Farm animal embryo technologies: Achievements and perspectives

Keith J. Betteridge*

Department of Biomedical Sciences, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada N1G 2W1

Abstract

Progress and changes in embryo technology in farm animals are briefly reviewed in terms of how well embryos can be made and used and what the subject has taught us about the maintenance of pregnancy and reproduction in general. Generalizations are made about the need to not accept dogma, how initially complex techniques always become simplified and thereby more applicable, and the need for the support of long-term and basic research. Personal views are offered on how best to prepare and motivate the next generation of scientists in the field, and the need for scientists to engage in the debate of how embryo technologies should be used responsibly in countering global inequalities.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Embryo transfer; Cattle; Sheep; Pigs; Horses

1. Introduction

Embryo technology or embryo transfer (ET) was as fashionable a term 30 years ago as it is today, but the procedures it conjures now were unimaginable then. Despite the enormous changes, however, the embryo itself, its survival of transfer, and the quality of the animal it produces still measure the success of any embryo manipulation. Therefore, progress in embryo technology can be gauged in terms of how well we reproductive biologists can make and use embryos, and how much they have taught us about the maintenance of
pregnancy and reproduction in general. The extraordinary progress and diversification in
what was initially a narrow field should also lead us to consider how well, or poorly, our
education prepared us for coping with rapid change, and how we might improve the
preparation of our successors. Thus the twin themes of progress made and lessons learned
will be explored as a means of giving perspective to the work that we do. Some parts of this
paper have been published in the Proceedings of the 23rd World Buiatrics Congress [1].

2. Progress made

In my opinion, the development and application of ET in cattle has been an excellent
example of how professionals should interact for the good of animal production. The
collegiality of veterinarians, animal scientists, and biologists in the International Embryo
Transfer Society (IETS) is exemplary and has paid important dividends, notably in the
elaboration of internationally accepted procedures for moving embryos around the world.
The history and current status of ET have been reviewed recently [2–4], describing how it
has evolved through “three generations”—the first with embryos derived from donors (in
vivo), the second with embryos produced in vitro, and the third including further in vitro
techniques, notably somatic cell nucleus transfer (NT) and transgenesis.

More than half a million (584,762) bovine embryos were reported to have been
transferred in 2003, 40% of them after freezing and thawing and 18% having been
produced in vitro [5]. North America is still the centre of most activity (45% of the
transfers) with Europe and South America each accounting for 20% of the transfers in
2003. Collection of these data is fraught with difficulties and so they are inevitably subject
to error [5] but the IETS data retrieval committee, chaired by Dr. Michel Thibier, deserves
great credit for assembling them and analyzing regional and technical trends. Regional
differences are striking. For example, 64% of bovine embryos collected in the USA in 2003
were from beef cattle whereas 80% of donor cattle in neighbouring Canada were of dairy
breeds. There is general agreement that a severe limitation to the more widespread use of
ET is the problem of reliably inducing superovulation in selected donors. Transvaginal
ultrasonically guided “Ovum Pick Up” (OPU) at frequent intervals, in combination with in
vitro fertilization (IVF), is proving a more efficient route for producing embryos from
individual donors where facilities and skills permit [6,7]. Embryo sexing is quite widely
practised; in Canada in 2003, almost 10% of embryos transferred were sexed, close to 2000
of them after freezing and thawing [5].

Bovine ET has directly involved large animal veterinary practitioners from the
beginning. However, their participation in routine field ET, which was absolutely essential
when ET first became commercial in the early 1970s and collection and transfer were
performed surgically, has gradually devolved, much as did their role in AI, earlier. It has
been predicted that the devolvement will continue, at least in North American ET practices
[3]. This is perhaps understandable, given that the challenge of putting a new technique to
practical use has long since passed, but it is important to note that the need for veterinary
involvement remains. Certification procedures demand it and, much more importantly, so
does the proper appreciation of the potential of ET in disease control and investigation
[8,9]. A particularly good example of the importance of such an appreciation is the
demonstration of how a non-cytopathic strain of BVD virus can adversely affect the production and manipulation of bovine embryos in vitro—essential components of the most recent reproductive technologies discussed below [8]. However, before embarking on that discussion, let us just remember that the proper transfer of embryos (however skilfully they may have been manipulated in vitro) to properly selected recipients is an inescapable component of most animal biotechnologies and deserves corresponding attention.

Bovine embryo production in vitro (IVP) is now a well-established and reasonably efficient procedure; more than 100,000 embryos produced in this way were transferred in 2003, almost 60% of them in South America [5]. Production in vitro has proved its worth in improving the yield of embryos from designated donors subjected to OPU (see above), salvaging irreplaceable genetics following slaughter in the face of infectious disease control or in culling for other reasons [3,6,7], and producing virtually all the thousands of embryos currently used for scientific research, including efforts to derive embryonic stem cells. The constituent oocyte maturation (IVM) and embryo culture (IVC) techniques are integral parts of the procedures for cloning and facilitating the production of transgenic cattle that produce valuable pharmaceutical proteins in their milk [9]. IVM by intracytoplasmic sperm injection (ICSI), so prominent in human assisted reproduction, is feasible in cattle, even with freeze-dried sperm [10] or cryopreserved oocytes [11], but is not yet widely applied. The fact that IVF embryos cultured in the sheep oviduct survive cryopreservation as well as do embryos collected in vivo [6] gives promise that it will eventually be possible to achieve similar survival of all frozen IVP embryos.

Farmers are canny and conservative consumers, unlikely to continue to use technologies that do not work well in their particular economic circumstances. Consequently, I believe that the data in Fig. 1, showing steady growth in the application of ET to dairy production in Canada at least, prove that ET’s usefulness in this context is firmly established. The continued growth during 2003 and 2004, when the industry suffered enormously from the prohibition of cattle export to the USA because of a single case of BSE in Canada, is added evidence of the resilience of ET’s acceptance. In assessing the contribution of ET to genetic improvement, it is important to note that of 1754 Holstein bulls admitted to the young sire testing program in Canada since the year 2000, more than 80% were produced by ET (Dr. Brian Van Doormaal, Canadian Dairy Network, personal communication, December 2004). Further, the full-sib families which can aid modern methods of selection for traits such as fertility and disease resistance, can only be produced by ET.

Embryo transfer in the other domestic species is less widely used: Thibier’s summary of the most recent data available [5] records 3700 transfers in small ruminants (which is considered an underestimate) and 11,775 in horses (64% of them in the USA and a further 31% in Brazil). Data from pigs have been particularly difficult to obtain but it is estimated that about 20,000 embryos were transferred in 2003 [5]. There has also been ET activity in buffaloes (especially in Vietnam) and in rabbits in Taiwan [5]. Recent reviews of the techniques and applications in these domestic species include those of Squires et al. in horses [12], Cognié et al. in sheep, goats and deer [13], and Hazeleger and Kemp in pigs [14].

By and large, then, ET has been put to good use in agriculture in developed countries and is undoubtedly contributing to the genetic improvement of dairy cattle as far as milk is
concerned, though this has been at the expense of fertility [15]. It is much less encouraging to read of its use to supposedly hasten Armageddon [16], and it is very discouraging that, globally, its impact on lessening the gap between rich and poor must be acknowledged, so far, to be negligible.

3. Lessons learned

Besides being a production tool in animal agriculture, ET has been from the beginning, and remains, an invaluable research tool. The basic principles of this use, together with examples of how it had been applied, were set out many years ago for cattle [17], sheep and goats [18] and pigs [19]. Since then, the value of ET as a research tool has been greatly amplified by advances in micromanipulation, cryopreservation, and molecular biology. Of the many advances that have resulted from using ET for research purposes, two seem to me to stand out for their agricultural and societal implications: our appreciation of (a) the active role of the embryo in maintaining pregnancy and (b) the vulnerability of the embryo to its early environments.

The path to our acceptance of the fact that the mammalian conceptus is an active participant in pregnancy, not a mere passenger in the uterus, I have traced before [20]. The first steps were at the end of pregnancy, with the unravelling of the role of the term fetus in...
its own birth. However, ET (particularly by Rowson and Moor in Cambridge in the 1960s) came very much to the fore in demonstrating the existence in ruminants of a “critical period” for the “recognition of pregnancy”—terms coined at the 1969 symposium on fetal autonomy [21]. It was recognition of the fact that very small embryos can influence very large mothers that led to investigations of active roles for the embryo earlier and earlier in pregnancy including, e.g., the control of its own transport through the oviduct in mares. Today, the idea of interaction with the maternal environment has even been extended back to the follicular oocyte in sheep [22]. It is well to remember that the total acceptance of the importance of signal exchange between mother and conceptus (or even gametes), now widely investigated at the molecular level, owes much to embryo transfer studies. The agricultural implications of research into the dialogue between the embryo and its mother have been wide and diverse; routine methods of estrus synchronization, for example, and all current programs that aim at reducing embryonic mortality, depend upon it.

The way in which we have come to appreciate the vulnerability of the embryo to its early environments is an example of how the failures that have accompanied the development of embryo technologies can be every bit as instructive as the successes. Early embryonic loss and the “Large Offspring Syndrome” [23–25], for example, have plagued IVP and cloning even though they can be largely avoided with appropriate culture systems [6,26]. However, they have also been invaluable in focusing attention on the epigenetic effects of the environment from the earliest stages of development. This, in turn, has made us aware that “insults” to an early embryo—even inadequate maternal nutrition in vivo—can have effects that become manifest very much later (e.g. [27]). Clinicians responsible for the care of recipients carrying fetuses derived from manipulated embryos, especially clones, therefore need to be prepared to encounter losses much later in pregnancy than they normally do. Investigation of these syndromes is bringing together studies of embryology, gene regulation, “hallmark pathologies” [26], physiology, and perinatology—a combination that is surely wide enough to accommodate scientists of many stripes.

Quite apart from the obvious agricultural importance of information on how the environment in utero can affect the outcome of pregnancy and subsequent development throughout life, the societal implications of this understanding are enormous. Medically, such knowledge underlies the hypothesized connection between the uterine environment during pregnancy and chronic diseases of later life [28]. Politically, it provides the biological grounds to reinforce efforts to remove pollutants from the air we breathe and the water we drink.

Looking back over more than 30 years of ET research allows one to arrive at some generalizations about the way the field has developed to date, generalizations which may be of relevance to the future of our subject as well. The first of these is that we must always be ready to rethink established dogmas in reproduction. Thus, early notions that “genotype is everything” (and the recipient relatively unimportant), which have underpinned the application of ET, clearly need to be tempered as our knowledge of epigenetics extends. Such extension may have both positive and negative effects. On the one hand, identification of “intrinsically superior recipients” [23] for transferred embryos should eventually bring benefits to all aspects of bovine reproduction. On the other, the “identical” phenotypes anticipated to result from cloning may exhibit more variation than expected and desired [26].

A second generality is that initially complex techniques become simplified as they are put to use in the field, often in veterinary practice. Collection and transfer procedures
themselves, embryo manipulation, and cryopreservation [29] are examples and cloning may follow [30]. The simpler the technique, the wider its applicability, and the brighter the prospects for using it for animal production in less advanced countries.

My third generalization concerns the financing and motivation of ET research, both of which have undergone radical changes over the past 30 years. This is hardly surprising, of course, given the transformation of simple ET for genetic improvement of livestock into a means of producing valuable pharmaceuticals and other products from the milk and other body fluids of farm animals. This has encouraged the influx of private capital at the same time as public funding has withered in most countries. Further, in many countries, university research has become heavily dependent on “research partnerships” with private industry. For some topics, such partnerships no doubt work well; but it needs to be stressed that the short-term objectives of industry (in themselves perfectly understandable and justified) may be at odds with those of academic research. An example that comes to mind is the investigation of the long-term effects of cloning; creating, maintaining and monitoring herds or flocks for a lifetime would seem an advisable project from the point of view of comparative medicine, but not a profitable one that an industrial company would want to undertake. There remains, in my view, a need for government funded long-term research of this kind, complemented by what Nobel laureate John Polanyi has described as “unshackled” basic research in universities [31,32]. In illustrating the value of “basic” research to an intensely “applied” and topical end, one could not have a better advocate than Mark Schena, one of the founders of the “microarray revolution”, who has described how “Chemistry and biochemistry, computer science and bioinformatics, physics, mathematics, mechanical engineering, material science, and the full gamut of life science disciplines and subdisciplines were joining forces to forge a new biology” and concludes by emphasizing “the value and prudence of funding pure basic research, a lesson that we must not forget as we move increasingly toward application” [33].

Changed motivations for working with embryos have been brought about by the advances that have been made in reproductive and developmental physiology and the molecular tools available for their study. Our discipline leads us to see the whole animal as only one component of the reproductive cycle. Whereas in times past those of us entering the field of farm animal ET did so principally out of interest in the animals themselves, the advent of in vitro manipulation of gametes and embryos, and their study at the molecular level, has shifted the emphasis of ET from the diploid to the haploid half of the cycle. While our comprehension of fertilization and early development has advanced enormously as a result of this shift, we cannot afford to forget that expertise on the whole animal side of the cycle needs to be maintained and fostered. To ensure this, the education and training of the next generation of ET scientists needs consideration.

4. Preparing our successors

Judging from the past, we have little idea of what a conference like this will be discussing in 30 years time. I will not be there but I will forecast that coping with the changes to come will require much flexibility. Consequently, those involved will need to have a broad educational background upon which they can build detailed expertise in
subjects as yet unknown. My biased view is that a veterinary education is second to none as a starting point for those keen to take the embryo technologies forward and to employ them for the public good. For a research career, that education will have to be extended of course, but whether the choice is to explore the wonders of the haploid or diploid generation, a broad appreciation of the indivisibility of the two is a decided advantage. Veterinary practitioners, enjoying respect for their knowledge of animals and their welfare, will play an increasingly important role in influencing public perception of reproductive technologies, potentially a major impediment to their use. As scientists involved in ET and as teachers, we should feel obliged to make good use of our privileged position both directly and through the students we educate.

5. Conclusions

It is the word “societal” in the title of this Symposium that I find especially pertinent. Surely there can be no more over-arching responsibility of biologists today than to explain to societies, and leaders of societies, that the root cause of the environmental problems confronting us is biological: a result of we humans behaving as might be expected of relatively recent descendants of hunter-gatherers. We are “running twenty-first-century software on hardware last updated 50,000 years ago or more” as Ronald Wright [34] has persuasively put it. In the past, the consequent human behaviour has led us into localized “progress traps” such as the clearing of trees off Easter Island and the irreversible salination of irrigated lands by the Sumerians [34]. Today, the price of falling into analogous traps by belligerence and greed would be paid globally. Whether our use of domestic animals in general, and manipulation of their embryos in particular, is more a part of the environmental problem than a part of its potential solution is debatable and we should be active in the debate. It remains certain, though, that the efficient provision of meat, milk, fibre and motive power by domestic animals remains essential; as long as our embryo work helps to improve the efficiency of this provisionment, we can have confidence in its value. Or can we? Why do we strive to increase milk production in the face of “milk lakes”, “butter mountains” and rampant obesity in western countries?

Perspective, like beauty, is in the eye of the beholder and none of us reading this is likely to be finding it difficult to get adequate meat, milk, fibre or motive power. In contrast, it has been pointed out that for the half of the world’s population that lives on less than $2 per day, it would be better to be a European cow that receives $2.20 daily in subsidies from the European community taxpayer [35]. How working with farm animals might change that situation is difficult to see but it does at least equip us with relevant practical knowledge and, I think, the obligation to use it in rational debate and implementation of possible solutions, as we have been encouraged to do at this Symposium.

Acknowledgements

My thanks to: Drs J.I. Raeside, T. Greve and R.O. Waelchli for their helpful comments on the manuscript; Dr. Michel Thibier for providing ET data before their publication;
Holstein Canada for the data in Fig. 1; Dr. Brian Van Doormaal for information about ET-derived AI sires; Dr. J.W. Wilton for genetic advice; and to NSERC and OMAF for support in my research.

References

[16] Wright L. Forcing the end: why do a Pentecostal cattle breeder from Mississippi and an Orthodox rabbi from Jerusalem believe that a red heifer can change the world? New Yorker 1998;(July 20):42–53.


