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Realizing the promise of IVF in cattle—an overview

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Abstract

The in vitro-produced embryo could play a central role in dairy and beef production systems because of its potential role in genetic selection strategies and crossbreeding schemes, and because it can be integrated into reproductive management strategies for improving pregnancy rates in herds with low fertility. The promise attendant upon use of in vitro-produced embryos is not being fully realized, however. Indeed, there are important technical limitations to their production that reduce the desirability of in vitro-produced embryos because of increased costs, sub-optimal embryonic and fetal survival, and offspring that are occasionally abnormal. Most technical problems associated with in vitro production of embryos can be overcome through research. Among the requirements for a successful research program will be renewed emphasis on conducting embryo transfer trials to determine effects of modifications to embryo production protocols on pregnancy rates and fetal development. Given the promise of in vitro embryo technologies, there is an urgent need for a concerted and sustained investment in research to improve these technologies. Developing a consensus that the study of embryo technology is one of the most important areas of agricultural research should be both an individual and organizational priority.

Keywords: Embryo transfer; In vitro embryo production; Cattle

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1. The promise of the in vitro-produced embryo

The in vitro-produced embryo could play a central role in dairy and beef production systems. Genetic selection and crossbreeding schemes can be optimized through strategies involving use of in vitro-produced embryos. In addition, prospects exist for using in vitro-produced embryos to improve pregnancy rates in herds with low fertility.

Rate of selection for quantitative traits can be increased by exploitation of in vitro embryo technologies to improve the accuracy and intensity of selection and to reduce the generation interval [1]. Reports that embryonic stem cells can differentiate into oocytes [2] and that oocytes can be derived from cells resident in bone marrow and blood [3], mean that future technological advances could make the pool of oocytes from genetically-superior females limitless. The in vitro-produced embryo can also play a major role in genetic selection schemes based on identification of allelic variants of specific genes. Embryos can be screened for inheritance of specific alleles so that genetic selection can be performed before pregnancy is established. Sexed semen is another genetic tool whose use can be amplified through in vitro embryo production systems because sex-sorted sperm can be used to fertilize more oocytes in vitro than would be the case with AI. Nuclear cloning and transgenesis, while currently facing technical, regulatory, and societal limitations, are additional genetic technologies that depend upon in vitro embryo technologies for success.

Crossbreeding has received renewed interest for dairy production systems [4] and Rutledge [5] has made a little-referenced but compelling argument that crossbreeding schemes can be improved through the transfer of F_1 crossbred embryos produced in vitro into F_1 females. Such a strategy eliminates the loss of heterosis and increased phenotypic variation that takes place when F_1 females are bred to either a purebred or crossbred sire.

Production of embryos in vitro also has promise as a method for bypassing infertility caused by ovulation and fertilization failure, early embryonic death and other causes. The historical decline in pregnancy rates of dairy cattle [6,7] makes the search for solutions to infertility compelling. Embryo transfer with vitro-produced embryos has been shown to improve pregnancy rate in lactating dairy cows exposed to heat stress [8], and further improvements in the system for production and transfer of embryos may make it useful for improving fertility in herds where fertility is compromised due to other causes. At the current level of technology, however, embryo transfer does not appear to improve pregnancy rate in lactating cows that are not subjected to heat stress [9]. The in vitro-produced embryo is potentially a more economical source of embryonic material for bypassing early embryonic losses than embryos produced by superovulation because of the low cost associated with embryo production using abattoir-derived oocytes.

2. Current status of in vitro-embryo production in the cattle industry

The promise that in vitro-produced embryos represents for the dairy and beef cattle industries is not matched by its actual use in cattle production systems. For 2003, the last year reported, the International Embryo Transfer Society Data Retrieval Committee recorded 106,220 transfers worldwide of embryos produced in vitro [10]. This is an impressive number and represents an increase from previous years. Nonetheless, it is evident that only a very small percentage of the hundreds of millions of female cattle worldwide receive an embryo produced in vitro. There are many reasons for the poor penetration of in vitro embryo technologies into the cattle industry, including many not directly related to the effectiveness of the technology itself. There are, however, important technical limitations, including increased costs, sub-optimal embryonic and fetal survival and occasionally, abnormal offspring, that reduce the desirability of in vitro-produced embryos. Cloning, which is not the topic of this symposium, has more undesirable consequences, including abortion, congenital abnormalities and postnatal death [11,12]. Moreover, there are regulatory and societal concerns attendant with production of cloned animals and animals containing transgenes [13,14].

3. Scientific advances toward the realization of the promise of IVF

Most technical problems associated with in vitro embryo production can be overcome through research. There are many points in the process of embryo production and transfer that are amenable to manipulation for improvement of success. The specific nature of the limits to embryo production in vitro and possible strategies for overcoming these limits will be expertly addressed by the authors of the papers for this symposium; no effort will be made to recapitulate their thoughts here. By way of introduction, however, a brief outline of some of the technical limits to efficient production and transfer of in vitro-produced embryos is presented. Scientific advances in these areas are likely to make the in vitro-produced embryo a more important component of cattle production systems than is the case at present.

One of the most important aspects of success of in vitro production systems is the starting materials, the ovum and sperm. The importance of the oocyte as a determinant of the resultant embryo's ability to develop is well known. Research is focused on the impact of the oocyte's environment while resident in the ovarian follicle and following its introduction into culture. In addition, there is the more prosaic, but still pressing question of supply. While transvaginal, ultrasound-guided recovery techniques allow for non-invasive recovery of oocytes from genetically-valuable animals, the cost of such oocytes is high. Slaughterhouse oocytes are much less expensive and, at the current level of technology, can often yield a higher percentage of blastocysts [15]. There is the perception that these oocytes come from genetically-inferior females even though Rutledge has shown that the genetic merit of animals sent to slaughter is only slightly less than the average cow in the herd of origin [16]. In the future, the advent of permanent electronic identification of livestock should make it possible to identify females at slaughter, and by cross-reference with production database, to select ovaries from animals with superior genetic ability for production traits.

The source of sperm used in IVF is also important. Sperm function in culture is especially important with sexed sperm because of the reduced performance inherent with this technique [17]. More generally, it may be possible to choose bulls that produce embryos with a higher competence for development to the blastocyst stage or for post-transfer survival. Palma and Sinowatz [18] have shown differences between bulls in the proportion of oocytes that became blastocysts when their sperm was used in IVF. Some differences between bulls may be related to how sperm cells react with capacitation factors in culture media. It is also possible, however, that there are genes transmitted by the bull (and cow) that determine the success of embryonic development. An analog of the preimplantation embryonic development (*ped*) gene identified in the mouse as controlling rate of embryonic development has been identified in cattle [19]. It is likely that there are several polymorphic genes controlling growth, apoptosis, gene expression, and epigenetic processes during development that affect embryonic function in culture, determine embryonic resistance to stress, and affect embryonic survival after transfer into recipients.

The embryo produced in vitro exhibits a host of aberrations in chromosomal content, morphology, gene expression, metabolism and incidence of apoptosis [1,20–22 and papers in this volume]. These abnormalities may result from embryos being derived from dysfunctional oocytes or because of perturbations caused by the culture systems employed. Both causes occur, as indicated by large differences in blastocyst development between embryos derived from different sources of oocytes [23] as well as by evidence that placing in vitro-produced embryos in the oviduct can improve rates of embryonic development [24] and freezability [25]. Elimination of these perturbations through manipulation of oocyte function, improved oocyte selection criteria, improvements in culture techniques, and advances in procedures for selecting embryos for transfer will result in greater commercial demand for in vitro-produced embryo.

As pointed out by Gabor Vajta (personal communication), the development of tools for embryology have not kept pace with the increase in interest in using embryos for agriculture, medicine, and biotechnology. One area in which new technology has been applied to embryo production systems is in the area of cryopreservation. Thus, two strategies for improving survivability of in vitro-produced embryos following cryopreservation exist: to alter the metabolism of the embryo to prevent alterations in cellular physiology that increase susceptibility to cryological damage, and to use physical principles to develop new procedures such as the open-pulled straw method of vitrification to avoid cryological damage.

One research area that should have been included in this symposium, but was not, is the issue of disease transmission with in vitro-produced embryos. A recent review on the potential for viral transmission using in vitro-produced embryos suggests that the risk is low, but not yet completely understood, and that proven methods for elimination of the risk do not yet exist [26].

The recipient itself is a limiting factor in any embryo transfer procedure. McMillan [27] has made a compelling case, based on statistical analysis of data sets where embryo transfer was used to establish potential twin pregnancies, that large differences exist among recipients in their ability to support development of transferred embryos. Thus, improvement of fertility of the recipient is an important area of research. Attainment of this goal will require more basic research into the physiological events responsible for establishment and maintenance of pregnancy. There is much work to be done in this area as it is not even known when embryonic mortality occurs in high-producing dairy cows. Most of the studies on the topic of timing of embryonic mortality predate the large increases in milk yield and the depression in fertility that has been recently identified in dairy cattle populations.

Another important consideration is the need for recipients to be cycling and for embryo transfer technicians to know the timing of the ovulatory cycle. Timed embryo transfer procedures, based on systems used for timed AI, have been developed for both dairy [28] and beef [29] cows to bypass problems of estrus detection and possibly induce cyclicity in anestrous females.

4. Advances in embryo technologies will be more rapid when embryonic potential for development can be assessed more accurately

It has long been a practice of embryo transfer practitioners to identify the embryo best suited for transfer, i.e., the embryo that is most likely to establish a pregnancy and develop normally to term. Advances in the technology of in vitro embryo production also require accurate measures to assess embryo competence for post-transfer development. If we do not know whether an alteration in some aspect of in vitro production procedures improves or reduces embryo competence, we cannot know whether that modification should be implemented. Most assessments of embryo competence, which are usually made on morphological criteria, are inadequate because they do not allow discrimination between embryos with good and excellent morphology that have potential for post-transfer development versus good and excellent embryos that do not. Other measures of in vitro embryo production, such as cleavage rate, blastocyst yield, blastocyst cell number, trophectoderm to inner cell mass ratio, and percent apoptosis, are of uncertain value in the assessment of embryonic competence for post-transfer survival. Certainly, the yield of blastocysts is an important measure when trying to improve the yield of transferable embryos that can be produced, but it may not provide much information about the ability of blastocysts to establish pregnancy. Similarly, there is no direct evidence that blastocyst cell number, ratio of trophectoderm to inner cell mass or percent apoptosis is related to embryonic competence for post-transfer survival.

More experiments where embryo competence is determined by transferring embryos to recipients are needed. At present, very few in vitro-produced embryos are transferred as part of the experimental design. A search of PubMed over an 18-month period (1 January 2004–30 June 2005) revealed only 13 papers in which embryos produced in vitro by IVF or somatic cell nuclear cloning were transferred to recipients. Performing an embryo transfer experiment is not a trivial exercise because of the large numbers of transfers that are required to make meaningful conclusions about treatment effects on pregnancy rates [30]. In parts of the world where large numbers of cows are not available, conducting embryo transfer trials to validate changes in embryo production procedures is not feasible. The difficulty of performing embryo transfer trials increases the importance of developing in vitro markers of embryonic competence.

5. Research to optimize in vitro embryo production technologies deserves more funding

In time, technologies based on the in vitro production of embryos will very likely influence animal breeding strategies to a similar degree as AI. Given this promise, there is an urgent need for a concerted and sustained investment in research to improve technologies for embryo production in vitro to eliminate the inefficiencies and problems that have limited their impact to date, and yet, there exists no large-scale effort by any major research funding organization to provide the amounts of funds required for embryo technology research. There is also a corresponding lack of effort by scientists involved in the field to convince the research funding agencies of the value of embryo research. Developing a consensus that embryo technology research is one of the most important areas of agricultural research should be a priority for scientific organizations and research scientists engaged in the embryo technologies.

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