving an increase of 5 percentage points in pregnancy rates would be very important. Unfortunately, experimental proof that a specific treatment leads to such a small improvement involves very large number of transfers. Very few

academic institutions or commercial programs can afford to conduct experiments on the scale necessary to provide statistical significance.

Embryo quality is well known as a significant factor in pregnancy rate. However, practitioners have little choice regarding this variable when it comes time to transfer, whereas numerous variables related to recipients provide the opportunity for influencing pregnancy rate. Comparisons between different studies regarding recipient factors are not always legitimate. Recently, however, it was shown that pregnancy rates were similar among beef cows and heifers of *Bos taurus* breeds and dairy heifers (6), while a substantially lower pregnancy rate was achieved using dairy cows as recipients.

It has long been know that the degree of estrus synchrony between embryo and recipient is related to pregnancy rate. Several early studies seemed to indicate that synchrony was more critical in beef recipients than in dairy recipients. However, when beef and dairy recipients were compared in the same study (6), there was no difference in synchrony requirements. Also, it appears that 24 h plus or minus asynchrony between donor and recipient does not compromise pregnancy rate whether fresh or frozen-thawed embryos are transferred (6).

There has been a great deal of effort directed at identifying a hormone (progesterone, hCG, rbST, GnRH) or drug (banamine, clenbuterol) that improves pregnancy rate in embryo transfer recipients. These agents have been utilized in numerous studies directed at improving pregnancy rates in recipients without any clear, consistent improvement being demonstrated. Recently, the use of a low dose (400 IU) of eCG has resulted in improved pregnancy rates in embryo transfer recipients in several field trials.

Embryo Freezing

Largely as the result of pioneering work at Cambridge, embryos freezing of cattle embryos became a dependable and commercially viable tool in the early 1980s. Primarily utilizing glycerol as a cryoprotectant, the only disadvantage of this technology was that a microscope, specific thawing media and a trained embryologist were necessary at the time of thawing. Using ethylene glycol as a cryoprotectant instead of glycerol made possible the direct transfer of embryos directly from the straw in which they were frozen and provided a very significant improvement in the field of embryo transfer in the early 1990s. Pregnancy rates appear to be very similar between embryos frozen in glycerol and ethylene glycol. As a consequence, ethylene glycol is now the predominant cryoprotectant used in most commercial embryo transfer programs. Pregnancy rates resulting from transfer of frozen-thawed embryos are currently only approximately 10 percentage points lower than fresh embryos of similar quality.

IVF

In the early 1990s a number of embryo transfer businesses started offering IVF procedures on a commercial basis. This resulted largely from academic research breakthroughs in defining in vitro maturation conditions, capacitation procedures and temperature requirements for IVF. Commercial production of IVF-derived embryos became highly successful and as a result, many thousands of pregnancies were established, primarily under conditions involving in vitro culture with serum and coculture. Unfortunately, a significant number of pregnancies were characterized by early abortion, calving difficulties, perinatal deaths or calf abnormalities (5).

As a result, the demand for IVF services in North America has declined significantly. However, there is evidence that the use of semi-defined culture systems may result in an improvement in the percentage of normal pregnancies. In fact, it has been suggested that in the future, IVF procedures for embryo production may replace traditional embryo transfer involving superovulation and flushing (2,4).

Embryo Manipulation

The first successes in cloning cattle involved the division of embryos into two half or demi embryos. This technique can be accomplished either with the aid of a micromanipulator or by hand and results in both an overall increase in the number of calves produced from a group of embryos and also in the production of identical twins from some embryos. However, this technology is being utilized less frequently today than during the period when in first became technically feasible in the mid 1980s.

Another utilization of manipulation involves the removal of a few cells from embryos with the use of a micromanipulator and PCR analysis of sex or the presence of certain genotypes. In addition to the need for relatively sophisticated and expensive equipment, this technology requires a high level of skill and consequently has not been widely adopted by the commercial embryo transfer industry.

Cloning of adult cattle by the transfer of somatic cell nuclei is an area that is currently receiving enormous attention in the press and in academic research laboratories. The actual commercialization of cattle cloning is proceeding on a somewhat limited scale (3). In the USA, continued growth of cattle cloning is dependent on a clearly defined policy decision by the Food and Drug Administration.

Biosecurity

There has been little attention focused on the relationship between disease and embryo transfer on a domestic basis within countries. As the international trade in frozen embryos grew rapidly during the 1980s, however, this subject received a good deal of attention and very specific protocols have been developed for the production and handling of embryos destined for movement between countries. These protocols have proven quite effective and there are no indications that any identified microbes have been transported internationally in association with embryos. The protocols in place do not necessarily apply to embryos produced by in vitro procedures and more research is necessary to develop effective sanitary regulations for the production of in vitro, cloned and transgenic embryos (9). In light of recent international outbreaks of foot and mouth and BSE, it is highly likely that the use of media containing no products of animal origin will be mandated for the handling and freezing of all cattle embryos.

Abstract

Embryo transfer in cattle has grown into a mature, international business with high success rates. Future improvements will involve small incremental changes that are difficult to prove experimentally.

Résumé
Le transfert embryonnaire est rendu à un niveau commercial mature et qui atteint des niveaux de succès élevés. Les améliorations que connaîtra cette technologie seront minimes et difficiles à démontrer expérimentalement.

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