Possible impact of New Technologies On Dairy Cattle Breeding

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INTRODUCTION

The aim of this paper is to draw together a variety of existing and emerging technologies and to look at how they interact and how they may impact on genetic improvement.

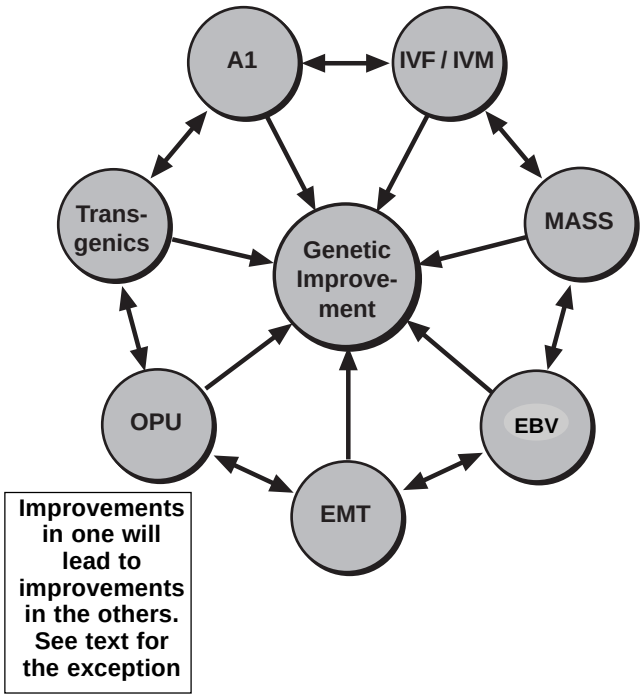


FIGURE 1

AI – Artificial Insemination (e.g. x- and y- sperm separation)
EBV – Estimated Breeding Values (Breeding Worth)
EMT – Embryo Multiplication and Transfer (Embryo cloning)
OPU – Oocyte Pick-Up (Eggs from juvenile heifers)
MAS – Marker Assisted Selection (DNA tests to help select animals)
IVF – In vitro Fertilisation (fertilising eggs in the lab)
IVM – In vitro Maturation (growing embryos and eggs in the lab)
Transgenics – Moving individual genes from one organism to another

ARTIFICIAL INSEMINATION

AI is a mature technology and has changed remarkably little over the last 20 years. Considerable effort has been directed towards the improvement of conception rates but there have been no startling break throughs. Sexed Semen has been talked about for many years but I see little prospect for the wide spread adoption of current technologies such as Fluorescence Activated Cell Sorting (FACS). The concept was investigated in the mid 1970s but with cattle semen the wastage rate is very high and with experimental animals such as the rabbit, we see high rates of abortion and foetal losses. Sperm cells that have been separated into those bearing x- and y- chromosomes could play a part in the lab where only small numbers of sperm cells are needed. Recently individual human eggs have been injected with a single sperm cell as part of the treatment of low male fertility and under these circumstances the wastefulness of the FACS sexing process is not important.

For the last ten years I have heard claims that immunological methods of sexing semen are imminent, but I am not expecting anything to happen suddenly.

ESTIMATED BREEDING VALUES (BREEDING WORTH)

Even with well run AI breeding programs, genetic improvement happens slowly typically at 1% per year. Generally the changes are highly cost effective, partly due to the fact that genetic changes are permanent, unlike fertilisers for example. The other reason why genetic improvement in dairy cattle is cost effective is that the cow expresses her genes each year. Carcass traits in beef animals are expressed only once, when the animal is slaughtered.

There are a number of points in a well run AI breeding program where fine-tuning can improve the rate of progress slightly, say, from 1.0% per year to 1.01%. The methods of estimating breeding values of animals have been evolving over the last 50 years, largely as a consequence of the improving performance : price ratio of computers. These changes in rate of change are usually quite small but can be achieved with relatively little cost. I would be surprised if the effort that has been put into the development of the Breeding Worth calculation does not generate a big payback over the next decade or two.

EMBRYO MULTIPLICATION AND TRANSFER (EMBRYO CLONING)

It has been well known that an embryo can be split into two halves at about 6 days after fertilisation and the two parts transplanted into two recipient cows. This process can result in the production of identical twin- or in my terminology a clone family of two members. Attempts to divide embryos into more than two fragments have not been successful; the survival rate of small fragments is too low. Some Laboratories have had success in producing clone-families with up to fifteen members, using a technique which involves breaking an elite early embryo into its individual cells, and persuading these to develop into complete individuals.

Typically, the process involves fusing these tiny cells with another unfertilised egg from which the genes have been removed. Each of the tiny cells contains a complete

set of genes and these take control to the new embryo. Because each of the tiny cells contains the same set of genes, every calf which develops from the original elite embryo will be genetically identical.

It is important to realise that just because two calves are genetically identical, it does not follow that they will perform identically. Many of the production traits are about 30% heritable. This means that 70% of a variation that we see between animals in the same farm, is due to non-genetic factors. Some important traits such as fertility or mastitis, are less than 10 percent heritable so more than 90% of the variation is due to non-genetic factors. This means that even if every animal in the herd was genetically identical, we would see almost the same amount of variation as we see at present.

The typical process involves taking an embryo at about four days after fertilisation when it is composed of about forty identical cells. Each of these identical cell is fused with an egg taken from the ovaries of a slaughtered cow, and about seven of the forty will develop normally. In the 4 days following fusion, the egg starts by dividing into two cells, each of these cells divides into two more genetically identical cells, so numbers develop from 1 to 2, 2 to 4 and so on to 8,16,32,64 etc.. Each of these seven new embryos can be broken apart into their individual cells and the whole process is repeated again. This process is sometimes called embryo recycling, and although in theory it may become possible to multiply up numbers by a factor of 7 every 4 days, it is not successful at present. The embryos appear normal after a number of rounds of recycling but so far most pregnancies do not seem to develop beyond 60 days.

A similar technique is being tested in which cells are encouraged to develop in 'tissue culture' in the lab and over a period of a week or two, millions of identical cells can be grown. These cells are even smaller than those taken from a 4 day-old embryo and present bigger electrofusion problems. Direct injection of the nuclei (genes) from these tissue-cultured cells into enucleated eggs produces what look like good quality embryos, but these have yet to demonstrate a high probability of developing into a viable calf.

Adult Cloning

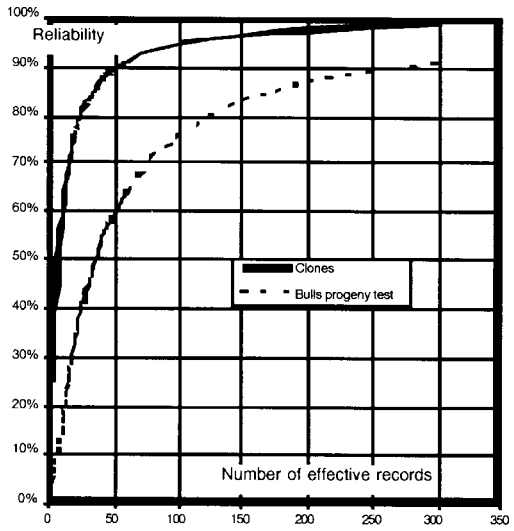
The ability to use tissue cultured cells derived from an adult sheep and to produce a viable lamb (Dolly) caused a rethink about the conventional wisdom. It had been thought that cells of mammals underwent permanent changes during foetal development and that it was only worth attempting cloning with cells from early embryos. Scientifically, Dolly represents a huge breakthrough, but from an agricultural perspective it is far more important that we develop a cheap and efficient system of making large numbers of identical individuals-It is of less importance whether these have been derived from an adult or from an embryo.

How can we use cloning technology in Agriculture

A central concept in making genetic improvements is simple. It involves testing large numbers of animals under commercial conditions and then selecting the best ones for further reproduction. The most important process in dairy improvement involves selecting the top bulls for extensive use through Artificial Insemination, based on the performance of a limited number of recorded daughters. On a Worldwide basis typically we see several thousands of dairy bulls each year, each being tested on the basis of 50 to 150 daughters.

The heritabilities of most conformation or production traits are in the order of 25% to 35% but traits like Fertility or Mastitis Susceptibility are about 5% heritable. Detection of reliable differences between bulls for these lowly heritable traits requires even more daughters. A clone family will also show considerable phenotypic variation so it is important to test the family on the basis of how a number of its members perform under a variety of real farming conditions. Figure 2 shows that for traits with a 25% heritability, clone testing will require fewer numbers of records for the same level of Accuracy.

FIGURE 2: The relationship between Reliability and Assessment and the number of records per bull or per clone family. Notice that it requires roughly four to five times fewer clone records to obtain the same level of Reliability as a Progeny Test.



The primary constraint on a testing program is often thought to be the total number of recorded cows in the herds of co-operating test farmers. To some extent this is true, and it must then be asked: is it better to have a few sires each with many daughters (high accuracy but low selection pressure). The alternative strategy is to have many sires each with correspondingly fewer daughters (low accuracy but high selection pressure).

Theoretically, it is better to test large numbers of families each with small numbers of test records, but this was also shown to be true for bull testing but was rejected partly because farmers generally dislike the associated unreliability of assessments, and partly because of higher bull purchase and maintenance costs. Clone testing of large numbers of families with smaller numbers of test records per family will not be so closely related to in-

creased costs and may become more acceptable. This is because the families would be maintained in liquid nitrogen for the 4 years that are required to assess the performance of the test representatives of each family.

At the conclusion of the first batch of testing, the best clone families would be thawed and replicated for use on a large scale by farmers. Perhaps in the early stages the top 5 out of 100 families might be selected for extensive reproduction.

Dollars and Family size

Once the best clone families have been identified, using limited numbers of representatives, there follows the widespread release of the best families. Small family size at this stage would be a serious problem in that it would prevent companies from recouping their investment in the family-testing phase. A potential family size of about 100,000 would be large enough to encourage the thorough testing of each family before the decision was made whether or not to release it for widespread use. Family sizes of a million would be too large if they were all located in Australia or New Zealand: there would be a danger of lack of diversity. So an intermediate clone family size seems desirable.

It is essential to be able to suspend a family line while some representatives are undergoing a thorough testing program. This now seems entirely feasible for example the team at Monash University, led by Alan Trounson, has demonstrated the viability of frozen cloned embryos.

If cloning became practicable using nuclei derived from cell cultures, then limited family size, and storage of frozen material should cease to be a problem.

If cloned embryos cost $30, and had a 50% pregnancy rate, it seems very likely that they would be attractive to most dairy farmers. Cloned replacement females could be F1s, for example Friesian X Jersey. Cows not needed for breeding replacements could be used to carry cloned embryos for other purposes.

At $300 per embryo, farmers might be more inclined to use cloned females to breed replacement cows. In this situation, there would be little advantage in producing F1 clones. At this price it would still be economical to breed beef bulls.

At $3000 per embryo, then there would be relatively limited scope for cloning. If it were possible to produce adult cloned embryos for $3000, then there would be a number of niche markets, particularly for male or females clones from show winners.

Farmers have become accustomed to the 50% (approximately) chance of getting a calf from a particular mating. If a cloned embryo had a lower chance of producing a calf then it would be possible to circumvent low conception rates by transferring several embryos into a recipient cow at one time. Of course this would increase the cost per calf born, and some twins might be produced which many dairy farmers do not currently favour.

Impact on genetic improvement programs

If the use of cheap cloned embryos became accepted in the dairy industry, it would become more difficult to keep the present progeny testing systems running for dairy bulls. It seems likely that this would also be happening in most countries around the world at the same time. As a result, continued genetic improvement would cease unless there was some sort of nucleus breeding program that incorporated the best genetics from around the world. These programs would be at a genetic disadvantage relative to the commercial cloned animals. The sires used would probably not be at the same genetic standard as the females. In addition, much of the superiority of the cloned females would be due to non-additive gene effects that would be lost in the next generation.

If an elite, tested adult bull could be cloned for, say, $2000, there would be a market for such bulls in both the dairy and beef industries, for use as natural service bulls.

Impact on production systems

The ability to clone dairy cows cheaply, and with satisfactory conception rates, would allow dairy farmers to breed sufficient replacement cows with a relatively small proportion of their herd. The remaining cows could be used to carry embryos of a different breed. For example complementary beef breeds might be used to produce cloned F1 females with good maternal traits. Alternately male cloned embryos could be used to breed terminal sires for use as beef bulls in commercial beef herds.

Cloning from Adult, rather than foetal cells

The ability to clone 'performance-tested adult animals' would be an advantage, but the ability to produce essentially unlimited numbers of cheap cloned embryos would be of greater importance to agriculture. Ideally both technologies would become commercially viable. However, suppose it was possible to create just one viable embryo from an adult animal (with considerable effort) then this could perhaps be used as the 'donor' embryo for a cheaper embryo/foetal-based multiplication technology. The ability to replicate an outstanding bull after his superiority had been recognised would be of particular importance to the beef industry. Replication of outstanding adult dairy cows could be of some advantage, but the lower accuracy with which such females can be identified would limit the attractiveness of this procedure. No doubt some cloned families derived from outstanding cows would be tested as described earlier; if they were good enough, then the clone family would be made widely available.

The establishment of Gene Banks in a variety of countries should be done now. Farmers should not be expected to be the custodians of living museums. Both gametes and somatic tissue cultures should be considered.

The simultaneous use of a number of elite families in each of the many production environments will ensure sufficient diversity. In addition, if cloning were cheap enough, we would expect to see F1 clones being adopted for dairy production. Far from leading to inbreeding problems, dairy cows would be more heterozygous than they have ever been.

Reproduction without the need for males

If embryologists can find a way of taking eggs from two different cloned female families and 'combining'

them to produce viable female embryos, it would greatly enhance our ability to sustain genetic improvement in the absence of conventional progeny testing programs. Conventional wisdom suggests that genetic imprinting makes this impossible, but conventional wisdom can change.

Impact of public perception

We have already seen the rejection of some new technologies (e.g. BST in Europe). There is a distinct nervousness in Japan concerning the use of transgenic plants. It is not difficult to imagine a situation where dairy products made from milk from transgenic cattle was not acceptable in certain markets. It would be very easy for technologies such as cloning to become confused with transgenics, both in the media, and in the mind of the public. In a species such as cattle where it takes many years to replace the national herd, farmers would be reluctant to do anything that would cause major markets to become inaccessible.

OOCYTE PICK-UP (EGGS FROM JUVENILE HEIFERS)

Oocyte Pick-Up (OPU) is unlikely to become used on a large scale to produce embryos for use by commercial farmers. However the technique could improve the effectiveness of other important technologies:-

- In conjunction with marker assisted selection, there is a real prospect for a dramatic acceleration in the rate of genetic gain; this is particularly true if oocytes can be extracted from mid-term foetuses.
- OPU could reduce the time required to test transgenic individuals.
- OPU offers AB centres the opportunity to use an outstanding new sire more rapidly than is possible with conventional breeding or embryo transfer (ET). The attractiveness of this will vary from year to year and breed to breed. While OPU is of borderline attractiveness with current success rates, it is reasonable to anticipate much higher efficiencies and hence lower costs
- OPU offers the prospect of rapidly multiplying up the number of offspring from a rare transgenic individual.
- OPU is being used in Holland to produce additional embryos from females born in their Nucleus Breeding Program (Delta). These embryos are sold to dairy farmers and have the added benefit of providing additional progeny data for females in the Delta Program.

If young calves could be screened cheaply (but with limited accuracy) using genetic markers, then it would become attractive to select them at, or before birth.

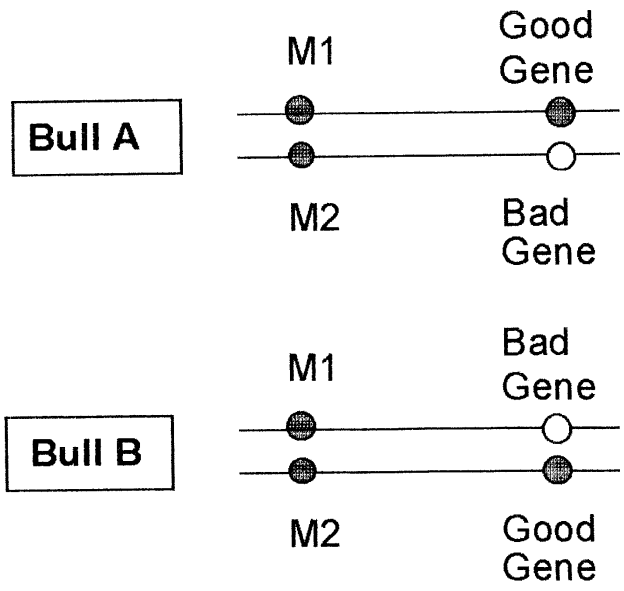
To really speed up the generation interval it would be desirable to harvest sperm from foetuses that had been screened on the basis of DNA markers. These sperm could be used to fertilise oocytes collected from foetal donors.

MARKER ASSISTED SELECTION (DNA TESTS TO HELP SELECT ANIMALS)

In the past it was assumed that traits like milk production were controlled by many genes each having a small effect. However if there are a handful of genes which have a larger effect then it would be useful to know a young bull's genetic make-up for these particular genes and to select on an index which combined pedigree information with this genetic knowledge. At present we are at a relatively early stage in our understanding, and know little about which variants of which genes are desirable. Instead, various labs have been looking for easily detectable regions of genetic variation (the marker) which it is hoped will lie close enough to the unknown genes of real importance (the Quantitative Trait Locus-QTL). There are two problems with this approach

1. Unless the marker region is very close to the QTL, there is a chance that the two regions may become uncoupled by 'crossing-over'.
2. It is necessary to know the desirable marker genes for each family separately.

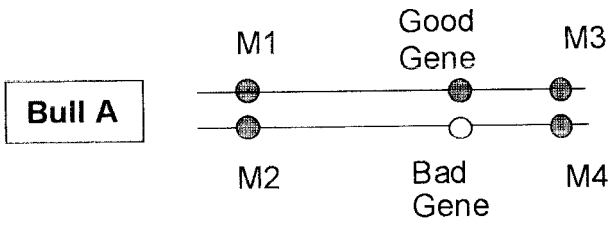
FIGURE 3



It would be a mistake to select sons of Bull B with the M1 Marker, but it would be the right thing to select for in the case of Bull A's sons. This mixing-up (crossing-over) happens during the formation of sperm and eggs and happens once or twice per chromosome. Clearly, the chance that this will take place is much smaller when the marker and the desirable gene are physically close to each other (closely linked). If there is really close linkage, it is reasonable to assume that sons of Bull A that have inherited the M1 marker from him, will be more desirable than those that have the M2

If we expect Bull A to normally transmit the M1 - M3 combination, but find a son with the M1 - M4 combina-

FIGURE 4



tion, we would not be sure whether the son had inherited the "Good Gene": it would depend where the cross-over(s) had occurred.

This process where marker genes which, in themselves have no value, but which point to the presence of desirable genes, is called Marker Assisted Selection. Many AI companies (including LIC) are interested in this technology as an aid in the selection of young bulls. The cost of testing would have to come down substantially before commercial dairy farmers would want to screen their heifer calf drop.

IN VITRO FERTILISATION (FERTILISING EGGS IN THE LAB)

It is essential to be able to fertilise eggs in the lab if one wants to harvest eggs from young heifers. It is possible to harvest eggs from a females even in the first two months of pregnancy. This technique is particularly useful if one is running an MOET Nucleus Breeding Program, because it is usually difficult to obtain enough eggs from each cow using conventional methods.

IN VITRO MATURATION (GROWING EMBRYOS AND EGGS IN THE LAB)

The ability to mature embryos in the lab is fundamental to any work involving cloning or embryo development. It is likely that companies will become more cautious about revealing the composition of cell-culture media to potential competitors.

TRANSGENICS (MOVING INDIVIDUAL GENES FROM ONE ORGANISM TO ANOTHER)

The term transgenics refers to the situation when some genetic material is transferred from one organism to another. In the case of plants, the gene that is transferred is often taken from a bacterium but in animals, it is more likely to be an animal gene. Recently, we have started to see transgenic animals being produced, mainly by companies who are interested in creating pharmaceuticals in milk. Generally speaking, pharmaceuticals are required in relatively small quantities, so large numbers of animals are not required. It is important to distinguish between the use of transgenics for the production of pharmaceuticals, as opposed to its uses in agriculture. In general, the financial rewards from the production of pharmaceuticals are vastly greater than we can expect from agricultural uses.

It seems likely that transgenic cattle will be produced in moderate numbers to make new dairy-based products for specialist markets, for example , infant feeding formulas (nutriceuticals).

Public perception must be taken into account when planning research in this area. It seems likely to me that the pharmaceutical uses of transgenics will be acceptable. However, it remains to be seen whether genetically modified milk would be acceptable to the general public.

The Australian Dairy Research Corporation is funding a research program which aims to incorporate additional copies of the casein gene complex into dairy cattle. The aim would be to increase the percentage of protein in the milk. In this situation the "foreign" gene is actually just a normal bovine gene, but additional copies of it would be present, and so we would hope for a higher level of expression.

It is not clear whether companies who invest in the creation of transgenic bulls will be able to recoup their costs. Consider the situation where a transgenic bull is being used. The price of his semen will largely determine the financial rewards going back to the owner of the bull. I rather doubt whether dairy farmers would accept a bull, if there was a legal requirement that a royalty be paid on any grand progeny which he wished to retain for herd replacement purposes. Initially such bulls would probably only transmit the transgenes to half his offspring so only a quarter of his grand progeny would carry the transgene.

CONCLUSION

There is a bewildering array of new tools which are on the verge of becoming available for use in cattle breeding. Claims were made by the biotechnologists in the early eighties, that animal breeding as we know it would soon be a thing of the past. It seems more realistic that the new technologies will simply add to the tool-kit rather than replacing it altogether.