INTRODUCTION

The theoretical potential for genetic improvement through the application of reproductive technology has not been achieved in many livestock industries. This is mainly because the use of the technology often requires intensive animal management, at least for limited periods. It also involves additional cost and often the use of specialist skills. The skill-use usually increases the cost of the technology, especially where it involves routine surgical procedures or drugs classified for veterinary use or supervision.

Factors which influence the incidence of use of well developed forms of reproductive technology should be identified. Otherwise the impact of recently developed or developing technology will not be effectively utilised to improve livestock production. In many cases, the emphasis has been on developing the techniques in controlled situations which are not duplicated in commercial herds or flocks. The limitation is more with the practical application of the technology than with the potential success of the technique.

In this paper we discuss factors affecting the application of existing technology and briefly outline some new technological developments.

ARTIFICIAL INSEMINATION

The use of artificial insemination (AI) is routine and widespread in many dairy cattle industries with consequent gains in the genetic merit of these populations. Yet Bindon (1988) reported that only 50 to 65% of cows were AI'd in Australia, France and USA. Britain (58%) also falls within this range (Milk Marketing Board 1988). This degree of use does not provide sufficient AI progeny to maintain these national populations. In contrast, in 1988, 86% of New Zealand dairy herd owners used AI presenting 89% of cows in their herds for insemination. Overall, 78% of New Zealand dairy cows were AI'd (New Zealand Dairy Board 1988).

The rate of genetic progress in a dairy herd can be increased by inseminating maiden heifers with semen from progeny tested sires. Yet frequency of AI use in this class of animal is low and they are often inseminated with semen from sires of recognised beef breeds. For example, only 13% of dairy heifers in New Zealand are inseminated and although the figure is closer to 30% in Britain, 60% of inseminations in Friesian heifers in that country are with "beef breed" semen.

Dairy herds in many countries provide a large number of animals which are reared for beef. In continental Europe, dual purpose breeds are quite common and the dairy-beef cross is a feature of British dairy farming. In 1987-88, 49% of all inseminations made by the British Milk Marketing Board were Friesian/Holstein, with another 49% as beef inseminations. Even within Friesian cows as the major dairy breed, 45% of inseminations were with semen from beef breed sires (Milk Marketing Board 1988).

AI use in beef cattle is much less than in dairy cattle and usually involves less than 5% of the population, except in France where it is 70% (Bindon 1988). The low incidence of AI use in beef herds even occurs in those countries with well established progeny and/or performance testing schemes for beef breed sires. For example, in Britain where beef breed inseminations with dairy cows equalled the total number of Friesian inseminations, less than 3% of these beef breed inseminations were with cows of the same breed as the sire from which the semen was processed (Milk Marketing Board 1988). Beef breed sires contribute more to the "genetic merit" of progeny produced by dairy cows than to the genetic improvement of their own breeds.
The use of AI in national sheep flocks is even less frequent on a proportional basis than in beef cattle. In Australia, it is estimated that there were 26,900 inseminations with ram semen in 1986-87 (Bindon 1988). Most of these were with Merino ewes using semen from Merino sires to produce stud or flock rams. In France and Ireland, sheep AI is used to utilise terminal sires for sheep meat production, especially for year round or out-of-season lambing. It is also widely used in France in milking sheep. In New Zealand, the technique is mainly used to produce stud sires and approximately 2% of performance recorded ewes (Animalplan or Flocklink) were inseminated in 1989 (Harvey and Binnie pers. comm.).

FACTORS INFLUENCING AI USE

AI use in lactating dairy cattle is facilitated by several practical features. These are that:

(i) the animals are congregated for milking at least twice daily and can receive specific "treatments" such as insemination or mastitis treatment without additional handling and herding;

(ii) cows are the only domestic livestock species which display homosexual behaviour when in oestrus. This simplifies oestrous detection and eliminates the need for teaser males;

(iii) the insemination process is relatively uncomplicated to achieve intra-uterine deposition of sperm;

(iv) bulls undergo a comparatively simple semen collection process by artificial vagina to obtain one or two ejaculates every 3 days with each ejaculate containing about 10 ml of semen with $1.2 \times 10^6$ sperm/ml;

(v) bull sperm are very tolerant to processing either in a liquid form which retains viability for at least 4 days, or as deep frozen semen which can be stored indefinitely; and

(vi) high semen dilution rates with as few as $2 \times 10^6$ total sperm/insenimation can be used. (This is the dose rate routinely used in New Zealand dairy herds; New Zealand Dairy Board 1988).

The combination of these advantages means that progeny tested dairy sires of high genetic merit can be very effectively utilised (for example, semen from individual sires has been used for over 150,000 inseminations per year; New Zealand Dairy Board 1988). Such extensive use has advantages through allowing the costs of accurate progeny testing to be spread over a large market demand. The consequences are a high potential rate of genetic improvement limited mainly by the extent to which AI is used within a dairy herd or within the industry.

In contrast, most other types of livestock have disadvantages which limit the use of AI. Beef cattle and dairy heifers are handled less frequently than dairy cows. They are also more likely to be anoestrus. Sheep, goats and deer are also handled less frequently, they do not display homosexual oestrous behaviour, they have distinct breeding and non-breeding seasons, insemination is less convenient, semen collection in deer is complex and semen processing in all three species is often less successful than in the bull. A major factor which also tends to diminish the use of AI in sheep and goats is the ability of an individual sire to naturally mate and settle a larger number of females than a bull during the breeding season.

Technical developments which should increase the application of AI in sheep, goats and deer in the immediate future include: small changes in AI protocols based on improved understanding of ovulation timing and control; development of an artificial vagina at Ruakura for collecting deer semen; improved fresh ram semen diluents which allow ram sperm to be stored at ambient temperature for several days; and improved methods for evaluating semen quality to predict fertility. Most of the classical tests of semen quality in bulls have a correlation with fertility of about 0.3. Correlations between sperm swimup, various measures of computer assisted sperm tracking, rate of induced acrosome reaction and fertility however are higher at 0.5 to >0.9. These new tests appear to be valuable predictors of fertility of individual males.
The dream of a practical method of sexing sperm seems as elusive as ever. It is possible that a method will be developed by combining technologies like flow cytometry, monoclonal antibodies and molecular biology.

OESTROUS SYNCHRONY

The artificial control of the oestrous cycle in farmed livestock has the objective of producing adequate precision in oestrous synchrony to allow all treated animals to be mated over a short period or inseminated at one time without detection of oestrus. There are two principle forms of oestrous cycle control. One involves the release of either a potent progestagen or progesterone from a subcutaneous or intravaginal device. The other induces premature luteal regression by injecting prostaglandin F$_2\alpha$ (PGF) or a potent analogue. These basic forms may be combined, used with another type of luteolytic agent (e.g. oestradiol), or supplemented with a gonadotrophin (e.g. RMSG). Progesterone treatments extend the cycle and control follicle development in the absence of a corpus luteum, whereas PGF reduces the length of the cycle. Sheep and goats are synchronised almost exclusively with progesterone/progestagen forms of treatment, whereas both basic forms are used with cattle.

In 1988 Bindon stated that, "It seems that AI cannot be successfully implemented without a reliable and cost-effective technique for synchronisation of oestrus of range cattle to the point where good fertility can be expected from timed insemination, preferably without detection of oestrus". A similar requirement exists for the effective application of AI in dairy heifers.

The currently available systems for synchrony in cattle can achieve a predictable and precise post-treatment decline in plasma progesterone, but none will consistently produce an adequate synchrony of oestrus and ovulation. Anoestrous animals may not respond to treatment and cycling animals exhibit variation in the length of pro-oestrus. The development and maturation of ovarian follicles in cows after a PGF induced luteolysis varies from 2 to 7 days (Scaramuzzi et al. 1980) even though the decline in plasma progesterone is synchronised (Macmillan et al. 1980). This variation is largely due to the 7 to 10 day cycle of ovarian follicles within the bovine ovary (Macmillan and Henderson 1983), recently elucidated using ultrasonography (Pierson and Sinther 1987; Sirois and Fortune 1988; Scaramuzzi et al. 1989). An example of the variation is provided by recent work (Macmillan et al. 1988) involving 1277 dairy cattle treated with a range of synchrony treatments. Only 1% ovulated without being detected in oestrus, but variation in oestrous onset patterns varied significantly between treatments and among herds within treatments. The results emphasised that none of the treatments could be expected to give sufficiently precise synchrony for single set-time insemination, but the tailpainting/aerosol technique did allow insemination on detection, even in large herds of synchronised cattle.

One method for increasing the precision of synchrony is to extend progestagen/progesterone treatment intervals to more than 12 days in sheep or at least 14 days in cattle. These treatment regimes do not require the concurrent use of a luteolytic agent at treatment initiation (e.g. oestrogen) or termination (e.g. PGF). The effects of treatment length on synchrony and fertility are summarised in Table 1 for a recent CIDR trial in 4 herds of dairy heifers.

<table>
<thead>
<tr>
<th>Treatment interval (days)</th>
<th>No. heifers</th>
<th>% inseminated at 48 h</th>
<th>% inseminated by 96 h</th>
<th>% pregnant to 1st insemination</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>105</td>
<td>50.0</td>
<td>96.2</td>
<td>64.4</td>
</tr>
<tr>
<td>14</td>
<td>119</td>
<td>80.6</td>
<td>98.3</td>
<td>48.7</td>
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<tr>
<td>21</td>
<td>122</td>
<td>96.7</td>
<td>100</td>
<td>36.7</td>
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<tr>
<td>Total</td>
<td>346</td>
<td>80.0</td>
<td>98.3</td>
<td>49.1</td>
</tr>
</tbody>
</table>

$^a$ = PGF at CIDR removal
Extending treatment interval improved synchrony but reduced fertility. New Zealand studies with suckling beef cattle (Smith and Tervit 1980) inseminated at a fixed time after progestagen removal have, in contrast, shown a decrease in incidence of cows in oestrus after long durations of progestagen treatment (14 or 20 days vs 10 days). However, as with dairy cows, the long durations were associated with reduced fertility. The reduction in fertility is most likely due to prolonged treatments producing unfavourable conditions for fertilisation (Roche and Ireland 1981) and/or fertilised ova which have retarded embryo development (Wlshart 1977). Current studies indicate this is associated with persistent ovarian follicles during treatment with exogenous progesterone or progestagens.

Synchronisation of oestrus and AI of beef cattle is far more difficult than dairy cattle. This is because the herds are geographically isolated, are seldom yarded and handled, and cows are usually suckling a calf. The level of anoestrum, particularly in the suckling cows, can be high. The results of extensive New Zealand trials were reviewed by Smith (1983) who concluded that: Cycling dry cows and maiden heifers could be effectively synchronised and gave satisfactory pregnancy rates after insemination twice on a fixed-time basis after PGF treatment, suckling beef cows, at least 40 days post-partum, were best treated with progesterone (PRID) for 10 days with oestradiol administered at insertion. At the time of PRID removal, the calves should be removed from the cows and remain separated until insemination at 56 h after removal. A major factor affecting the application of oestrous synchronisation in beef cattle is the high cost of the drugs and procedures.

Calf removal at the end of a progestagen synchrony treatment increases both the incidence of post-treatment oestrus and the pregnancy rate to AI in suckling beef cows (Wiltbank and Spitzer 1978; Smith et al. 1980; Rice 1987). The technique means cows and calves must be separated for 48 to 72 h and is most practical in herds which have had a concentrated calving producing a limited spread in calf age. Neither synchrony treatment nor calf removal reliably overcome the limiting effects of poor body condition, undernutrition and short post-partum periods.

Synchrony treatments in sheep usually involve intravaginal devices or sponges inserted for periods of 12 to 14 days. Although the post-treatment interval to oestrus is less variable than in cattle, the average interval and the variation differ between breeds of sheep, stage of breeding season or anoestrum, type of progestagen treatment and dose of PMSG used (Smith et al. 1989, Crosby et al. 1990). Smith et al. (1989) reported that in Coopworth ewes treated with a CIDR containing progesterone and with PMSG at device removal, the average interval to oestrous onset was 38.2 h compared to 45.1 h in ewes treated with a sponge containing MAP plus PMSG. The overall breeding season average was 34.9 h compared to 49.1 h in the non-breeding season. These differences may affect results of set-time AI, but not in natural mating if sires are introduced at device removal. Even with natural mating, pregnancy rates may vary from 57.7% (Smith et al. 1989) to 73% (Crosby et al. 1990). These results confirm that any recommendation on when to AI after device withdrawal should be based on local results taking into account breed, type of treatment and season. PMSG is frequently administered at the end of treatment, especially with out-of-season or early season programs. Dose related effects on interval to oestrus, synchrony, ovulation rate and pregnancy rate also vary with breed, type of treatment, stage post-partum and season (Smith et al. 1988, 1989; Crosby et al. 1990). Similar variability in response is recorded in goats, and protocols need to be developed which take into consideration breed, age, milk production and season (Corteel 1975).

Successful synchrony programs can be developed for use in many commercial herds and flocks. The development phase should involve monitoring of post-treatment responses to avoid failures due to inseminating animals either too soon or too late, or which are anoestrous. Set-time inseminating will continue to produce variable results in the absence of monitoring, or until more effective and reliable forms of treatment are developed to synchronise follicle development. Developments which increase the application of oestrous synchronisation are therefore likely to come through studies which increase knowledge of the physiology and endocrinology of follicle development, ovulation, seasonality and anoestrum.

ANOESTRUM AND POST-BREEDING TREATMENTS

Anoestrum due to nutrition and/or suckling is a major factor influencing reproductive efficiency in cattle. It also affects the effectiveness of out-of-season treatments in sheep and goats. Most treatment regimes involve progesterone priming preceding gonadotrophin stimulation, usually with PMSG. The variation in the success of these treatments arises from a lack of precision in diagnosing the degree of anoestrum either from one group of animals to another, or among animals.
within a group. Consequently a standard progesterone plus PMSG treatment regime may produce inadequate, adequate and/or over stimulation of ovarian follicles. The injection of PMSG antibodies may reduce the incidence of over stimulation but necessitates additional animal handling. Regimes which stimulate follicle development have been successfully used for super-ovulation but are too costly and intensive for routine use with normally synchronised animals.

Progesterone plus PMSG treatments are used to induce early cycling in immature domestic animals. The proportion of animals exhibiting oestrus and conceiving is often variable and usually low (Dry mundsson 1973). More success is expected the closer animals are to reaching puberty. If the induction is conducted well before puberty, the animals will not continue cyclic activity. Much detailed work is needed before reliable techniques will be available to advance puberty.

Most treatments to improve fertility in cycling sheep or cattle are applied during pro-oestrus or at the time of insemination. Their effectiveness is limited to increasing ovulation rate or fertilisation rate. Yet, the greatest loss rates in bred animals are due to failure of pregnancy recognition. Parr et al. (1987) showed that progesterone supplementation could prevent the decline in pregnancy rates in ewes which were overfed after mating. Subsequent studies have shown that the sheep embryo is particularly sensitive to reduced progesterone at days 11 and 12 of pregnancy (Parr et al. 1989). These progesterone effects may not be applicable to many normal management situations but are applicable were expensive technology (eg. embryo transfer) is being utilised.

Treatments which stimulate embryo development or delay the onset of luteolysis need to be applied around the time when a normal embryo is producing proteins which inhibit the luteolytic process (Bazer 1989; Thatcher et al. 1989). Cows injected once with a GnRH analogue from 11 to 13 days after first insemination had pregnancy rates increased by 11.5% (Macmillan et al. 1986) probably because this type of treatment altered follicle wave patterns (Thatcher et al. 1989) and reduced the incidence of cycle lengths of less than 20 days (Macmillan et al. 1985). Similar effects on corpus luteum lifespan have been demonstrated with PAF (Battye et al. 1989), bovine trophoblastic proteins (Helmer et al. 1989), trophoblastic vesicles (Heyman et al. 1987) and β-interferon (Plante et al. 1989). Further development of "pregnancy recognition" treatments will be particularly applicable to heat stressed dairy cows and ET recipients implanted with "sectioned" embryos.

The above procedures are also likely to synchronise returns to service. Failure to detect returns is a major factor contributing to extended calving intervals in dairy cattle (Macmillan 1985). This is reflected in inter-service intervals averaging over 35 days. CIDR insertion in cows from 14 to 17 days post-insemination, with removal on day 21 reduced the spread in returns to service from 7 days (18 to 24 day cycles) to 2 days (23 and 24 day cycles) and increased the detection rate from 75% to over 90% (Macmillan et al. 1987). This pattern of CIDR-use is also being evaluated with ewes in out-of-season breeding programmes.

SUPEROVULATION AND EMBRYO TRANSFER

The potential contribution of ET technology to genetic improvement is restricted by the high between-animal variation. Seidel and Seidel (1981) reported that 32% of cattle donors flushed after standard superovulation treatments did not produce any pregnancies. The average number of live calves still remains at 2 to 3 per donor flushed. This is similar to that achieved in sheep (Tervit 1989), but less than that routinely achieved in goats (Tervit 1987). The major variables are superovulatory response and fertilisation rate. Other variables include embryo recovery rate, embryo quality and pregnancy rate among recipients.

Different hormonal regimes have been tested to stimulate superovulation using gonadotrophins with FSH-like properties. Most treatments in cattle are applied from about mid-cycle for 4 to 5 days, followed by one or two inseminations after oestrous onset and embryo recovery by non-surgical flushing at 7 days post-oestrus. An average of 10 to 12 corpora lutea at flushing will have a coefficient of variation of 30% or more, and the final outcome is usually 4 to 5 transferable embryos per donor flushed. Sheep and goat treatments usually involve a lengthy progesterone treatment (12-17 days) commencing at any stage of the oestrous cycle with FSH, often in combination with PMSG, administered either as one injection or 6 to 8 injections over 3 to 4 days near the end of the progesterone treatment. The ovulation rates and harvests of embryos achieved is variable and depends very much on the breed of goat or sheep being treated.
Numerous superovulation regimes and cocktails have been used. Modifications in the treatments are often difficult to compare because of large variation. For example, FSH priming during metoestrous has been reported to have a beneficial effect (Ware et al. 1988), no effect (Hunton et al. 1989) and an inhibitory effect (Guilbault et al. 1989). Also, despite claims that preparations of FSH with low LH contamination or "optimal" FSH:LH ratios give superior harvests of transferable embryos, results are confusing. Indeed, the data of by Mapletoft and Murphy (1989) suggests that non-hormonal factors such as breed, age, year, nutrition, stress and temperature can have more effect on superovulation than hormonal factors.

Advances in superovulation will arise through improved understanding of the endocrinology and physiology of follicular and ovulation development and control processes. For example, the inconsistency of superovulation responses achieved may well be associated with the stage of the follicular wave existing on the ovary when superovulation treatment is initiated. Grasso et al. (1989) reported that treatment initiation in the absence of a dominant follicle produced twice the response of treatment in the presence of a dominant follicle (13.5 CL’s v 7.1 CL’s).

Concepts of follicle wave management have only recently been applied in cattle superovulation programmes and developments are likely to occur now that the hormonal effects on follicular dynamics can be monitored by ultrasonography (Thatcher et al. 1989; Ginther et al. 1989). If the termination or stimulation of follicle waves can be controlled, then donor treatment programmes need not be restricted by stage of cycle at treatment initiation. The treatment programmes will usually involve concurrent use of a progestagen implant (eg. SMB) or a progestagen intravaginal device (eg. CIDR-B). Extensive Canadian trials involving over 200 SMB-treated donors have shown that animals can be implanted at any stage of the cycle so that FSH treatment can be commenced 7 days later. Mapletoft (1987) concluded that "cows implanted with SMB had numerically more ovaulations after treatment with FSH-P than did cows superovulated on a normal cyclic CL". To date however, this has not resulted in a higher yield of transferable embryos.

Concepts of corpus luteum and follicle management may also be applicable to recipient management. Sequential 3-day injections of a GnRH analogue have been used to facilitate asynchronous embryo transfers in dairy cattle (Thatcher et al. 1989). In this trial, the recipients were in oestrus 3 to 7 days before the donor. The feasibility of the reverse sequence has been demonstrated by Lawson and Cahill (1983) in sheep which were treated with progesterone during metoestrous. A potential feature of this latter result is its applicability for use with recipients implanted with embryos which may have either less mass due to splitting, or slower initial rates of development after freezing and thawing.

There have been a number of recent advances in sheep and goat embryo transfers which improve the practicality of the technique. These include: widespread adoption in Australia of single injection FSH/PMSG regimes (eg. Maxwell and Wilson 1989); development of improved surgical techniques enabling donors to undergo at least 11 surgeries (Tervit et al. unpublished); increasing use of laparoscopic techniques which enable the donor uterus to remain within the abdominal cavity during flushing (McKelvey and Robinson 1986); laparoscopic transfer of embryos to recipients; and development of effective regimes for use in the non-breeding season (Tervit et al. 1989).

However, it is the basic research on donor treatment and recipient management which has the potential to substantially increase the number of pregnancies per donor flushed and increase the pregnancy rate with frozen or divided embryos. The combination of improved donor responses and embryo splitting will radically alter the potential contribution of ET technology to livestock breeding programmes at substantially less cost. The results will also be applicable to other "embryo manipulation" programmes and will increase the demand for embryo sexing.

**EMBRYO MANIPULATION**

Remarkable advances have recently been made in embryo manipulation technology.

Appropriately treated embryos with an intact zona pellucida are generally regarded as disease free and are readily transported between countries. Cattle, sheep and goat embryos are now routinely deep-frozen, and embryo splitting is increasingly being used in the cattle industry to increase the number of calves born from each embryo recovered. In our experience split sheep embryos, give variable results. However, Herr et al. (1988) appear to have overcome many of
the problems with the technique and report satisfactory results from sheep (and cattle, goats and deer) embryos. This group also reports a successful DNA Y probe technique for sexing embryos (Matthews et al. 1987) and is now offering a commercial service.

A number of additional technologies will become available either shortly or in the longer term. These are:

**In vitro fertilisation (IVF):** In this technique oocytes are usually collected from the ovaries of slaughtered cattle (or sheep), matured and then fertilised in vitro and then either cultured in vivo (in the sheep oviduct) or in vitro to a stage where they can be transferred or deep-frozen (Lu et al. 1987; Lelbried-Rutledge, 1984). The technique is being used commercially in dairy cows in Ireland and about to be used in Britain. The IVF embryos are from half-bred exotic beef cow oocytes fertilised with pure-bred exotic semen and are twinned into the poorer producers in dairy herds. In this way, dairy cows produce more beef calves (which are three-quarter bred) than if they were inseminated with beef semen. The overall IVF technique is not particularly efficient but large numbers of embryos can be generated. It is anticipated that each cow inseminated with twin embryos should produce a profit of about £32. It is not envisaged that the technique will be used in this form in New Zealand. It could however be used to: supply a cheap source of cross-bred embryos for export to tropical countries; evaluate bull fertility; produce embryos from high-genetic merit cows subjected to repeated oocyte recovery by laparoscopy (Siarid et al. 1985); and to supply eggs for various further manipulations.

**Cloning:** This can be achieved by either bisecting morulae or blastocysts, separation of the blastomeres of early embryos, or nuclear transfer. The first method has already been mentioned and is being used commercially. The second has produced identical twins, triplets and quadruplets in horses, pigs, cattle and sheep (Willadsen 1982). Also, up to 5 identical offspring have been produced in sheep after the blastomeres of an 8-cell embryo were separated and each combined with a blastomere from a 4-cell embryo (Fehilly and Willadsen 1986). The potential of these methods for producing identical individuals is surpassed by the technique of nuclear transplantation (Willadsen 1986). With this method blastomeres from relatively late stage embryos (eg. 32-cell) are separated and each transferred to an enucleated unfertilised oocyte. The blastomere and oocyte are fused by electro- or viral-fusion and then grown to the 8- to 32-cell stage at which time they can be recloned. The overall efficiency of nuclear transplantation is currently low, but the technology should be available commercially in about 3 years (Polge 1989).

Already one commercial company, Granada Genetics, has produced up to 7 clones from one embryo (Van Brunt 1988).

It must be remembered that the clones are of an embryo, not an adult. Thus it is anticipated that most clones will be frozen while a few are transferred to assess the merit of the offspring. In the case of nuclear transplantation, possible cytoplasmic effects on the genetic identity of the clone will need to be evaluated. The technology will enable adult animals to be cloned, not by taking nuclei from the adult, but by thawing embryo clones which had been stored since the original clones were produced.

**Chimera production:** This powerful embryological technique involves the aggregation of blastomeres from a number of embryos or the injection of single or multiple blastomeres into the blastocoel of an embryo. The chimeras can be formed from embryos of a single species or the species barrier can be transgressed as has been done for sheep-goat and goat-sheep chimaeras (Willadsen 1985). These interspecies chimaeras were constructed so that goats gave birth to sheep and sheep to goats. Chimaeras are useful for immunological and developmental studies (eg. Bos taurus and Bos indicus chimaeras; Summers et al. 1984) as well as for studying gene expression (eg. Charolais x Jersey chimaera; Church 1987), modifying genetic makeup through injection of transfected stem cells (Bradley et al. 1984) and for rescuing uniparental embryos (Anderegg and Markert 1986).

**Gene transfer:** Techniques are now available for the isolation of a gene from one animal, its multiplication and modification in the laboratory and its transfer to another animal of the same, or a different species. The power of this technique is immense, as it means that animals with unique genotypes, unable to be produced by normal selection, can be produced (eg. cows producing pharmaceuticals in their milk). The techniques have been used extensively in mice (eg. Palmiter and Brinster 1986) and transgenic sheep, cattle and pigs have also been produced. The technique is very complex and has many problems. First there is the difficulty of identifying genes capable to having the desired effect on animal production. Then there is the problem of incorporating the gene into embryos. This is usually done by injecting the gene into a pronucleus of a fertilised egg (Hamner
et al. 1985). There is also the possibility of using retroviruses (Jacher et al. 1985) and sperm to carry the gene into the egg (Lavitrano et al. 1989). If totipotent stem cells can be isolated from domestic animal embryos, the injection of transfected stem cells into blastocysts will form chimeras. Alternatively, transgenics could be formed by either fusing a transfected stem cell with an enucleated egg (as in cloning) or transferring a group of transfected stem cells to a blastocyst which has had its inner cell mass removed. The next problem is to ensure the foreign gene works as intended. To do this the gene must be introduced with, or next to, a gene which controls its effect. This regulatory gene (or genes) ensures that the foreign gene can be switched off as required and acts only on the targeted gland or organs.

To date most domestic animal transgenics have been produced following pronucleus injection. The low overall success rate (about 1%) is not necessarily a problem as, provided the gene is present in the germ cells, new technology like cloning should soon be able to be used to rapidly multiply the new genotype. A bigger problem with the most commonly produced transgenic, the Growth Hormone transgenic, is their poor vigour (eg. they die young and have reduced ability to fight infection). Also, the sheep transgenics producing Human Clotting Factor IX or Q1 Antitrypsin in their milk (Simons et al. 1988) do so at low levels.

Transgenic research is in its infancy, and much has been learned. There is little doubt that as the techniques become more precise, the possibilities for genetic engineering are endless.

EMBRYO HANDLING IN VITRO

The above very detailed techniques rely on efficient in vitro gamete and embryo handling techniques and efficient culture techniques. Compared to the rodent, relatively little is known of the metabolic and other requirements of domestic animal embryos. This will change in the near future as our laboratory (Thompson et al. 1989) as well as a number of laboratories overseas, conduct extensive research into factors affecting embryo development in vitro. It will be interesting to see whether improved understanding of conditions for maintaining and for assessing embryo viability in vitro will enable the pregnancy rate from transferred embryos to be increased from the generally accepted maximum of about 60%.

SUMMARY

The application of the relatively simple technology of AI in dairy cattle has not been as widespread as expected. Practical problems with oestrous synchronisation discourage farmers from inseminating their maiden heifers and farmers may still need to be convinced that the advantages of the technology outweigh the costs. The application of AI technology is less commonly adopted in other domestic animals. This is because of problems with animal handling, high levels of anoestrus and the need for oestrous synchronisation techniques which give satisfactory and reliable pregnancy rates after a single insemination at a fixed-time without recording oestrus. This is not consistently achieved and so AI is often viewed as a costly procedure. Increased adoption of the technique will come through further farmer education, but mainly as a result of studies which increase knowledge of follicle development, ovulation, seasonality and anoestrus. The adoption of embryo transfer is limited by the expense of the technology and the variability of superovulation and fertilisation rates. The adoption of intrauterine insemination techniques and small technical refinements has increased the application of the technique in sheep and goats. However, major advances in understanding ovulation control, fertilisation and pregnancy establishment are needed before substantial increases in use of the technique will occur.

What then is the future for the new wave of embryo technology such as IVF, cloning, chimeras and gene transfer? IVF is likely to be applied readily because it will supply cheap embryos. The other techniques will be very expensive, and their main value in the short term may be in elucidating principles of physiology rather than for the direct use of the technology to increase production (eg. use of clones to simplify experimental design and transgenics to provide unique information about the effect of genes on physiological processes). Once the efficiency of the embryonic manipulations improves, the techniques would be expected to be increasingly adopted in commercial breeding programmes.
REFERENCES