MODERN REPRODUCTIVE TECHNIQUES IN CATTLE BREEDING

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INTRODUCTION

Since Robert Blakewell established the basis of selection as a method to improve livestock, many techniques which augment genetic selection programmes, have been developed. These include artificial insemination, detection of oestrus and ovulation, control of the breeding cycle, rectal palpation of the genitalia, laparoscopy, induction of parturition, sex determination and embryo manipulation.

Artificial Insemination (AI.)

The late Sir John Hammond of Cambridge stated that the greatest development in agriculture in his lifetime was the use of AI. In the developmental years, AI., although a resounding technical success , proved disappointing as a method of genetic improvement. The use of bulls selected on ancestry performance alone did not automatically improve production. Later, ancestry performance, coupled with progeny testing schemes, have been shown to be a better way of selecting superior sires. However, the process takes a long time and in the end many of the major differences between top and lower yielding herds using AI., were found to be due to improved management rather than genetic differences.

The genetic improvements in dairy herds cannot be expected to be rapid, as the selection potential for milk production is limited to females. However, this is not so for beef production as important characteristics can be assessed in both sexes and their heritabilities are relatively high. Thus, with the judicious use of superior sires, rapid improvement in productivity can be expected. Unfortunately, this has not been the case, as "fad and fancy" type sire selection has been utilized rather than soundly tested superior beef sires. Artificial insemination has failed therefore to achieve its full potential as a technique for the genetic improvement of cattle, especially beef cattle. 'This must be blamed on the application and not the technique.

The current techniques of insemination are well established, and detailed descriptions are available (Cole and Cupps 1969). Maximum fertility will depend upon: accurate oestrus detection, the potential fertility of the semen, proper handling of the semen prior to AI., insemination at the correct time during oestrus, and semen deposition by an experienced inseminator.

The Detection of Oestrus and Ovulation

The detection of oestrus in cattle, especially Bos indicus, can be difficult and unless provisions are made for the accurate detection of oestrus artificial insemination should not be undertaken. Most cows may be detected by observations, in the early morning and late afternoon, for a period of 30 minutes (Baker 1967; Donaldson 1968). However, because oestrous periods of many Bos indicus cows have been shown to last less than 8 hours (Baker 1967) and also because some dairy cows tend to have a "silent oestrus," it is desirable to utilize aids for the detection of oestrus,

If vasectomized bulls (teasers) are used it is important to remember that

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teasers are capable of transmitting venereal diseases. To avoid the spread of diseases, teaser bulls with a penectomy or surgical deviation of the penis laterally or posteriorly have been used successfully (Royes and Bivin 1973; Jillella et al. 1977).

The use of the heat mount detectors which are fastened by adhesive to the lumbo-sacral region of the cow, has been described by Baker (1965), and although a proportion of false positives may occur, checking for other signs of oestrus will increase reliability of the method. The detectors are less reliable when used in pens or in certain field situations, when they may be triggered by shrubs, brush and objects in the pens.

In conjunction with teasers a chin harness incorporating a large ball-point marker is now readily available or a marking crayon or coloured paint such as the "sire-sine", may be fastened by a special head stall to the submaxillary region of a vasectomized bull or steer. This method is effective provided adequate time is spent to accustom the bull or steer to the harness. Similarly the cow can be painted on the butt of the tail and the mounting teaser rubs and disturbs the paint. Alternatively, the teaser can be painted on the sternum and the cow is marked when mounted, the painted sternum is pushed across the loin of the cow. The method can be adapted to hormone-treated cows or steers or nymphomaniac cows. Used on steers or bulls or cows, it effectively marks cattle for AI. or experimental purposes (Lang et al. 1968).

Examination of cervical mucus. Mucus is taken at or around oestrus from the midcervical canal. It is dried on a glass slide and examined under a microscope. A fern leaf pattern can be identified. If the cow is not in oestrus there will be very little or no pattern (Alliston et al. 1958, Howes et al. 1960).

Attempts have been made to make the cervical mucus test more quantitative (Lamond and Shanahan 1968); this has involved analysis of the cervical mucus for chloride and total protein content and from this an index has been developed. Another analysis of mucus involves the measurement of the electrical conductivity or impedence of the ionic flow. An instrument has been developed to measure this and can indicate the time of ovulation as the period with the least impedence to ionic flow (Carter and Dufty 1980).

Rectal and vaginal examination Rectal palpation as a procedure for the examination of the reproductive organs, provides invaluable information for the veterinarian. When using this technique it should be remembered that there are risks to both the cow and the operator. These are minimised by an experienced operator who should ensure that there is adequate restraint of the cow and minimal discomfort or damage to sensitive tissues. As a preliminary to rectal palpation, examination of the vagina by means of an illuminated speculum and external reproductive genitalia will often reveal some of the classical signs of oestrus, particularly swelling of vulval lips, reddening of the mucous membrane of the vagina and the presence of a copious, clear vaginal mucous discharge.

Rectal, palpation of the uterus and ovaries during oestrus should reveal a uterus with increased tone and a tense follicular swelling in the ovary.

At ovulation, the tense and distended ovary collapses, thus decreasing its size. This change is quite apparent when a sequence of observations is made, thus delineating the time of ovulation. In general, the rectal examination is used to check oestrus detected by other means, in special situations, or in AI. Detection of ovulation does not necessarily mean that oestrus has occurred or vice versa, but such checks can be of considerable value in cases where the cow has not been observed in oestrus.

Pregnancy diagnosis by rectal palpation is considered to be an accurate and reliable technique if carried out by experienced operators (Zemjanis 1970; Ball 1980). The associated techniques of "membrane slipping" and palpation of the amniotic vesicle, as described by Zemjanis (1970), are important for accuracy in certain situations although the former technique may possibly cause embryonic loss if performed injudiciously (Ball 1980).

Direct Visual Examination of the Reproductive Organs

This involves the insertion of an endoscope through a small incision in the paralumbar fossa of the cow (Megale et al. 1956). This technique was modified by Lamond and Holmes (1965) and Baker (1966). When repeated observations of the ovary over a short period are required, a plastic or stainless steel canula can be fitted into the incision. While the cannula is in place, the ovaries may be observed at any time over a two-day period. It is possible to see the entire surface of the ovary in most instances, both ovaries being examined through one side of the animal. The technique may result in complications such as peritonitis.

Baker (1968a) developed an intravaginal technique which enabled up to four successful ovarian sightings per cycle in individual cows. No apparent deleterious effect on ovarian function was observed, but adhesions developed around the ovaries in most cows.

Control of the Breeding Cycle

A successful application of AI. in beef cattle is its use in conjunction with the control of the breeding cycle. The control can be achieved by management procedures such as restricted breeding season, strategic weaning and restricted suckling periods or by the use of exogenous hormones which control ovarian activity,

Hormonal control The endocrine control of the oestrous cycle and ovulation in cattle has been reviewed (Smith 1976). Attempts at controlling the oestrous cycle are aimed at inducing oestrus and ovulation at a pre-determined time. This has the greatest application in the associated use of AI., particularly if the degree of control is such that fixed time insemination can be used without regard to the onset of oestrus.

Following initial experiments, numerous investigations reported the suppression of oestrous behaviour and ovarian activity in cattle by daily injections of 20–100 mg progesterone for 7 to 24 days. Oestrus occurred 3 to 6 days later. Although it reliably controls the cycle, the conception rate at the synchronized oestrus is usually lowered. The use of oestrogens in conjunction with progesterone improved synchronization but with no improvement in fertility (Ulberg and Lindley 1960).

Progestins have also been used successfully to synchronize oestrus and ovulation by oral administration in the form of feed additives to dairy cows for 15 to 20 days (Cole and Cupps 1969). Such progestins include medroxyprogesterone acetate (MAP) (Zimbelman 1964; Hansel et al. 1966), melengestrol acetate (MGA) (Zimbelman and Smith 1966) and progesterone (Wiltbank et al. 1967). However, conception rates were lower than in control animals. Fertility was not improved by injections of pregnant mare serum gonadotrophin (PMSG) and human chorionic gonadotrophin (HCG) after the last feeding of MAP (Jainudeen and Hafez 1966).

Control of the oestrous cycle has also been accomplished by the use of intravaginal polyurethane foam sponges or silastic intravaginal devices (PRID), both impregnated with a progestin, or subcutaneous silastic implants impregnated

with progesterone (Carrick and Shelton 1967; Shimizu et al. 1967; Smith 1974; Roche 1976). Experimentally this method has not produced higher conception percentages than orally or intramuscularly administered progestins.

Research more recently has been directed towards the use of luteolytic agents such as prostaglandin PGF or one of its analogues. An intra-uterine or intramuscular injection in the luteal phase of the oestrous cycle, in a normal cycling cow will result in oestrus within 4 days of treatment (Rowson et al. 1972; Cooper and Furn 1974; Hernshaw et al. 1974) or, if the stage of the cow's cycle is not known, double injections of PGF 11 days apart will ensure that the animal will be within the luteal phase and should therefore respond. This effectively synchronizes cycling cows, but it does not have any effect on anoestrous animals (Nancarrow and Radford 1976). There is no evidence to suggest that, in normal cycling animals, fertility is impaired following PGF treatment (Donaldson 1977).

Baker et al. (1980), in an AI. trial involving over 1,200 cows, showed that there was a significant difference in conception rates between PGF treated cows (42%) and PRID treated cows (50%). This was in agreement with previous reported trials (Roche 1976; Smith et al, 1978).

The Induction of Parturition

This is a technique which was developed from the experimental work of Liggins (1973). The application of the technique has reached significant commercial proportions in New Zealand and some southern states of Australia (Bailey et al. 1973; Welch et al. 1973).

One method consists of an intramuscular injection of a long-acting glucocorticoid approximately 4-6 weeks before natural calving is anticipated. This results in the birth of a calf about 10–14 days later and can therefore be used to shorten the pregnancy by 3-4 weeks. By the use of short-acting corticosteroids, calving can be induced within 5 days of injection. Sometimes a combination of long-acting and short-acting corticosteroids are used. Also oestrogen has been used in conjunction with the corticosteroids (Grunert et al. 1975).

Prostaglandins have also been used to induce calving up to 18 days prematurely. A high percentage of induced cows (70%) calve within 30-70 hours after injection (Johnson et al. 1982). This shortening of the gestation period allows the dairy farmer to manipulate time of calving to coincide with the flush of feed and the maximal price for milk.

From observations of over 70,000 cows in New Zealand and Australia, the signs of vulval swelling, relaxation of pelvic ligaments and enlargement of the mammary glands are normal. The most serious disadvantage of induced parturition, particularly with the long-acting corticosteroids, is the increased perinatal mortality. This is primarily due to the calf being unable to absorb the immunoglobins of the colostrum and so has no disease resistance. This is due to a direct effect of the corticosteroid and is not related to prematurity of the calf (Bailey et al. 1973). On some farms losses have reached as high as 80% and on others with better management losses were nil. In some instances a high incidence of retained placenta can also be a problem, especially if the short-acting costicosteroids or prostaglandins are used to induce calving.

Sex Determination

All attempts at sex determination by identifying the X and Y carrying spermatozoa have failed (Bhattacharya et al. 1966). Some experiments have

involved the effect of alkaline or acid media on the cow's reproductive tract in the belief that there is a selective effect on X or Y bearing spermatozoa. Other techniques have involved the assumption that because there is a difference in size between the X and Y chromosomes there must be an overall difference in mass of the two types of spermatozoa. Experiments in counter stream centrifugation, electrophoresis, sedimentation in selective media, and the immunological techniques have been attempted (Hegde et al. 1981). Although claims of success have been made these have not been substantiated.

REPRODUCTIVE MANAGEMENT PROCEDURES IN CONTROL OF BREEDING

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Reproduction is one of the most important economic traits in commercial beef production. The greatest reproductive losses are due to cows failing to become pregnant.

Restricting the breeding season and calving seasons to relatively short periods is the first step in achieving and maintaining a high level of reproductive performance in beef herds. Many of the problems contributing to poor reproductive efficiency can be attributed to a "strung out" or year-round breeding and calving pattern. In most regions of the world there is an optimal period for calving. The aim is to achieve as many calves born during this period as possible.

The concept of a limited breeding season presupposes an inter-calving interval of approximately 12 months. To achieve this, there is a limited post-partum period of 75 to 90 days in which females must cycle and become pregnant. The greatest limitations to achieving re-mating during this period are levels of nutrition (both pre and post calving), the effects of lactational stress and interactions between the two.

The nutritional requirements of pregnant and post-partum beef females have been well documented (Wiltbank et al. 1962; Dunn et al. 1969; Corah 1978) and are beyond the scope of this review. However, recent developments in simple management practices to increase cyclic activity in post-partum cows have shown that such practices, in conjunction with adequate nutrition, have great potential for exploitation within the beef industry.

Weaning Stratagems

Suckling delays post-partum reproductive activity in cows (Baker 1969; Short et al. 1972; Bellows et al. 1974; Berndtson 1977). This effect is most evident in younger females where growth requirements compound the problem. It is also more evident in Bos indicus crossbred cattle in comparison with Bos taurus cattle. Baker (1969) showed that suckled Bos indicus crossbred cows, provided they did not have unduly low bodyweight, demonstrated oestrus between 90–140 days post-partum irrespective of when the calves were weaned at varying periods between 95 and 188 days. If weaned at 10–14 days, the post partum oestrus occurred within 50 days, and if weaned at 3 days or less, within 35 days.

It was also found that if the liveweight of the lactating cows was low at the commencement of the lactation (mean liveweight 303 kg) and maintained at this level during the lactation period there was a high correlation between the length of the suckling period and the interval to first oestrus.

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However, it is impractical to recommend early weaning as a universal tool for improving reproductive efficiency of young beef cows as considerable management effort is required. Nevertheless, under some conditions, early weaning of calves as early as 45 days of age may be advantageous (Rose et al. 1963; Armstrong et al. 1968; Baker 1968b, c).

One alternative to early weaning is once-daily suckling. Research with Brahman cross females in the U.S.A. has established a regimen whereby calves are penned at 30 days of age and the free grazing cows are allowed to suckle their calves for one 35-40 minute period per day. At the end of 45 days, the calves are returned to their dams for normal rearing. Trials with first-calf heifers showed that this regimen reduced the post-partum interval to oestrus; there was no difference in the weaning weights of the treatment and control groups (Randel et al. 1976, 1978). As with early weaning, the greatest effects of once-daily suckling were shown to be in the first-calf heifers.

In an experiment to define further nutritional interactions with once-daily suckling it was shown that, although interactions did occur, the manipulation of suckling had the greatest effect on time to return to oestrus post-partum (Randel et al. 1977).

These and other trials established that once-daily suckling by calves from 21 or 30 days of age dramatically decreased post-partum interval in first-calf heifers at either high or low levels of energy intake. At high energy levels, 100% of once-daily suckling heifers had at least two opportunities to become pregnant by 90 days post-calving. In the low energy heifers 71% had at least one chance to become pregnant during this period. Overall milk production, calf weight at weaning and calf health were not affected by the treatment.

Another management procedure which has been shown to effectively reduce lactational anoestrus in beef females is temporary calf removal. This technique was used as an adjunct to oestrous synchronisation with progestagen implants in lactating females (Wiltbank and Mares 1977). Temporary calf removal for 48 hours at the time of implant removal increased oestrous response and pregnancy rates.

Subsequent trials (Smith et al. 1979) confirmed that temporary calf removal in conjunction with progestagen treatment increased the percentage of cows in oestrus and pregnant after 4 and 21 days of mating. The use of temporary calf removal in conjunction with prostaglandin synchronization trials has not been well documented. In a trial with Santa Gertrudis cows, 48 hour calf removal in conjunction with a two injection PGF_2 treatment, achieved an advantage of 7.2% in pregnancy rates Qver the conventional (non-calf removal) treatment (Chenoweth unpublished data). In contrast to employment with progestagen regimens, temporary calf removal with prostaglandin treatments should be achieved either before or immediately after the first prostaglandin injection. Although the optimal duration of calf removal has not been fully resolved, the present recommendation is for 48 hours, with lesser periods showing greater variation in cow response and longer periods being of no apparent benefit. Temporary calf removal has also been shown to be advantageous in inducing cyclic activity in lactating anoestrous females not subjected to oestrus synchronization.

Temporary calf removal therefore provides a useful mechanism for inducing oestrus in lactating females. However, best results are obtained with cows on an adequate energy level and whose calves are at least 50 days old.

Biostimulation (Presence of the Male)

There is considerable evidence from a number of species that the male has a

stimulatory effect on oestrus and ovulatory responses in females. This effect has been termed biostimulation (Fraser 1968). Biostimulation can accelerate the onset of puberty in females undergoing seasonal or lactational anoestrus and alter the time periods associated with oestrus and ovulation. Although the neuro and endocrinological mechanisms are unknown, biostimulation may be caused by: (i) direct genital stimulation, (ii) allelomimetic cues, or (iii) pheromones or similar biochemical substances.

In domestic animals of economic importance the effects of biostimulation are most evident in sheep and swine where management techniques are commonly employed to exploit this phenomenon. Such techniques include the sudden introduction of rams to ewes during the transitional periods from the "non-breeding" to the "breeding" season to stimulate group cyclic activity, and the exposure of peripuberal gilts to boars to advance and synchronise puberty.

In cattle, the effects of biostimulation are less dramatic. Randel et al. (1973) have suggested that cows may not be completely spontaneous ovulators and that various stimuli such as cervical and clitoral stimulation and the presence or introduction of males can influence the timing of LH peaks, oestrus and ovulation. Data suggestive of a biostimulatory effect have come from breeding programs where AI. and natural breeding were compared. Although there are grave difficulties in comparing pregnancy and/or calving rates due to AI. and natural service, a number of studies have reported an advantage for the latter (Stewart 1952; Mattner et al. 1974; Elving and Govers 1975; Langley 1978). This advantage, which has not been discerned in other studies (Donaldson 1968; O'Farrell 1977; Williamson et al. 1978) could be caused by a number of factors of which biostimulation may or may not be of greatest importance.

In those studies showing an advantage for natural service over AI., part of this advantage could be caused by genital stimulation of the female by the bull either before or during service. Nuzzling, nudging and licking of the perineal region of the female by the bull can act to induceoestrousbehaviour (Fraser 1968) and also to prepare the female genital tract for optimal gamete transport. The act of intromission provides the female with an important source of genital stimulation which, in some species (e.g. the cat and the rabbit) is necessary for ovulation and in others (e.g. the laboratory rat) is necessary for inducing the "progestational state" necessary for pregnancy (Clemens and Christensen 1975). That genital stimulation can favourably influence pregnancy rates was shown in several studies on the effects of clitoral stimulation during AI. (Randel et al. 1975; Short et al. 1979). In these studies, clitoral stimulation improved pregnancy rates by 6.3 to 7.5 per cent in cows but no advantage was seen with yearling heifers.

Other reports have shown positive effects from the presence of bulls or bull-like behaviour on oestrous behaviour and pregnancy rates in females. Fraser (1968) stated that teaser bulls, when run with newly calved cows, caused oestrous signs to be shown much earlier than in controls which were not run with bulls. Similar findings were reported by Kiddy (1978), whereas Weston and Ulberg (1976) observed that control cows responded with oestrous behaviour similar to their progestagen-synchronized herdmates when both were run together. In this latter study, this effect could be caused by either an allelomimetic type of response in the control cows, or a response in these cows to some unidentified environmentalor pheromonal factor associated with the treated cows (Britt 1978). Pexton et al. (1977) reported that in two trials employing oestrus-synchronized females, the presence of teaser bulls during the period after implant increased both oestrous and pregnancy rates significantly above control animals which were not placed with teaser bulls. However, Fields et al. (1975) found no effect of bull presence on pregnancy rates of multiparous cows inseminated at first service, although

oestrous detection and repeat breeder pregnancy rates were enhanced ($P < 0.0005$). Chenoweth (in press) showed that a significant improvement occurred in oestrous response in heifers synchronized with prostaglandins when androgenised cows were placed with the heifers during the interval between injections. Skinner and Bonsma (1964) found that the mean time to mating after introduction of a bull to cows run alone was 19.3 days compared with 9.5 days in cows which had been previously run with a vasectomized bull. Work with swine and sheep indicates that in these species, biostimulation is most effective when intact males areintroduced as a novel stimulus and that the female response is largely influenced byolfactory cues (Signoret 1980). It remains to be determined whether bulls of high libidoare more effective as biostimulators than bulls of low libido, as has been shown to be the case with sheep (Lindsay and Signoret 1980).

TECHNIQUES INVOLVING EMBRYOS AND OOCYTES

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Embryo Transfer

Embryo transfer has been described in many farm species and the techniques have been reviewed by Betteridge (1977).

In Australia, embryo transfer has been used in sheep, cattle and goats in which the techniques are similar in essence. Multiple ovulation of donor animals is stimulated by pregnant mare serum gonadotrophin (PMSG), horse anterior pituitary extract (HAP) or follicle stimulating hormone (FSH). These are given late in the luteal phase of the oestrous cycle, in mid-cycle followed by prostaglandin, or towards the end of a period of progestagen treatment. Prostaglandin or progestagen controls the time of oestrus with resultant greater predictability of the ovarian response to gonadotrophin. Pituitary extracts and purified FSH have some advantages over PMSG (Moore and Shelton 1964; Moore 1975; Armstrong et al. 1981). The persistence of activity of PMSG causes continued follicular growth and associated oestrogen production which is detrimental to fertilization and embryo development. There is evidence that this effect may be significantly reduced by anti-serum to PMSG (Bindon and Piper 1977).

In superovulated donors abundant numbers of spermatozoa are necessary to ensure high rates of fertilization; this can be achieved by multiple inseminations, or,in the ewe,by surgical insemination (Trounson and Moore 1974), or by laparoscopy (Killeen and Caffery 1982).

In the ewe and doe,laparotomy is necessary for collection of embryos and there seems little prospect of a non-surgical approach. This limits the number of times a donor may be used. Nevertheless, Moore and Shelton (1962) used the technique to speed up development of a Poll Merino flock, and it has made a significant contribution to Angora goat breeding in Australia (Moore 1974).

In the cow the most significant recent development is the emergence of non-surgical techniques for the collection and transfer of embryos. Non-surgical collection was attempted several decades ago (Dowling 1949) but only recently has it become a practicable technique. Itsdevelopment was stimulated by the realisation that animals do not become refractory to repeated use of PMSG in superovulation regimens. Thus the loss is not important if an attempted collection is not successful. There now is ample evidence that embryo collection rates approaching those of the surgical method can be achieved by competent operators

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(Elsden et al. 1976; Newcomb et al. 1978; Baker and Jillella 1978; Shelton et al. 1979). These methods have changed embryo transfer from a sophisticated surgical procedure requiring elaborate facilities to an on-farm operation. It would be a mistake, however, to conclude that the requisite level of technical skill is less; in fact, it is greater. The published data provide clear evidence that operator differences exceed other sources of variation in results.

Since the first report of successful freezing and thawing of embryos (Whittingham 1971), many investigators have examined the effects of cryoprotectants, freezing rates, thawing rates and their interactions on survival of sheep and cattle embryos (Wilmut and Rowson 1973; Willadsen et al. 1976; Bilton and Moore 1977; Lehn-Jensen et al. 1981). Until recently the simplest successful technique involved two-step freezing and rapid thawing with step-wise addition and elution of the cryoprotectant. However, Leibo et al. (1982) described one step freezing of bovine embryos in 1.5 m glycerol in 1/4 cc plastic insemination straws. After rapid thawing the glycerol was diluted with sucrose within the straw which was then used for non-surgical transfer with a resultant pregnancy rate of 36.7 per cent from 313 transfers (Leibo 1983). Most commercial interest involves frozen cattle embryos which are being used in international transport and in special breeding programmes. There are quarantine protocols for theimportation of frozen embryos from several countries, and one wonders whether adequate cognisance is taken of this technology in considering the alternatives for facilitating the import of genetic material from many other countries.

With non-surgical methods and embryo freezing techniques the stage is now set for a scenario of banks of frozen embryos which can be transferred on-farm to surrogate dams in the same way as artificial insemination now is performed. It is likely that this exercise could not be justified at present by the results which are the consequence of multifactorial variables such as embryo quality, survival of freezing-thawing, endocrine status of recipient and skill of technician. While the efficiency of each step remains at 40-60 per cent the end result is not commercially attractive. Clearly more developmental research is needed in these areas.

Genetic Aspects of Embryo Transfer

Embryo transfer increases the intensity of selection on the female side and the resultant increase in annual genetic improvement can range from 5 per cent to 100 per cent depending on species, breed, trait, extent of use of AI. and other management procedures. This aspect of embryo transfer has been reviewed by James (1974), Bradford and Kennedy (1980) and Rathie (1981). In a large population of dairy cattle where AI. is used effectively, embryo transfer is likely to add less than 10 per cent of the rate of genetic gain. Rathie (1981) suggests that in a radical scheme using bulls and donor females for one breeding season only, selecting bulls on full and half sib information and females on pedigree information, an annual genetic gain of 2 per cent can be achieved. This response is similar to that expected in a national dairy breeding programme using progeny testing.

The potential of embryo transfer for increasing the rate of genetic improvement appears to be much greater in beef cattle because of their low fecundity and the high heritability of growth rate. In addition to selection programmes there are other situations in which embryo transfer may be helpful in a genetic context. These include rapid multiplication of a rare genotype and progeny testing for genetic defects.

Embryo Transfer in Research

Embryo transfer allows the research scientist to place an embryo (or embryos) of selected genotype in a surrogate dam of selected genotype. This can aid in the study of development and differentiation in the embryo, interactions between embryo and mother and between embryos. Physiological events, elucidation of which may be aided by these studies, include maintenance of pregnancy, foetal growth and endocrinology of parturition. In a more practical context, we have a powerful tool for the study of embryonic mortality and the transmission of certain diseases.

Twinning

Rowson et al. (1971) demonstrated production of twins in cattle by embryo transfer and more recently twin pregnancies have been induced by the non-surgical transfer of two embryos or transfer of one embryo to a previously inseminated cow (Anderson et al. 1979). Survival of these contrived twins, pre-natal and post-natal, has been inconsistent. The results of Summers, Shelton and Edwards (Anim. Reprod. Soc., in press) strongly suggest that in induction of twinning by embryo transfer, it is desirable to use embryos of similar potential duration of gestation because the earlier maturing calf regulates the onset of parturition and a co-twin of longer potential gestation will be premature with concomitant hazards to survival.

Twinning probably would appeal to only a small segment of breeders and would require an assured supply of embryos. In future these might be obtained by in vitro fertilization of oocytes from slaughter animals. The final proof of in vitro fertilization is the birth of viable young and only recently Brackett et al. (1982) reported the birth of a normal calf from an embryo derived by in vitro fertilization of an ovum. Clearly a great deal of developmental work will be necessary before in vitro fertilization becomes a useful technique in animal production.

Cloning

No one has succeeded in activating an undifferentiated mammalian cell by transfer of nuclear material from an adult cell. Perhaps this is not possible. Nuclear transplantation between embryonic cells has been performed (Illmensee and Hoppe 1981) and this approach permits the synthesis of limited clones. Another method of doing this is by selection of embryos before development to the blastocyst stage. Willadsen and Polge (1981) have produced monozygous twins and triplets by this method.

At this time, a stud breeder cannot synthesise replicates of his valuable and fashionable breeding animals. It is possible, however, to divide a 5 or 6 day cow or sheep embryo into four masses of cells, each of which can be cultured to a blastocyst which may be frozen for future use. Alternatively, one "part embryo" may be transferred immediately while identical sibs are stored in liquid nitrogen. This is potentially useful if one can predict that a certain mating has a high likelihood of producing outstanding progeny.

Homozygous Livestock

Because they are not homozygous, no farm animal can produce offspring the same as itself. There may be instances where homozygosity would be desirable. In-bred strains of laboratory animals can be produced by brother-sister matings for 20 generations but this is not a practicable procedure in species with long generation intervals. Markert and Petters (1977) produced homozygous mouse

embryos by removing one pronucleus shortly after fertilization and then by cytochalasin B treatment transforming the remaining nuclear material from the haploid to the diploid state. These embryos survived to a few days before term but it has not been possible to discern whether the mortality was due to technical problems or to low viability or completely homozygous embryos. Hoppe and Illmensee (1977) produced seven live female mice from 135 manipulated eggs. Six of these were fertile and gave birth to progeny corresponding only to the pronuclear genotype of the mother. If this synthesis proves technically feasible, it could have some interesting applications in livestock breeding.

DNA Transfer

Genetic engineering is revolutionising many facets of applied biological science. Engineered bacteria are producing a variety of protein hormones and there is speculation about the use of genetic engineering techniques in human patients to correct defects attributable to single genes. Foreign genes have been injected into mouse eggs with subsequent demonstration of the gene product in the resultant animal, and in some cases the gene has been incorporated into the germ cell line as evidenced by its transmission to the next generation. The most spectacular demonstration to date of this technology is the dramatic growth of mice that developed from eggs microinjected with metallothionein - growth hormone fusion genes (Palmiter et al. 1982). There are some technical difficulties in applying gene transfer to livestock and, while there are significant single gene effects, many of the economically important characters are under polygenic control. Transfer of multiple genes into the genome would be a formidable task even if they could be identified and a further hurdle might be the "turning-on" of their expression. Thus, while this technology offers exciting prospects, the realisation of practical application in animal breeding may be some time off.

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