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New reproductive Technologies in Cattle: A Veterinary Perspective

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Whether practitioner in the field, research worker in the laboratory, or educator of the next generation of veterinarians, delegates to this Congress are more or less bound to be confronted with questions – both technical and ethical – about how the "new reproductive technologies" (NRTs) are advancing cattle breeding, and how we veterinarians assess these developments. In this paper I aim to briefly answer the first question and offer some personal views on the second.

It is safe to say that virtually all people entering the veterinary profession do so as a result of their interest in animals. Later, that interest may become modified into a preoccupation with other organisms or factors affecting animals – parasites, microorganisms and disease processes for example – but the whole animal remains a principal focus of their work. The same used to be true of those of us who chose to specialize in reproduction. However, Figure 1 illustrates the fact that, uniquely, our discipline leads us to see the whole animal as only one component of the reproductive cycle. The advent of in-vitro manipulation of gametes has shifted the emphasis of the NRTs from the diploid to the haploid half of the cycle, and this, I think, can affect the perspective of scientists who use them; the temptation is to share Samuel Butler's view that "A chicken is only an egg's way of making another egg". In my opinion there are dangers in adopting such a narrow viewpoint – dangers that veterinarians are in a privileged position to avoid. A broad review of the NRTs, paying special attention to their implications for the control and investigation of bovine diseases, gives opportunity to explain how and why I think we should.

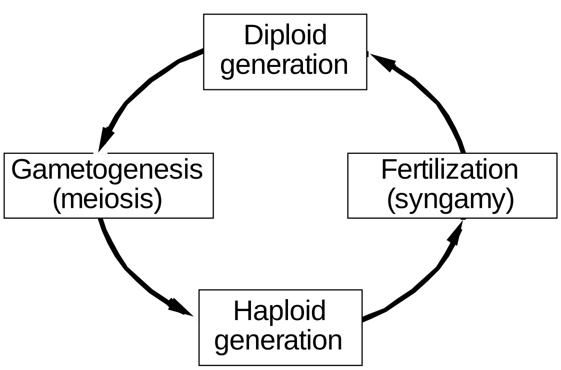

Figure 1. The alternation of generations.

Artificial insemination

The oldest, the most widely used and the most beneficial of all the NRTs, AI is still developing. There are abundant reviews of how AI, in conjunction with intense selection and progeny testing, has had such an enormous effect on improving dairy production (e.g. Vishwanath, 2003; Hansen and Block, 2004) and how the advents of "fixed time" AI (Bo, this

Congress), and of sperm "sexing" (the separation of X- and Y-bearing sperm; Seidel, 2003) are beginning to extend its usefulness even further. It has even been speculated that the major route of transgenes into the cattle population is likely to be, not through transgenic embryos as might be thought, but through transgenic spermatogonia transplanted into the testes of disseminating bulls by refinements of techniques pioneered in the laboratory of Brinster and his colleagues (Kane, 2003). Sometimes forgotten is the crucial role played by AI in all but eliminating so many venereal diseases in cattle (Rasbech, 1993) – a role facilitated by the ability to hold frozen semen pending health checks on bulls. Thus, the reintroduction of the use of fresh semen in the interests of maximizing the number of AI doses per ejaculate (in New Zealand for example) is not without risk. Improving sperm cryopreservation so that the yield of straws per ejaculate is not reduced by freezing could eliminate any such risk.

A concerning corollary of the increase in milk production that has been brought about through AI is the concomitant decline in fertility (Hansen and Block, 2004; Bousquet, this Congress). Manifestations of this confront veterinary practitioners every day and it is they who can bring signs of infertility to the fore. More or less elaborate schemes to counteract the problem, including the use of various NRTs, have been proposed but, in the long run, it may be more profitable to seek remedy through a fuller understanding of the causes of the decline, which can only come from research.

Embryo transfer

In my opinion, the development and application of embryo transfer (ET) in cattle has been an excellent example of how the veterinary profession should interact with sister organizations 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 (Betteridge, 2003; Hasler, 2003 and this Congress), describing how it has evolved through "three generations" – the first with embryos derived from donors (in vivo), the second with embryos produce in vitro, and the third including further in-vitro techniques, notably somatic cell nuclear transfer and transgenesis.

More than half a million (538,312) bovine embryos were reported to have been transferred in 2002, more than half of them (52%) after on-farm freezing and thawing and 15% of them having been produced in vitro (Thibier, 2003). North America is still the centre of most activity (35% of the transfers) but this is static or declining in contrast to South America where ET is expanding and accounted for 22% of the world's transfers in 2002. Europe and Asia each reported about 17% of the total number of transfers in 2002. 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, as will be discussed (Hasler, Mapletoft, Evans) elsewhere in this Congress. Transvaginal ultrasonically guided "Ovum Pick Up" (OPU) at frequent intervals, in combination with in-vitro fertilization (IVF), is proving a more efficient route to producing embryos from individual donors where facilities and skills permit (Galli et al., 2003). Embryo sexing is quite widely practised; in Canada in 2002, almost 3800 sexed embryos were transferred, one-third of them after freezing and thawing (Thibier, 2003).

ET is the NRT that has most directly involved large animal veterinary practitioners. 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 AI did earlier. In North American ET practices at least, it is suggested that the devolvement will continue (Hasler, 2003). 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, for one thing, but, much more importantly, so do the proper appreciation of the potential of ET in disease control and investigation (Stringfellow et al., 2004; Wrathall et al., 2004). 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 NRTs discussed below (Stringfellow et al., 2004). However, before embarking on that discussion, let us just remember that the proper transfer of embryos (however skillfully they may have been manipulated in vitro) to properly selected recipients, is an inescapable component of most animal biotechnologies and deserves corresponding veterinary attention.

Production of embryos in vitro

Bovine embryo production in vitro (IVP) is now a well-established and reasonably efficient procedure. It 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 (Galli et al., 2003; Hasler, 2003), and producing virtually all the thousands of embryos currently used for scientific research, including efforts to produce embryonic stem cells. The constituent oocyte maturation (IVM) and embryo culture (IVC) techniques are integral parts of the procedures for cloning by somatic cell nuclear transfer (NT) and facilitating the production of transgenic cattle that produce valuable pharmaceutical proteins in their milk (Niemann and Kues, 2003). IVF by intracytoplasmic sperm injection (ICSI), so prominent in human assisted reproduction, is feasible in cattle, even with freeze-dried sperm (Keskintepe et al., 2002), 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 (Galli et al., 2003) indicates that it will eventually be possible to achieve similar survival of all frozen IVP embryos. Rather that try to review this immense field even briefly, let us consider four generalities that have emerged from the work and which are, I think, of special veterinary relevance.

The first generality is that the failures that have accompanied the development of IVP are proving every bit as instructive as the successes. Early embryonic loss and the “Large Offspring Syndrome” (Peterson and Lee, 2003; Farin et al., 2001 and this Congress) for example, have plagued IVP and cloning even though they can be largely avoided with appropriate culture systems (Galli et al., 2003; Lee et al., 2004). 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. Bloomfield et al., 2003). 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" (Lee et al., 2004), physiology, and perinatology – a combination that is surely tailor-made for veterinary scientists of many stripes.

The second generality is that research makes us rethink some 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" (Peterson and Lee, 2003) 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 (Lee et al., 2004).

Third in the list of generalities is the truism that initially complex techniques become simplified as they are put to use in the field, often in veterinary practice. ET itself, embryo manipulation and cryopreservation are examples and cloning may follow (Vajta et al., 2003). The simpler the technique, the wider its applicability, making the prospects for NRTs benefiting animal production in less advanced countries a little brighter.

My final generality is that a veterinary education is second to none as a starting point for those keen to take the NRTs to the next stage of newness, whatever that may be. 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. Practitioners, enjoying respect for their knowledge of animals and their welfare, will play an increasingly important role in influencing public perception of the NRTs, potentially a major impediment to their use. As a profession and as teachers, we should feel obliged to make good use of our privileged position both directly and through the students we educate.

Conclusion

The NRTs are already an integral part of the beef and dairy industries and an increasingly important component of pharmaceutical biotechnology. The broad perspective provided by a veterinary education should be especially useful in furthering the development of the techniques, combating problems and envisaging new applications for them.

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Résumé

L'auteur présente en premier un bref sommaire des "nouvelles technologies de la reproduction" qui peuvent toucher la vie buiatrique quotididienne. Il fait ensuite part de la situation privilégiée du médecin vétérinaire avec son champ d'expertise très large. Cette situation offre des avantages particuliers à la profession qui devront être exploités pour le bénéfice de la production animale et ainsi de la société agricole.

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