The future impact of new opportunities in reproductive physiology and molecular biology on genetic improvement programmes.

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ABSTRACT

The possible impact of developments in artificial insemination (AI), multiple ovulation and embryo transfer (MOET), sexed semen and embryos, cloning, physiological markers, genetic markers and transgenic livestock on rates of genetic change are reviewed and discussed. Previous theoretical predictions have exaggerated the potential increases in rates of genetic change available from MOET, particularly in small, closed MOET nucleus schemes. Sexing of semen and/or embryos has a relatively small effect on rates of genetic change, but sex control can potentially have a dramatic effect on the efficiency of farming systems. Developing techniques to produce large clone families in livestock will increase rates of genetic change, particularly through faster dissemination of superior genotypes to commercial populations. A cost-benefit analysis of some different strategies for increasing genetic change in the New Zealand dairy cattle population has identified that useful contributions could come from AI (by doubling current bull coverage) and from MOET and genetic and/or physiological markers. Equally important economic returns were identified from increasing the number of bull mothers continuously bred by AI.

The impact of transgenic animals, gene mapping and genetic markers (linkage) on rates of genetic change is likely to take much longer (10-20 years) than the reproductive techniques.

Keywords Genetic gain, artificial insemination, multiple ovulation and embryo transfer, cloning, genetic markers, transgenics.

INTRODUCTION

This paper reviews some of the recent developments in reproductive physiology and molecular biology and assesses their likely impact on animal breeding theory and genetic improvement programmes. Where pertinent the impact of these new opportunities is assessed relevant to current genetic improvement programmes and possibilities that exist for modifications to present systems.

REPRODUCTIVE PHYSIOLOGY

The current state of the art in animal reproduction and embryology has been reviewed recently by Woolliams and Wilmut (1989), Macmillan and Tervit (1990) and Tervit *et al.* (1990).

Artificial Insemination

Artificial insemination (AI) with fresh or frozen semen is a widely used technology in dairy cattle populations all around the world. Coupled with progeny testing and intensive sire selection it has led to genetic improvement in milk production (yield and composition) in many dairy cattle populations over the last 30 years (Foote, 1981; Van Vleck, 1981). In addition to its role in genetic improvement, AI also permits control of certain diseases, especially venereal diseases, and the reduction in frequency of recessive lethal genes (Foote, 1981).

AI in most dairy cattle improvement programmes uses frozen semen with large amounts of sperm (20 to 25 x 10^6/insemination) to maximise conception rates (Foote, 1981). Innovative research by the New Zealand Dairy Board has shown that fresh semen diluted in Caprogen and stored at ambient temperatures can be used at very high dilution rates (2 x 10^6/insemination) without loss of fertility (Shannon, 1968). This has led to much wider use of proven bulls over a short mating period (about 3 months), as is required for the seasonal production pattern in New Zealand. This has resulted in 3 times the coverage from top proven sires in New Zealand than in other countries (Shannon, 1989), with

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desirable implications for genetic improvement programmes (Stichbury, 1968). The rate of genetic progress could be further increased by inseminating dairy heifers with progeny tested sires. Currently only 13% of the dairy heifers in New Zealand are inseminated (Macmillan and Tervit, 1990). Recent research (Shannon, 1989) suggests that further increasing the shelf life of fresh semen and reducing the dose rate of frozen semen could double current bull output.

AI in other livestock 'species' (e.g. beef cattle, sheep, pigs, goats, etc) is technically feasible (Foote, 1981), but much less common than in dairy cattle (Macmillan and Tervit, 1990). Progeny testing and AI as an aid to selection is most useful for sex-limited traits, or lowly heritable traits. Limited AI over small numbers of cows in a herd, linked with a reference sire progeny testing programme and on-farm performance testing, can increase genetic improvement for growth in beef cattle by 30-40% (Willham, 1979; Morris *et al.*, 1980; Parnell *et al.*, 1986) and is being successfully implemented in North America (Koch *et al.*, 1986).

AI in sheep is becoming more common in a number of countries, including France, Ireland, Norway, Sweden, Australia and New Zealand (Baker and Parratt, 1988). In some cases (e.g. Australia and Ireland), AI is mainly being used to disseminate highly regarded breeds and/or sires more widely. In Norway, Sweden, France and New Zealand, reference sire programmes have been implemented to improve rates of genetic progress as well as disseminate semen from proven sires. A recent workshop has summarised the current state of sheep sire reference schemes and across-flock genetic evaluation in New Zealand (Garrick, 1989). About 2% of performance recorded ewes were inseminated in 1989 (Macmillan and Tervit, 1990). Theoretical predictions by Blair (1989) indicate that sire reference schemes in sheep could increase rates of genetic progress per year by 4-25% for moderately heritable traits (e.g. fleece weight) and 20-40% for lowly heritable traits (e.g. number of lambs born). A substantial proportion of the ewes must be mated to the best proven sires to get the highest rates of genetic improvement. Genotype-environment interactions can be a major hindrance to effective across-flock/herd breeding value estimation (Wickham, 1989). A long-term experiment at Massey University indicates that there are large sire by environment (years and/or stocking rate) interactions for live weights in sheep but wool traits are not greatly affected (Newman, 1988). Sire by environment interactions have also been investigated in beef cattle but do not appear to be of major significance to the effective implementation of sire reference schemes for growth (Parnell *et al.*, 1986).

More widespread dissemination of genetic improvement from proven sires in sheep and beef cattle is limited because of problems with animal handling, additional labour requirement, sometimes high levels of anoestrus (beef cattle) and lack of reliable oestrous synchronisation techniques (Macmillan and Tervit, 1990). In addition in sheep there is considerable scope for improving semen technology (e.g. dose rates, storage time, etc) and insemination techniques and success rates for both fresh and frozen semen.

Multiple Ovulation and Embryo Transfer (MOET)

MOET is a composite technology and includes superovulation, fertilisation, embryo recovery, short-term *in vitro* culture of embryos, embryo transfer and/or embryo freezing. There is still considerable scope for improvement and appropriate research on all these component parts (Macmillan and Tervit, 1990; Tervit *et al.*, 1990).

Benefits from current MOET technology include obtaining more offspring from valuable females; obtaining offspring from infertile females; exporting or importing animals as fresh or frozen embryos; testing for mendelian recessive traits; introducing new genetic material into specific pathogen-free farms; increasing the population base of rare or endangered breeds or species; and as a research tool, especially for distinguishing effects of the uterus from those of the embryo (Seidel and Seidel, 1981). Other applications of MOET which could be realised with further technological developments and associated reduced costs are: twinning (transfer of one embryo to the contralateral horn of single-pregnant cows or transfer of two embryos to unmated recipients), especially in cattle; obtaining progeny from prepubertal females and progeny testing females. Twinning rates in pregnant recipients of more than 70% have been achieved (Anderson *et al.*, 1979).

During the 1970s a multi-million dollar embryo transfer industry developed in North America resulting

in about 17 000 bovine pregnancies being recorded in 1979 (Seidel, 1981). This activity was mainly to increase the reproductive rate of 'valuable' cows or to multiply-up new imported breeds and was largely unrelated to genetic impovement. As pregnancy rates from MOET improved, the potential benefits of MOET for genetic improvement began to be evaluated both theoretically and more recently in practice.

Currently, the average number of live progeny born per donor flushed is still quite low (2-3) for sheep or cattle and somewhat higher (6-8) in goats (Macmillan and Tervit, 1990). Higher numbers of progeny (6-10) can be achieved in sheep and cattle with multiple flushing (superovulations) of donors (Tervit, pers comm.). Increased numbers of transferable embryos per flush may also be attainable with improved hormonal products (Dieleman *et al.*, 1989). While these results are an improvement on those in the past, the full potential of the MOET technology remains to be developed and exploited. The developments in this field, including *in vitro* oocyte maturation and *in vitro* fertilisation, have been reviewed by Woolliams and Wilmut (1989).

MOET in Progeny Testing Schemes

The possibility that cows can produce more than the usual one offspring per year can affect genetic progress in a number of different ways. Initially, methods of incorporating MOET into conventional dairy cattle progeny test schemes were investigated and have been reviewed by Ruane (1988). The three main ways of doing this are through modifying selection intensities on the cows-to-breed-sons pathway and cows-to-breed-daughters pathway and through increases in selection accuracies. While some improvements in rates of genetic progress are possible, usually these are relatively small (5-20%) when compared with genetic progress possible in an efficient progeny testing scheme (Ruane, 1988). These advantages are much less or negative when the costs of MOET are taken into account (Van Vleck, 1981).

Van Vleck (1981) also investigated the value of MOET and sexed semen, separately and jointly, and predicted genetic progress could be increased by up to 60% (Table 1). However, his results assumed widespread use of MOET in all breeding herds which at current costs of embryo transfer is probably uneconomic. In addition, the effects of inbreeding were not considered and as will be shown later this can be of critical importance, particularly when very high (1-5%) selection intensities are being applied.

MOET Nucleus Schemes

MOET nucleus schemes involve creating a nucleus herd of elite males and females, concentrating testing and selection in the herd, selecting at an early age using

TABLE 1 Possible effects of embryo transfer and sexing semen on genetic gain for milk yield in a conventional progeny testing programme (adapted from Van Vleck, 1981)

Generation interval Path[a]	(yr)	Accuracy	Percent selected: Normal AI	Sexed Semen	Embryo Transfer	Both	Both (few bulls)
SS	6.5	0.79	4	4	4	4	2
SD	7.5	0.79	20	20	20	20	2
DS	6.0	0.65	6	3	1	0.5	0.5
DD	4.0	0.65	90	45	10	5	5
Genetic gain/year[b]		0.09	0.10	0.12	0.13	0.15	

[a] SS = Sire-son; SD = Sire-daughter; DS = dam-son; DD = Dam-daughter

[b] Phenotypic standard deviation units.

family information and then using MOET on a small number of female stock. The increased genetic response in these schemes is largely due to reducing the generation interval while tolerating less accurate selection. These schemes require a much smaller number of recorded dairy cattle than in conventional national breeding programmes, and could replace the need for expensive progeny testing.

Land and Hill (1975) were the first to investigate this approach using beef cattle. The generation interval was reduced to 2 years and it was shown that the rate of genetic progress for 400-day weight could be doubled compared with conventional, within-herd, performance test programmes (i.e. not considering any AI or progeny testing).

Nicholas (1979) was the first to examine the impact of MOET nucleus schemes on dairy cattle improvement. This was further expanded by Nicholas and Smith (1983) who considered the impact of two MOET schemes and embryo splitting (i.e. production of small clones). The juvenile scheme involved embryo transfer from 1-year-old females, with males and females selected at this age on the dam's first-lactation record and information available at that time from the dam's relatives. This results in a generation interval of 1.8 years. In the adult scheme, embryo transfer takes place at the end of the first lactation, with selection at this time based on first lactation records plus full-sib, half-sib and dam's records. This results in a generation interval of 3.7 years and both schemes have generation intervals considerably less than the average of 6 to 7 years in conventional progeny test programmes.

Response to selection in these two MOET schemes was evaluated in terms of both annual genetic change and inbreeding rate, with varying numbers of donors per male and progeny per donor. While the juvenile scheme gave a greater response rate than the adult scheme it also led to a much higher inbreeding rate (now known to be underestimated), but both had higher annual genetic gains (by 30-80%) compared with conventional selection (Table 2). Nicholas and Smith outlined what they considered a feasible adult MOET scheme with current technology, involving 512 females milk-recorded per year and 1024 embryo transfers from 64 donors (16 embryos per donor). This was predicted to sustain annual genetic gains about 30% greater than conventional national progeny-testing, and at a tolerable rate of inbreeding. Some interesting advantages of such a scheme are apparent (Table 3). Woolliams and Smith (1988) made some corrections to the base rate of genetic change possible in progeny test schemes (0.104 now 0.133 phenotypic standard deviation per year). They then concluded that efficient field progeny testing schemes could be competitive with adult MOET nucleus schemes. They also modified the juvenile scheme to include information on the sire's family to add to the accuracy of selection which increased the genetic responses shown in Table 2 by 25-35%.

TABLE 2 Annual genetic change (in phenotypic standard deviations) and inbreeding rate (percent) for two MOET nucleus schemes (from Nicholas and Smith, 1983)

	8 donors per male		16 donors per male	
Response	8[a]	16	8	16
Genetic change[b]				
Juvenile scheme	0.14	0.16	0.15	0.18
Adult scheme	0.13	0.15	0.14	0.17
Inbreeding				
Juvenile scheme	0.72	1.91	1.91	2.97
Adult scheme	0.13	0.27	0.25	0.81

[a] Progeny per donor

[b] Compared with annual genetic change of 0.10 theoretically possible in conventional dairy cattle progeny test programmes and 0.05-0.07 actually being achieved.

The publications by Nicholas (1979) and Nicholas and Smith (1983) generated much attention and interest around the world, resulting in additional theoretical papers on the subject as well as implementation of a number of MOET nucleus schemes starting in 1984.

On the theoretical front the idea of using MOET nucleus herds to produce young dairy bulls for progeny testing as first suggested by Nicholas (1979) has been further developed by Christensen (1984), Colleau (1985, 1986, 1989) and Christensen and Liboriussen (1986). These have been called MOET hybrid schemes and have been shown to give useful increases in rates of genetic gain (20-80%) while annual rates of inbreeding are much lower than in juvenile schemes due to longer

generation intervals.

TABLE 3 Some advantages and disadvantages of MOET nucleus schemes (Nicholas and Smith, 1983)

ADVANTAGES	
1.	Higher genetic response
2.	Bull performance test (e.g. growth rate, feed efficiency, etc)
3.	Better control of the breeding programme
	- better recording
	- higher genetic parameters in a single herd
	- selection on optimum index (using all relative information)
	- selection on other criteria (e.g. efficiency of milk production)
4.	Possibility of selecting on 2-3 lactation records (minimal culling)
5.	Benefits recouped sooner
6.	Lower national cost
7.	Provision of young sires for AI proofs and use
8.	Quicker utilisation of new technology (e.g. cloning)
DISADVANTAGES	
1.	Availability of technology
2.	Cost of implementation
3.	Disease risk to a single nucleus

While most emphasis has been on theoretical rates of genetic response of MOET nucleus schemes in dairy breeding programmes, very similar results have been found for beef cattle (Land and Hill, 1975; Gearheart *et al.*, 1989) and sheep (Smith, 1986; Colleau and Elsen, 1988; Toro *et al.*, 1988). The value of MOET in pig improvement is likely to be small (Smith, 1981) because the pig has a high reproductive rate and short generation interval. While the principles of applying MOET in beef cattle and sheep are similar to dairy cattle, there are some important differences. In particular, except for reproductive traits, the males express the traits under selection (e.g. growth or wool) so selection accuracy on the male side can be increased. The success of MOET nucleus schemes in sheep investigated by Smith (1986) was dependent on good embryo transfer rates at 6-8 months of age. This is not the case currently (Quirke and Hanrahan, 1977; Rangel-Santos *et al.*, 1990) and there is need for further research and development. Once moderate rates of embryo transfer (5 progeny per donor) are achieved in ewe lambs then gains of 50-70% in genetic response are predicted (Smith, 1986), relative to within-flock rates. It is also suggested that in a closed selection flock a minimum of 100-200 donor ewes (1000 progeny) is required to limit the rate of inbreeding to acceptable levels (0.5% per year).

D.J. Garrick (unpublished) extended the theoretical predictions on the value of sire reference schemes in sheep (Blair, 1989) to include a number of MOET schemes, that did not involve using MOET on ewe lambs. The basis for comparison was the same as that used by Blair (1989), a closed 500-ewe flock using 5 rams per year which are replaced annually. The rams were used at 18-months of age when selecting for fleece weight (FW) and at 6 months of age when selecting for number of lambs born (NLB). When selecting for FW (heritability of 0.30) 66 two-tooth (18-months of age) ewes were selected out of 250 available (1-stage selection). Alternatively 66 donor ewes were selected consisting of 46 selected as two-tooths and a further 20 selected as six-tooths out of the 46 based on progeny test using the hogget (1-year) fleece weight of 8 offspring (2-stage). A similar 2-stage selection process was considered for NLB (heritability of 0.10) with selection on both the ewes' own record and the dam's records. In all schemes 8 live progeny per donor ewe were assumed. The rate of inbreeding was calculated using the methods presented by Woolliams (1989a) which he showed to be about double the rates previously estimated for MOET schemes using the classical Wright (1931) formula. Predicted genetic gains were also adjusted for inbreeding depression using -0.02 kg/1% inbreeding for FW and -0.008 lambs per ewe/1% inbreeding for NLB (Clarke, 1982).

Responses were evaluated after 10 years of selection (Table 4). While genetic responses were increased by 50-60% in the MOET schemes this was accompanied by a 2-4 fold increase in inbreeding. After adjusting genetic response for expected inbreeding depression (net genetic change) there was no advantage in MOET schemes selecting for FW and only a small advantage (9%) for NLB.

Computer simulation of MOET schemes

Genetic response rates from computer (stochastic) simulated MOET based breeding schemes which did not account for the reduction in additive genetic vari-

TABLE 4 Genetic change, inbreeding, and net genetic change in MOET nucleus schemes for fleece weight (FW, kg) and number of lambs born per ewe (NLB) (Garrick, 1990)

Response (10 years of selection)	Within flock[a]		MOET		
			1-stage	2-stage	
	FW	NLB	FW	FW	NLB
Generation interval (yr)	2.7	2.7	2.0	2.3	3.2
Inbreeding (%)	10.0	10.0	42.0	36.0	26.0
Genetic change	0.68	0.30	1.09	1.06	0.45
Net genetic change[b]	0.48	0.22	0.25	0.34	0.24

[a] Mass selection in a closed flock of 500 ewes

[b] Adjusted for inbreeding depression (see text)

ance due to inbreeding or inbreeding depression have ranged from 9 to 61% lower than corresponding deterministic predictions, while rates of inbreeding were higher than predicted (Juga and Maki-Tanila, 1987; Ruane and Thompson, 1989; Toro *et al.*, 1988; Toro and Silio, 1989). The achievement of responses competitive with progeny test schemes and with acceptable rates of inbreeding (0.4-0.5% per year) was shown by Toro and Silio (1989) to require 2-3 fold larger MOET schemes (32-48 sires and 256-286 dams) and low rates of embryo transfer (4 progeny per donor) in dairy cattle.

It is now clear that theoretical predictions of selection response in closed nucleus breeding schemes have been too high (Keller *et al.*, 1990a) and predictions of rates of inbreeding are too low (Woolliams, 1989a). It has been generally acknowledged that there are a number of factors which will affect additive genetic variance and hence selection response (e.g. Ruane, 1988). These include genetic drift (the sum of inbreeding and sampling effects), linkage disequilibrium (Bulmer, 1971; Falconer, 1981), inbreeding depression and the effects of population size and structure on realized selection differentials (Hill, 1976).

The most definitive study on this subject has recently been completed by Keller *et al.* (1990). They developed a deterministic computer model to predict selection response over time in closed nucleus breeding schemes which accounted for linkage disequilibrium, inbreeding, inbreeding depression, finite population size and relationship structure. They used this model to assess the relative importance of these factors in reducing selection response as affected by length of planning horizon, population size, selection intensity and heritability.

Adjusting for reduced selection differentials or sampling losses caused a small (less than 5%) immediate and constant reduction in selection response. Linkage disequilibrium decreased response rates by 10-15% and fairly rapidly (within 5-10 years) led to new lower equilibrium levels. Excluding inbreeding depression, linkage disequilibrium was the most important factor decreasing response rates. Inbreeding effects on additive genetic variance as well as on potential phenotypic depression increased in importance with longer planning horizons; after 20 years they could decrease response by 10 and 30%, respectively. The authors suggest that discounting selection response over time would increase the importance of early effects of linkage disequilibrium and decrease the importance of the cumulative effects of inbreeding. The total percentage reductions in average selection response per year by adjusting for all effects combined ranged from 13 to 35% when inbreeding depression was ignored and 15 to 90% with inbreeding depression (0.5% per 1% inbreeding), for alternative mating structures and population sizes. Reductions in response were largest for small nucleus herds, and previous theoretical predictions have clearly exaggerated the competitive merits of small, closed MOET nucleus herds. The total reduction in response due to all factors combined now led to predicted rates of genetic response which agreed well with comparable results from the simulation studies cited earlier.

It is pertinent to ask how large a MOET nucleus

should be to maximise net genetic change. This has been investigated by D.J. Garrick (unpublished) for one of the MOET scenarios he considered in Table 4, accounting for all the factors considered by Keller *et al.* (1990). A minimum of 100-250 donor ewes and 10-20 rams per year were required to give an increased net genetic response of 50-80% in the 1-stage MOET for FW. With 8 live progeny per donor ewe transferred this translates into a flock which is performance-recording between 800 and 2000 ewes. This suggests that the efforts and costs are probably beyond the scope of individual breeders.

The rate of inbreeding in closed MOET nucleus schemes has been clearly established as a major factor in their effectiveness. In addition to leading to depressed performance in economically important traits it may also critically affect the production of good-quality embryos by the donor females (Woolliams, 1989a). While one solution to this problem is to increase the size of the nucleus, there are also modifications to the mating systems which can be applied to reduce inbreeding (Woolliams 1989a). There are also advantages in maintaining an open rather than a closed nucleus in terms of reducing the rate of inbreeding. However, while inbreeding may have undesirable effects in the nucleus herd, it is likely to have relatively little impact on the commercial population when this involves crossbreeding. When sires and/or semen from the nucleus are outcrossed into the commercial population this may reduce inbreeding in the short term (1-2 generations), but then inbreeding will accumulate with the same undesirable effects as in the nucleus.

Putting MOET into Practice

MOET is currently used to produce a high proportion of young dairy bulls for progeny testing in some countries, e.g. 58% in Canada, 50% in the USA and 50% in France (Ruane, 1988).

Implementation of MOET nucleus schemes and MOET hybrid schemes for cattle breeding in Britain and Europe was summarised recently in an EAAP seminar (Kalm and Liboriussen, 1989). Hybrid schemes are clearly the most common application of MOET to date (Table 5). Just two closed MOET nucleus schemes are presently operating (one dairy scheme and one beef scheme) and these are both adult schemes. The size of these schemes in terms of number of sires and number of donor cows (Table 5) is probably too small to be competitive with national progeny test schemes, given the results of Keller *et al.* (1990). For example, in the well documented Premier Breeders' dairy cattle adult MOET scheme in Britain, an annual genetic improvement rate for milk production of 0.11 phenotypic standard deviations (1.6% per year) has been predicted (McGuirk, 1989). We have used the approach of Keller *et al.* (1990) to adjust this response rate for all factors they considered, including inbreeding depression of -0.32% per 1% inbreeding (Falconer, 1981). This would reduce the predicted response for this scheme by about 20% after 5 generations (20 years) with an inbreeding rate of 1% per year. The Premier nucleus scheme (now called Genus) has recently been purchased by the British Milk Marketing Board and has changed from a closed to an open nucleus scheme (McGuirk, pers. comm.).

Sexed Semen and Embryos

The main genetic advantage of being able to use only female producing sperm is to increase the selection intensity on the female to breed female pathway. Van Vleck (1981) compared the advantage of using sexed semen in the dairy industry with regular AI and showed an increase of 15% in genetic gain in milk yield (Table 1). To obtain a net financial return from this modest increase in genetic gain, the processing costs for producing sexed semen would have to be minimal.

Similar modest increases in genetic gain (1-6%) have been found from using embryo sexing in MOET nucleus schemes (Woolliams and Wilmut, 1989; Kinghorn and Smith, 1990).

While sex control might not have dramatic effects on rates of genetic progress, it can potentially have very dramatic effects on the efficiency of farming systems. For example, Taylor *et al.* (1985) compared the efficiency of the total herd food utilization in traditional and sex-controlled systems of beef production. A single-sex (all female), once-bred heifer system with a reproductive rate of unity (attained by embryo transfer) was 50% more efficient than the highest achievable traditional system. It was suggested that if multiple sexed-embryo transfer became a relatively inexpensive routine operation similar to AI, then this system of beef production would become competitive with pig and

TABLE 5 Commercial applications of cattle MOET schemes in Europe[a]

Country	Year initiated	Breed	Type of MOET	Number of Sires	Donor cows[b]
France	1984	Dairy	Hybrid - open		140
East Germany	1985	Dairy	Hybrid - open		100
Denmark	1986	Dairy	Hybrid - open	2	100
West Germany	1987	Dairy	Hybrid - open		100
Britain	1987	Holstein/Friesian	Adult nucleus - closed	8	32
Poland	1988	Friesian	Adult nucleus - open	4	80
Holland	1989	Holstein	Hybrid - open		100
Austria	1989	Pinzgauer	Hybrid - open	2	20
Britain	1989	Beef (Simmental)	Adult nucleus - closed	6	16

[a] Extracted from Kalm and Liboriussen (1989)

[b] 5-10 live offspring per donor being achieved

poultry production in terms of efficiency of food utilisation.

Cloning

Identical twins are naturally occurring examples of clones, being derived from a single cell. They occur at a low frequency in cattle and humans (0.1%) and probably other mammals as well. Small clones have now been produced in horses, pigs, cattle and sheep, initially by embryo splitting, and more recently by nuclear transfer (Macmillan and Tervit, 1990). Both nuclear transfer, and perhaps in the near future, embryo stem cells, offer the potential of creation of very large clone families (Woolliams and Wilmut, 1989).

Once techniques are developed to produce large clones then rates of genetic improvement can be considerably increased as shown for dairy cattle by Van Vleck (1981), Nicholas and Smith (1983) and Teepker and Smith (1989) and for beef cattle by Smith (1989).

The benefits of cloning have to be considered for specific time periods as illustrated in Figure 1 adapted from Nicholas and Smith (1983). An initial genetic lift equivalent to 4-5 years of response in a progeny testing scheme can be produced by selecting a small number of elite bulls and cows and cloning embryos from them. Testing this set of clones and selecting the best clones for commercial use could give an additional genetic lift equivalent to about 15-17 years of normal annual genetic response. Both these genetic gains are obtained only once. With the constraints of limited testing facilities there is then a conflict between selecting the best clone or clones for use in the commercial population and of selecting and rebreeding clones for genetic improvement of the breeding unit. Possible solutions have been discussed by Teepker and Smith (1989) and Woolliams (1989b), with the general conclusion that cloning of embryos may not increase genetic response in MOET nucleus schemes, but that cloning does offer considerable advantages for fast dissemination of superior genotypes to the commercial population.

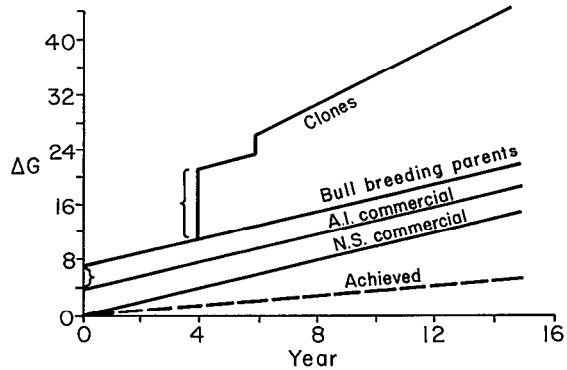

FIG 1 Possible genetic response (G = years of annual genetic change possible by progeny testing) from the breeding and use of selected clones compared with that possible from conventional progeny testing (adapted from Nicholas and Smith, 1983).

Smith (1989) came to very similar conclusions when considering how cloning might affect the genetic improvement of beef cattle. The need for both terminal clones and maternal clones was identified, with heterosis being exploited by using crossline clones. Both Smith (1989) and Teepker and Smith (1989) note that the very high genetic merit possible by selecting the best clones for commercial use will reverse the normal improvement lag between breeding and commercial stocks.

A number of novel breeding schemes which involve production of large clones and the production of bulls with only sires as parents have been investigated (Van Raden and Freeman, 1985; Kinghorn and McClintock, 1990). At present this approach is not biologically feasible, as a gamete from each sex is required to produce viable embryos (Surani *et al.* 1987).

Kinghorn and Smith (1990) investigated a breeding strategy which involved using both male and female gametes, with all selected males mated to all selected females in a completely cross-classified mating design. Rates of genetic response in a dairy cattle scenario were at least double those in conventional progeny testing schemes with acceptable annual rates of inbreeding (0.27 to 0.71% for different population sizes). The mating strategy described, while biologically feasible, cannot be achieved with current reproductive techniques since it would require sufficient oocyte collection from each cow to generate 100 viable candidates for selection and efficient *in vitro* fertilisation.

Some of the other possible applications of cloning were discussed by Woolliams and Wilmut (1989) and include more efficient evaluation of genotype by environment interactions; more efficient beef production from dairy herds; and testing and/or dissemination of transgenics.

Genetic Markers

The term genetic marker has been used to describe indirect predictors of genetic merit in two quite different categories (Blair *et al.* 1990). On the one hand these may be physiological or metabolic traits (sometimes termed indicator traits), while on the other hand genetic linkage at the chromosome level is involved. The first category is considered here while the second category is discussed in a later section.

Indicator traits can improve genetic response by increasing selection accuracy, by offering new opportunities for selection and by reducing generation intervals. The value of an indicator trait in increasing selection response will depend largely on the magnitude of the co-heritability which is a function of the heritabilities of the indicator trait and of the economic trait to be improved (e.g. milk yield) and the genetic correlation between them (Falconer, 1981; Woolliams and Smith, 1988).

Physiological and/or metabolic characteristics which might be considered as potential indicator traits have been reviewed by Blair *et al.* (1990). There has been quantitative assessment of indirect selection for reproduction in sheep (Walkley and Smith, 1980) and beef cattle (Hammond and Grasser, 1987). Traits such as testicle size in young rams or bulls or FSH in ewe lambs (Bodin *et al.*, 1986) have considerable potential as indirect predictors of reproductive rate in female offspring.

Woolliams and Smith (1988) evaluated the possible role of physiological indicator traits in the genetic improvement of dairy cattle for milk yield. With high co-heritability, selection for the indicator trait alone can result in greater rates of response than those feasible with progeny testing. Even better responses are obtained with combined selection, but when breeding values are accurately measured by progeny testing the advantage may be small. When breeding values are less accurately measured, as in juvenile MOET nucleus schemes, the extra rates of response can be appreciable, even with moderate to low co-heritabilities. A possible useful indicator trait considered by Woolliams and Smith was blood urea nitrogen measured in young animals after a short fast, with a co-heritability of 0.27. This could double response rates in a juvenile MOET scheme compared to responses in conventional progeny test programmes, but there is still the problem of high inbreeding levels in juvenile schemes. When and if such juvenile schemes are established it could be advantageous to run several schemes concurrently and exchange breeding stocks to minimise inbreeding. The economic benefits of this would need to be clearly established.

Economic evaluation of new opportunities

Most of the evaluations of new opportunities discussed

here have been carried out for one trait only (e.g. milk yield) and benefits assessed in terms of genetic gain per year both with, and more often without, accounting for the full effects of inbreeding.

Fewson (1989) considered some economic aspects of dual-purpose cattle MOET breeding schemes for an aggregate breeding goal which included milk, beef and secondary traits. The results of these analyses were given in terms of average annual genetic gain, cumulative discounted breeding returns, discounted costs and profit, but did not account for inbreeding. These calculations were carried out for the complete breeding and production unit which included AI and MOET centres as well as the commercial population. It may not be appropriate to make generalisations from this study since the findings are likely to be specific to the costs and returns and particular population structures that were modelled. In this study it was found that in large populations (500 000 cows) that genetic gain per year and profit were higher for AI progeny testing programmes than for a MOET scheme. In a small population (20 000 cows) the MOET system was superior in terms of both genetic gain and profit.

In New Zealand a computer model has been developed by the New Zealand Dairy Board to evaluate different breeding strategies taking into account both costs and returns (Shannon and Jackson, unpublished). Results are expressed as the difference in returns compared to a benchmark level. The benchmark is an AI scheme servicing 750 000 cows with 5% of service dedicated to a sire proving scheme. The expected returns for a 150 cow herd obtaining all its replacements from AI, are based on a milkfat price of $5 per kg and take into account any change in costs.

A number of different strategies for increasing genetic gain in the New Zealand dairy cattle population have been evaluated using this model (Shannon, 1989). These have included not only use of the new technologies such as MOET, improved semen technology to further increase bull coverage through AI and genetic (physiological) markers but also possible changes to the structure of the current breeding scheme. The results are summarised in Table 6.

Achievement of the potentially useful gains identified for all new technologies evaluated (MOET, semen technology, genetic markers) depends on further research before implementation in the breeding scheme. Increased gains from manipulating the size of the sire proving scheme are extremely modest, but certain. In Shannon's opinion the biggest restriction on increased genetic gain in the New Zealand dairy population is the small pool of cows continuously bred by AI. Increasing

TABLE 6 Economic evaluation of some different strategies to increase genetic gain in dairy cattle in New Zealand (from Shannon, 1989)

	Maximum Returns ($ per farm)	Further Research	Likelihood of success
Increase Sire Proving Scheme to 7% (vs 5%)	110	No	Certain
Double bull coverage (AI)	2 000	Yes	Good
Increase number of bull mothers from	5 000		
x 2	1 000	No	Certain
x 4	2 000	No	Certain
x 12	3 500	No	Good
MOET			
1. to produce sons	2 000	Yes	Good
2. Nucleus herd (2000 cows)	2 400	Yes	Good
Genetic markers			
Moderate correlation	2-4 000	Yes	Moderate
Perfect correlation	10 000	Yes	Low

the identification of such animals is currently occurring and this can contribute at least as much to increasing genetic gain as the application of more sophisticated techniques. It is also pertinent to note that although the independent effect of each strategy was evaluated, these are not necessarily mutually exclusive.

MOLECULAR BIOLOGY

Transgenic animals, gene mapping and genetic markers could all have an impact in the future on genetic improvement programmes. We believe that the practical implementation of these technologies is likely to take considerably longer (10-20 years minimum) than most of the reproductive techniques discussed previously. We review briefly the potential role of genetic markers and transgenic livestock.

Genetic markers

In the past, genetic markers have been measurable traits that have shown genetic linkage with another unobserved but economically important trait. For example, various blood type traits have been employed in selecting against halothane sensitivity in pigs (Vogeli *et al.* 1984). More recently, rapid advances in molecular biology have led to the ability to cut the genome (using restriction enzymes) into many small pieces called Restriction Fragment Length Polymorphisms (RFLPs). These many pieces of DNA can then be separated using electrophoretic techniques. If one (or several) of these RFLPs can be shown to be associated with the level of performance, these can be used for selection. This process of selection has become known as Marker Assisted Selection (MAS). In addition to RFLP's there are other DNA marker technologies being utilised in both MAS and gene mapping (e.g. variable number tandem repeats - VNTR).

Various authors (Soller, 1978; Beckman and Soller, 1987; Smith and Simpson, 1986; Lande and Thompson, 1990) have suggested that the MAS system has the potential to increase the rate of genetic gain per year. The benefit is greatest for traits of low heritability and when the marker explains a larger proportion of the additive genetic variance than the economic trait. For example, Lande and Thompson (1990) suggest about 50% additional genetic gain if the marker explains 20% of the additive genetic variance and the economic trait has a heritability of 0.2. An additional pathway through which MAS can increase genetic gain is by allowing measurement in young stock, thereby minimizing the generation interval.

A problem of MAS is that linkage groups will be broken during chiasma, thereby leading to incorrect selection decisions. To minimise this problem, it is necessary to have the distance between the marker and the locus coding for the economic trait as small as possible.

At the current time, the application of MAS using RFLPs in livestock is not feasible, but the approach has been applied in the tomato (Paterson *et al.* 1988). It is first necessary to produce a sparse map of the genome with the positions of various marker RFLPs widely distributed throughout the genome. It is expected to take between 5 and 10 years just to complete this first step in cattle or sheep, but this may proceed quicker in the pig (Haley, 1990). Then it will be necessary to define the linkage groups, i.e. which RFLP is associated with an economic trait. In time, this process will lead to the complete mapping of the genome, and the position of each locus will be known. When this occurs, selection will be directly for the allele of interest and the breaking down of linkage groups will not be of concern. However, complete genome maps of livestock species are likely to be 20 or more years in the future.

Transgenic livestock

Since the early 1980's there have been dramatic advances in the technology of gene transfer to produce transgenic animals, firstly in mice (e.g. Palmiter *et al.*, 1982) and more recently in farm livestock. These developments have been comprehensively reviewed at this conference (Clark *et al.*, 1990; Bullock, 1990). For the purposes of this discussion we will assume that viable and potentially commercially useful transgenic animals can be produced, although this has not yet been achieved.

With the excitement created by the first reports of the production of transgenic mice there were some very diverse opinions on how this might affect current animal breeding theory and genetic improvement programmes. Ward *et al.* (1982) believed it would result in the total reorganisation of conventional animal breeding theory. Robertson (1982) pointed out that we still

know very little about the mechanisms of physiological or biochemical differences between animals so that gene transfer technology may be available before we know how to utilise it most effectively. Transgenic technology was seen by Schuman and Shoffner (1982) as an extension of current animal breeding practices, in broadening the gene pool to make new and novel genotypes available for selection.

In our opinion the most lucid and comprehensive evaluation of the use of transgenic stocks in livestock improvement is that of Smith *et al.* (1987). Applications to pharmaceutical products are not considered here. Smith *et al.* (1987) examined some strategies for developing, testing, selecting, breeding and disseminating transgenic stocks in the context of quantitative changes in economic traits. Since each founder transgenic individual will be unique (at least until control of site incorporation and number of copies of the gene incorporated is achieved), breeding tests will be required. These will need to test the transmission, stability, transgene expression (which may be different in different backgrounds), target trait performance and overall economic performance and merit of each transgene. This will need to take place first in the hemizygous form, and then if useful in the homozygous form. This whole process could take a minimum of 3 generations with some advantages from both MOET and cloning to shorten this period a little. Once a transgene with a useful effect has been identified then there are a number of options available to utilise it commercially. These include incorporation into a purebreeding stock (i.e. make the stock homozygous); incorporation into an interbreeding pool which is then selected on total economic merit; and dissemination through an AI programme.

An evaluation of the costs of developing and testing transgenic lines has not yet been attempted but with the time periods required could be considerable. Smith *et al.* (1987) considered such analyses premature since there is not yet enough information available to carry out a comprehensive cost-benefit analysis.

It seems highly likely that sometime in the future transgenic stock will be of benefit to rates of genetic progress for economically important traits or breeding for specific goals (e.g. disease resistance), despite the very valid reservations about transgenics expressed by Fennessy (1990). While we may be seen to be biassed we think it will be equally true that there will be the need for quantitative genetics skills to bring about these exciting developments.

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REFERENCES

Anderson, G.B.; Cupps, P.T.; Crost, M. 1979. Induction of twins in cattle with bilateral and unilateral embryo transfer. *Journal of Animal Science 49*: 1037-1042.

Baker, R.L.; Parratt, A.C. 1988. Evaluation, selection and diffusion of sires using artificial insemination and/or natural mating. *Proceedings 3rd World Congress on Sheep and Beef Cattle Breeding 1*: 121-140.

Beckmann, J.S. and Soller, M. 1987. Molecular markers in the genetic improvement of farm animals. *Bio/Technology 5*: 573-576

Blair, H.T. 1989. Comparison of genetic gains per year from a variety of selection schemes. *Proceedings of a Seminar on Sire Reference Schemes and Across-Flock Genetic Evaluation*, Massey University, pp 15-29.

Blair, H.T.; McCutcheon, S.N.; MacKenzie, D.D.S. 1989. Physiological predictors of genetic merit. *Proceedings of the Australian Association of Animal Breeding and Genetics 8*: 133-182.

Bodin, L.; Bibe, B.; Blanc, M.R.; Ricordeau, G. 1986. Parametres genetiques de la concentration plasmatique en FSH des agnelles de race Lacaune viande. *Genetique, Selection, Evolution 18*: 55-62.

Bullock, D. 1990. Development and use of gene transfer in New Zealand. *Proceedings of the New Zealand Society of Animal Production 50*:

Bulmer, M.G. 1971. The effect of selection on genetic variability. *American Naturalist 105*: 201-211.

Clark, A.J.; Archibald, A.L.; McClenaghan, M.; Simons, J.P.; Whitelow, C.B.A.; Wilmut, I. 1990. The germ line manipulation of livestock : Progress during the last five years. *Proceedings of the New Zealand Society of Animal Production 50*:

Clarke, J.N. 1982. Mating plans and their effects in sheep improvement. In: *Sheep Production : Breeding and Reproduction*, Eds G.A. Wickham and M.F. McDonald, New Zealand Institute of Agricultural Science and R. Richards, Publisher Vol. *1*: 111-142.

Christensen, L.G. 1984. Breeding problems in small cattle populations.

35th Annual Meeting of the European Association of Animal Production, The Hague. 18 pp.

Christensen, L.G.; Liboriussen, T. 1986. Embryo transfer in the genetic improvement of dairy cattle. In: *Exploiting New Technologies in Animal Breeding*. Eds. C.Smith, J.W.B. King and J.C. McKay. Oxford University Press, Oxford, U.K. pp 37-46.

Colleau, J.J. 1985. Genetic improvement by ET within selection nuclei in dairy cattle. *Genetique, Selection, Evolution* 17:499-538.

Colleau, J.J. 1986. Genetic improvement by ET within an open selection nucleus in dairy cattle. *Proceedings of the 3rd World Congress on Genetics Applied to Livestock Production*, Lincoln, Nebraska, U.S.A. *12*: 127-132.

Colleau, J.J. 1989. The genetics of dairy MOET's. In: *New Selection Schemes in Cattle: Nucleus Programmes*. Eds. E. Kalm and T. Liboriussen, EAAP Publication No. *44*, PUDOC, Wageningen, Netherlands. pp 55-63.

Colleau, J.J. and Elsen, J.M. 1988. Potentialities of embryo transfer for improvement of beef cattle and sheep productivity. *Proceedings of the 3rd World Congress on Sheep and Beef Cattle Breeding 1*: 141-157.

Dieleman, S.J.; Bevers, M.M.; Worth, Y.A.; Gielen, J.H.; Willemse, A.H. 1989. Improved embryo yield and condition of donor ovaries in cows after PMSG superovulation with monoclonal anti-PMSG administered shortly after the preovulatory LH peak. *Theriogenology 31*: 473-

Falconer, D.S. 1981. Introduction to Quantitative Genetics. Longman Group Ltd. England.

Fennessy, P.F. 1990. Animal industries in the 21st century - A New Zealand perspective. *Proceedings of the New Zealand Society of Animal Production 50*:

Fewson, D. 1989. Economic aspects of MOET breeding schemes. In: *New Selection Schemes in Cattle: Nucleus Programmes*. Eds. E. Kalm and T. Liboriussen. EAAP Publication No. *44*, PUDOC, Wageningen, Netherlands, pp 143-160.

Foote, R.H. 1981. The artificial insemination industry. In *New Technologies in Animal Breeding*. Eds B.G. Bracket, G.E. Seidel and S.M. Seidel. Academic Press, New York, pp 13-39.

Garrick, D.J. 1989. Sire Reference Schemes and Across-flock Genetic Evaluation. *Proceedings of a Seminar at Massey University*. 52 pp

Gearheart, W.W.; Smith, C.; Teepker, G. 1989. Multiple ovulation and embryo manipulation in the improvement of beef cattle: Relative theoretical rates of genetic change. *Journal of Animal Science 67*: 2863-2871.

Haley, C.S. 1990. Current and future considerations for breeding programmes in pigs. *Proceedings of the 5th Asian-Australasian Association of Animal Production. Vol 2*: 211-246.

Hammond, K.; Graser, H.U. 1987. The value of indirect selection for female reproduction in beef cattle using scrotal circumference. *Proceedings of the Australian Association of Animal Breeding and Genetics 6*: 232-236.

Hill, W.G. 1976. Order statistics of correlated variables and implications in genetic selection programmes. *Biometrics 32*: 889-902.

Juga, J.; Maki-Tanila, A. 1987. Genetic change in a nucleus breeding dairy herd using embryo transfer. *Acta Agriculturae Scandinavica 37*: 511-519.

Kalm, E.; Liboriussen, T. 1989. New Selection Schemes in Cattle: Nucleus Programmes. *European Association of Animal Production Publication No. 44*. PUDOC, Wageningen, Netherlands.

Keller, D.S.; Gearheart, W.W.; Smith, C. 1990. A comparison of factors reducing selection response in closed nucleus breeding schemes. *Journal of Animal Science 68*: 1553-1561.

Kinghorn, B.P.; McClintock, A.E. 1990. A strategy for use of sires only as parents. *Proceedings of the Australian Association of Animal Breeding and Genetics 8*: 353-357.

Kinghorn, B.P.; Smith, C. 1990. Potential genetic gains in dairy cattle with gamete harvesting. *Journal of Dairy Science* (in press).

Koch, R.M.; Gibb, J.B.; Gosey, J.A. 1986. Evaluation of beef cattle industry breeding programs : breeders and breed associations. *Proceedings of the 3rd World Congress on Genetics Applied to Livestock Production 9*: 398-409.

Land, R.B.; Hill, W.G. 1975. The possible use of superovulation and embryo transfer in cattle to increase response to selection. *Animal Production 21*: 1-12.

Lande, R. and Thompson, R. 1990. Efficiency of marker-assisted selection in the improvement of quantitative traits. *Genetics 124*: 743-756.

Macmillan, K.L.; Tervit, H.R. 1990. Available technologies and prospective developments in reproduction and embryology. *Proceedings of the Australian Association of Animal Breeding and Genetics 8*: 9-18.

Morris, C.A.; Jones, L.P.; Hopkins, I.R. 1980. Relative efficiency of individual selection and reference sire progeny test schemes for beef production. *Australian Journal of Agricultural Research 31*: 601-613.

Newman, S.A.N. 1988. Genetic and environmental variation and genotype by environment interaction in New Zealand Romney sheep. *Ph.D. Thesis*, Massey University, Palmerston North.

Nicholas, F.W. 1979. The genetic implications of multiple ovulation and embryo transfer in small dairy herds. *Proceedings of the 30th Annual Conference of the European Association of Animal Production*, Harrogate, U.K.

Nicholas, F.W.; Smith, C. 1983. Increased rates of genetic change in dairy cattle by embryo transfer and splitting. *Animal Production 36*: 341-353.

Palmiter, R.D.; Brinster, R.L.; Hammer, R.E.; Trumbauer, M.E.; Rosenfeld, M.G.; Birnberg, N.C.; Evans, R.M. 1982. Dramatic growth of mice that develop from eggs microinjected with metallothionene-growth hormone fusion genes. *Nature 300*: 611-615.

Parnell, P.F.; Baker, R.L.; Foulley, J.L. 1986. The efficiency and place of multi-herd animal evaluation procedures for beef cattle. *Proceedings of the 3rd World Congress on Genetics Applied to Livestock Production 9*: 373-388.

Paterson, A.H.; Lander, E.S.; Hewitt, J.D.; Peterson, S.; Lincoln, S.E.; Tanksley, S.D. 1988. Resolution of quantitative trait into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nature 335*: 721-726.

Quirke, J.F.; Hanrahan, J.P. 1977. Comparison of the survival in the uteri of adult ewes of cleaved ova from adult ewes and ewe

lambs. *Journal of Reproduction and Fertility 51*: 487-489.

Rangel-Santos, R.; McDonald, M.F.; Wickham, G.A. 1989. Practical difficulties of a juvenile MOET scheme in sheep. *Proceedings of the Australian Association of Animal Breeding and Genetics 8*: 543-544.

Robertson, A. 1982. Genetic engineering in animal improvement. *Proceedings of the 2nd World Congress on Genetics Applied to Livestock Production 6*: 139-145.

Ruane, J. 1988. Review of the use of embryo transfer in the genetic improvement of dairy cattle. *Animal Breeding Abstracts 56*: 437-446.

Ruane, J.; Thompson, R. 1989. Simulation of an adult multiple ovulation and embryo transfer (MOET) nucleus breeding scheme in dairy cattle. In: *New Selection Schemes in Cattle: Nucleus Programmes*. Eds. E, Kalm and T. Liboriussen, EAAP Publication No. 44, PUDOC, Wageningen, Netherlands, pp 72-80.

Schuman, R.; Shoffner, R.N. 1982. Potential genetic modifications in the chicken, Gallus domesticus. *Proceedings of the 2nd World Congress on Genetics Applied to Livestock Production* 6: 157-163.

Seidel, G.E. Jr. 1981. Superovulation and embryo transfer in cattle. *Science 211*: 351-358.

Seidel, G.E.; Seidel, S.M. 1981. The embryo transfer industry. In *New Technologies in Animal Breeding* Eds. B.G. Brackett, G.E. Seidel Jr. and S.M. Seidel. Academic Press, New York, pp 41-80.

Shannon, P. 1968. Advances in semen dilution. *Proceedings of the New Zealand Society of Animal Production 28*: 23-31.

Shannon, P. 1989. Increasing rates of genetic gain. *Proceedings of the Ruakura Farmers' Conference* pp 6-11.

Smith, C. 1981. Levels of investment in testing the genetic improvement of livestock. *Livestock Production Science 8*: 193-201.

Smith, C. 1986. Use of embryo transfer in genetic improvement of sheep. *Animal Production 42*: 49-62.

Smith, C. 1989. Cloning and genetic improvement of beef cattle. *Animal Production 49*: 49-62.

Smith, C.; Meuwissen, T.H.E.; Gibson, J.P. 1987. On the use of transgenes in livestock improvement. *Animal Breeding Abstracts 55*: 1-10.

Smith, C. and Simpson, S.P 1986. The use of genetic polymorphisms in livestock improvement. *Journal of Animal Breeding and Genetics 103*: 205-217.

Soller, M. 1978. The use of loci associated with quantitative effects in dairy cattle improvement. *Animal Production 37*: 133-139.

Stichbury, J.W. 1968. Genetic implications of advances in semen dilution. *Proceedings of the New Zealand Society of Animal Production 28*: 32-39.

Surani, M.A.H.; Barton, S.C.; Morris, M.L. 1987. Experimental reconstruction of mouse eggs and embryos: an analysis of mammalian development. *Biology of Reproduction 36*: 1-16.

Taylor, St. C.S.; Moore, A.J.; Thiessen, R.B.; Bailey, C.M. 1985. Efficiency of food utilization in traditional and sex-controlled systems of beef production. *Animal Production 40*: 401-440.

Teepker, G.; Smith, C. 1989. Combining clonal response and genetic response in dairy cattle improvement. *Animal Production 49*: 163-169.

Tervit, H.R.; Peterson, A.J.; Thompson, J.G.E. 1990. Developments in domestic animal embryo manipulation technology which support the application of molecular biology to animal production. *Proceedings of the New Zealand Society of Animal Production 50*:

Toro, M.A.; Silio, L.; Rodrigarez, J.; Dobao, M.T. 1988. Embryo transfer and genetic improvement of wool yield in sheep. *3rd World Congress on Sheep and Beef Cattle Breeding 1*: 214-216.

Toro, M.; Silio, L. 1989. Genetic simulation of juvenile MOET breeding nucleus schemes in dairy cattle. In: *New Selection Schemes in Cattle: Nucleus programmes*. Eds. E. Kalm and T. Liboriussen, EAAP Publication No. 44, PUDOC, Wageningen, Netherlands, pp 64-71.

Van Raden, P.M.; Freeman, A.E. 1985. Potential gains from producing bulls with only sires as parents. *Journal of Dairy Science 68*: 1425.

Van Vleck, L.D. 1981. Potential genetic impact of artificial insemination, sex selection, embryo transfer, cloning and selfing in dairy cattle. In: *New Technologies in Animal Breeding*. Ed. Brackett, B.G., Seidel, G.E. Jr. and Seidel, S.M. Academic Press, New York. pp 221-242.

Vogeli, P.; Stranzinger, G.; Schneebeli, H.; Hagger, C.; Kunzi N.; Gerwig, C. 1984. Relationships between the H and A-O blood types, phosphohexose isomerase and G-phosphogluconate dehydrogenase red cell enzyme systems and halothane sensitivity and economic traits in a superior and an inferior selection line of Swiss Landrace pigs. *Journal of Animal Science 59*: 1440-1450.

Walkley, J.R.W.; Smith, C. 1980. The use of physiological traits in genetic selection for litter size in sheep. *Journal of Reproduction and Fertility 59*: 83-88.

Ward, K.; Sleigh, M.J.; Powell, B.C.; Rogers, G.E. 1982. The isolation and analysis of the major wool keratin gene families. *Proceedings of the 2nd World Congress on Genetics Applied to Livestock Production 6*: 146-156.

Wickham, G.A. 1989. The possible impact of genotype-environment interactions in across-flock breeding programmes. *Proceedings of a Seminar on Sire Reference Schemes and Across-Flock Genetic Evaluation*, Massey University, pp 30-40.

Willham, R.L. 1979. Evaluation and direction of beef sire evaluation programs. *Journal of Animal Science 49*: 592-599.

Woolliams, J.A. 1989a. Modifications to MOET nucleus breeding schemes to improve rates of genetic progress and decrease rates of inbreeding in dairy cattle. *Animal Production 49*: 1-14.

Woolliams, J.A. 1989b. The value of cloning in MOET nucleus breeding schemes for dairy cattle. *Animal Production 48*: 31-35.

Woolliams, J.A.; Smith, C. 1988. The value of indicator traits in the genetic improvement of dairy cattle. *Animal Production 46*: 333-345.

Woolliams, J.A.; Wilmut, I. 1989. Embryo manipulation in cattle breeding and production. *Animal Production 48*: 3-30.

Wright, S. 1931. Evolution in mendelian populations. *Genetics 16*: 97-159.