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EGG TRANSFER AS AN ADJUNCT TO TWINNING IN CATTLE

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Egg transfer has fascinated scientists throughout the world for many years and more recently has received a new impetus from the international trading of "superior genes", whereby fertilized eggs may be transferred from "exotic" breeds to incubator cows. In this fashion genetic improvement can be achieved without the expense and disease hazard of importing the live animal. Improved techniques for storage, transfer and precise timing of ovulation between donor and recipient have also played a major role in this renewed upsurge of interest.

The earliest recorded success with egg transfer was the report of Heape (1890) who used the technique to show that the offspring were not influenced by the genetic characters of their foster parents. Since this pioneering work a number of comprehensive reviews and papers have been published on the subject (Schilling, 1965; Dzuik, 1969; Foote & Onuma, 1970; Gordon, 1969; Baker, 1973; Polge, 1965, 1972; Rowson, 1970, 1971a, 1971b; Polge & Rowson, 1973). The technique of egg transfer as employed by Hunter, Adams & Rowson (1954) laid the foundation for most of the subsequent work in sheep and cattle. More recently it has been shown that satisfactory results can be obtained from egg transfer in cattle if strict attention is paid to synchronization of ovulation of donor and recipient, egg culture medium and the transfer technique itself (Rowson, Moor & Lawson, 1969; Rowson, Lawson, Moor & Baker, 1972). It has also been reported that a high incidence of twin-pregnancies (73%) can be induced using egg transfer (Rowson, Lawson & Moor, 1971). It was further demonstrated by Rowson, Moor & Lawson (1969) that, following the surgical transfer of two cow eggs to the uterine horn adjacent to the ovary containing the corpus luteum, the percentage of resulting pregnancies was high (91%), but the percentage of twins actually born was low (12,5%). It was postulated that the low percentage of twins might be due to:

1. Failure of one of the eggs to migrate to the other uterine horn and consequent implantation of both embryos within the same uterine horn leading to competition for survival, or

2. Occurrence of migration but failure of pregnancy to continue in the contralateral horn owing to the absence of a corpus luteum in the ovary adjoining that horn. Such a unilateral relationship is known to exist in both cattle and sheep if the embryo is actually confined to one horn.

Gordon, Williams & Edwards (1962) reported that twins occur naturally at a higher proportion when a *corpus luteum* is present in each ovary than when two *corpora lutea* are present unilaterally. Erdheim (1942) also reported that out of thirty-six cases of twinning in beef and dairy cattle, only five were unilaterally pregnant. Perkins, Olds and Seath (1954), upon examination of 255 cattle pregnancies, revealed only four cases in which an egg had migrated to the contralateral horn. Gordon – unpublished data, found that the incidence of twinning was influenced by ovulation location.

Table 1

The Influence of Ovulation Location On Twinning in Cattle [a]

	Location of Ovulations		
	Both in left ovary	One ovulation in each ovary	Both in right ovary
Cows	7	39	21
Pregnant with twins	2	24	6
% with twins	28,6	61,5	28,6

[a] Gordon – Unpublished data.

With the knowledge at our disposal several conditions must be adhered to before transferred eggs survive and grow to viable offspring. Oestrus of donor and recipient must be synchronized, fertilized eggs must be produced and recovered, recovered eggs must be stored *in vitro* for a certain duration and a reliable transfer technique must be employed.

Timing of Ovulation

Another important aspect of egg transfer is the exact synchronization of oestrus between the donor and recipient. Because of the vagaries of ovulation date, some form of control is imperative for synchronization. The most widely used technique for control of oestrus has been through the withdrawal of the inhibitory effect of progesterone or a progestagen-like compound for a few days prior to the desired breeding time (Scanlon, Burgess, Neville, Wilton, Stone & Macpherson, 1971). In the past emphasis was placed on orally active progestagens, eg. Medroxyprogesterone acetate (MAP, Upjohn) Melengesterol acetate (MGA Upjohn or Chlormadinone acetate (CAP Syntex). However, despite the degree of synchrony obtained, the conception rates to subsequent heats were very encouraging

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but the degree of synchrony was less than satisfactory. Other techniques being used employ synchronization using a relatively short term (9 day) progestagen feeding regime in conjunction with an injection of oestrogen on the day of treatment (Wiltbank & Casson, 1968; Scanlon, Neville, Burgess & Macpherson, 1971). A promising technique currently being tested involves the Searle implant in combination with an oestradiol/high progestagen injection given at the time of insertion. The oestrogen/progestagen combination appears to be effective in inducing corpus luteum regression irrespective of the stage of the cycle. This enables a short-term progestagen treatment to be employed which is considered a necessary prerequisite for high fertility at the controlled oestrus.

The recent introduction of prostaglandin (PGF 2 α) appears to have exciting possibilities for precise timing of ovulation and oestrous control and could well be instrumental in the widespread application of egg transfer and twinning in cattle. (Liehr, Marion & Olson, 1972; Louis, Hafs & Morrow, 1972; Rowson, Tervit & Brand, 1972; Shelton, 1973; Tervit, Rowson & Brand, 1973; Neville, 1974). Prostaglandin (PGF 2 α) exerts its action by causing regression of the corpus luteum prior to the natural physiological onset of luteolysis. To be effective it must be administered between days 5 and 16 of the oestrous cycle (Rowson *et al.*, 1972). However, this inconvenience can be circumvented by administration of PGF 2 α to a group of cows picked at random and another treatment 11 days later. In this fashion all cows will be at the appropriate stage of the cycle and oestrous control can simply be reduced to a two day treatment (Lamming, 1974 – personal communication). The pregnancy results obtained using PGF 2α) – were significantly higher than those obtained from natural onset of oestrus. It was also shown that breeding to a predicted time-table resulted in conception rates comparable to A.I. under conventional conditions. These findings have tremendous significance for controlled breeding of beef cattle, especially in Zebu breeds where it has been suggested that the duration of heat is very short and hence the difficulty in detection of oestrus (Avis & Ehret, 1974 – personal communication). Likewise, precise control of ovulation would greatly facilitate egg transfers. (Rowson *et al.*, 1972).

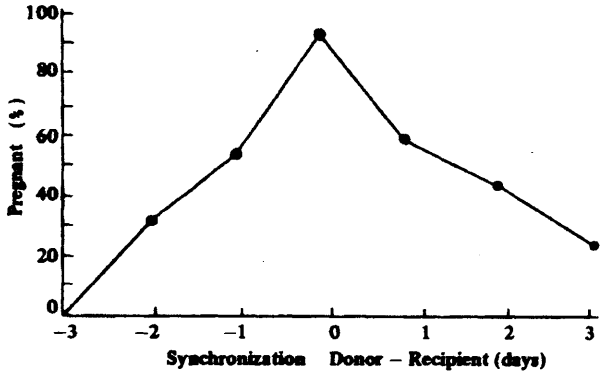

Fig. 1 *Pregnancy rates obtained following synchronous and asynchronous egg transfer to recipient cows (Rowson et al., 1972).*

Production of Fertilized Eggs

The primary prerequisite of any egg transfer programme is the ready supply of fertilized eggs from suitable donors. Fertilization in itself is the culmination of a series of complex and diverse phenomena and a concerted effort must be made to insure that only cattle of normal reproductive status are used. Despite the wealth of information on increasing ovulation rates, there is still no reliable method available for obtaining eggs consistently and continuously. Because of the problems encountered in superovulation and oestrous synchronization, it frequently happens that only 50% of transfers planned are completed.

There are a number of approaches to the problem of producing fertilized eggs, namely, superovulation, repeated or super-super-ovulation, gonadotrophin treatment in the prepuberal calf and the use of follicular oocytes from slaughtered or live animals, which have been matured and fertilized *in vitro*.

Superovulation

A number of authors have reported on cattle superovulation (Casida, Nalbandov, McShan, Meyer & Wisnicky, 1940; Casida, Meyer, McShan & Wisnicky 1943; Dowling, 1949; Rowson, 1951; Umbaugh, 1951; Hafez, Sugie & Gordon, 1963; Scanlon, Screenan & Gordon, 1968; McGaugh & Olds, 1971.

The administration of PMSG during the late stage of the oestrous cycle as a means of inducing a limited number of additional ovulations (doses of 1000 to 2000 I.U.) has been reported by Hammond Jr. & Bhattacharya (1944), Hammond Jr. (1949) and Gordon, *et al.* (1962). The most consistent feature of these reports was the great variability which occurred in the number of ovulations after administration of any given dose (Gordon – unpublished data, Table 2).

Table 2

Superovulatory response to PMSG [a]

PMSG(IU)	% Multiple Ov.	Range of Ov.	Mean Ov.
1000	36,6	1–15	1,8
1500	65,8	1–23	4,2
2000	56,5	1–25	4
3000	96,0	1–58	19,5

a. Gordon – Unpublished data.

One factor which can markedly influence superovulatory response between individual cattle is the interval which elapses between injection of the follicle stumilating preparation and the time of oestrus (Scanlon *et al.*, 1968 and Gordon – unpublished data, Table 3).

A number of workers have used PMSG treatment in conjunction with expression of the corpus luteum by mani-

Table 3

Ovulatory Response as Affected by Interval Between PMSG Injection and Breeding [a]

	Interval-PMSG Injection to HCG and Breeding (days)				
	1	2	3	4	5
Cattle	1	6	36	57	81
Ovulations	1	14	272	540	1486
Average Ovulation rate	1,0	2,3	7,6	9,5	18,3

a. Gordon – Unpublished data.

pulation per rectum (Dowling, 1949; Avery, Fahning & Graham, 1962). More recently PGF 2α in conjunction with PMSG has been used with good success (Tervit *et al.*, 1973). Luteolysis can also be induced even in cows with retained *corpora lutea* by administration of PGF 2α and Estradiol benzoate simultaneously (Neville, 1974). However, it has been reported that the superovulatory effect of PMSG in cattle can vary markedly with the particular batch employed (Baker, 1973; Polge & Rowson, 1973). Similar findings have been reported for other species (Neville, 1970).

Gonadotrophin treatment in the prepuberal calf

Pregnant mare serum gonadotrophin and follicle stimulating agents have been used to induce multiple ovulation in the calf from a very early age (Onuma & Foote, 1969a, b; Onuma, Hahn, Maurer & Foote, 1969; Onuma, Hahn & Foote, 1970; Seidel, Larson & Foote, 1971; Seidel, Larson, Spilman, Hahn & Foote, 1971). The use of prepuberal calves has not been particularly successful as a source of eggs for egg transfer.

Super-super-ovulation

Reduced response to repeated superovulation has been reported (Willett, Buckner & McShan, 1953). However, Dzuik, Donker, Nichols & Peterson (1958) failed to find evidence of any consistent reduction in the response of cows treated on more than one occasion with gonadotrophins. More recently, Scanlon (1972) reported the induction of substantial superovulatory responses after the second of two PMSG treatments applied during two successive oestrous cycles.

Follicular Oocytes

An eventual alternative to superovulation as a means of providing a ready flow of fertilized cattle eggs may prove to be the *in vivo* or *in vitro* maturation and fertilization of follicular oocytes. Numerous studies have been conducted in this area (Sreenan, 1968, 1970; Crosby & Gordon, 1971; Crosby, Ryan & Gordon, 1971; Quirke & Gordon, 1971a, b; Hunter, Lawson & Rowson, 1972; Shea, Bedirian & Baker, 1973).

Despite the abundance of literature available on follicular oocytes it appears that the ovarian oocyte is still not an acceptable alternative to the supply of eggs from superovulation in cattle. Sreenan, Scanlon & Gordon (1968) & Sreenan (1969) reported that the most successful method of maintaining continued development of cow eggs was to transfer the eggs to oviducts of recipient rabbits for storage periods up to 5 days. Also some eggs continued to develop in rabbits following storage at 10°C for 20–46 hr. It was also reported that cattle oocytes could undergo maturation *in vitro* resulting in subsequent cleavage after transfer of the oocyte to oviducts of oestrus ewes which contained bovine semen. The work of Quirke & Gordon (1971) in sheep, opens the possibility of obtaining literally thousands of oocytes awaiting fertilization. However, the problems lie in achieving a maturation that is similar to that occurring in the ovary, for experience with laboratory and domestic animals has shown that there is something deficient in these oocytes and they fail to develop to full term foetuses after fertilization. Nevertheless, the increasing attention being paid to this problem in laboratory species could auger well for similar studies in farm animals (Edwards, 1972).

Egg Recovery

Surgical recovery of eggs

When the precise timing of ovulation is known, the recovery of eggs can be comparatively easy as the egg can be located in the oviduct or upper uterine horn. Egg recovery has been based on flushing of the uterus or oviducts with a suitable medium thereby carrying the eggs along simultaneously. Eggs are flushed under rigid aseptic conditions and stored in an incubator held at 30° to 37°C until transfer to the recipient is called for.

The importance of egg media, recovery and transfer techniques is well borne out by the work of Rowson *et al.* (1969). The most consistent results have been obtained from surgical recovery, surgical transfer and use of tissue culture medium (TCM 199) in transferring eggs between donor and recipient. Eggs are most readily recovered by using the mid-ventral laparotomy procedure described by Rowson *et al.* (1969). In this method donor cattle are anaesthetized using an initial pentobarbitone sodium injection followed by closed circuit anaesthesia (halothane and oxygen). An alternative to general anaesthesia is the local anaesthetic method as employed by Avery *et al.* (1962). The possibility of failure of transplantation after general anaesthesia cannot be discounted.

Non-surgical recovery of eggs

While non-surgical recovery of eggs is desirable the results to date have been discouraging. This area of endeavour has been the subject of intensive investigation and

several devices for non-surgical recovery of eggs have been developed (Rowson & Dowling, 1949; Dracy & Peterson, 1950; Dzuik *et al.* (1958). Sugie (1970) and Sugie, Soma, Fukumitso & Otsuki (1972) have reported encouraging results from non-surgical recovery of superovulated cattle using a two-way cannula device.

Results with slaughter recovery of eggs have been very discouraging (Rowson *et al.*, 1969). However, if slaughter material could be used the possibility of using follicular oocytes and/or superovulation could greatly facilitate at least one aspect in the egg transfer chain.

Recovery Media

Homologous blood serum alone or with equal portions of some physiological buffer or salt solutions has been the basis of most media for egg transfer purposes. Homologous blood serum does not appear to be useful for cattle egg storage during transfer when compared with TCM 199 as evinced by the work of Rowson *et al.* (1969). An essential prerequisite for egg transfer is that the cow eggs remain viable *in vitro* for several hours. Cattle eggs have been successfully stored at 10°C and appeared to resume normal development when transferred to the rabbit oviduct (Sŧreenan, 1969).

The work of Wilmut & Rowson (1973) in which they reported the birth of a live calf from an egg that had been stored in liquid N_2 (–196°C), marks a turning point in egg storage techniques. Freezing of fertilized ova would enable scientists to explore techniques for super-superovulation with the eventual goal of establishing frozen egg banks. Another exciting report has been that of Tervit, Whittingham & Rowson (1972) in which they suggested that a satisfactory combination of medium and gas phase enabled sheep and cattle eggs to be cultured in vitro from the early cleavage stages to morula and blastocyst stage.

Another important aspect of the egg transfer chain is the evaluation of eggs prior to transfer. The most one can hope for in this regard is that the eggs appear to have cleaved normally and that distinct blastomeres are apparent. Hafez (1961) has detailed in a very comprehensive review some of the common abnormalities which have been found in mammalian eggs.

Egg Transfer

Non-surgical Transfer

Non-surgical transfer of cattle eggs would greatly speed up the operation. However, the success rates from non-surgical transfer have been discouraging (Rowson *et al.*, 1969; Sugie, 1965; Rowson & Moor, 1966; Sŧreenan, 1969). The easy access to the uterus via the cervix as in A.I. of cattle offers the most exciting possibility. Rowson & Moor (1966) reported a 20% success rate (five calves born) after transfer through the cervix and subsequent insufflation of the uterus with CO_2. Sugie *et al.* (1972) using non-surgical transfer obtained 12 calves from 83 fertilized eggs transferred to 68 recipients. One of the major reasons for the low success rate with non-surgical transfer of eggs through the cervix in the possibility of infection as the bovine uterus is highly susceptible to infection during luteal phase of the oestrous cycle. (Lamming & Rowson, 1953; Rowson, Lamming & Fru, 1953). Another important aspect of this failure rate was evident from the finding that "artificial-resin-eggs" were ejected from the uterus readily by muscle contractions after cervical transfer (Bennet & Rowson, 1961; Harper, Bennet & Rowson, 1961).

Surgical transfer

The surgical approaches in the cow are by flank or mid-ventral laparotomy in a similar manner to that for egg recovery. The fertilized egg is drawn up into a pipette and the egg is placed either in the oviduct or uterine horn in accordance with the stage of synchrony of oestrus. Rowson *et al.* (1969; 1972) demonstrated that an acceptable pregnancy rate could be obtained by surgical transfer of eggs and the incidence of pregnancy was in fact greater than natural (91% vs. 65%).

While surgical transfer of eggs may be quite laborious, it appears at this juncture that it is the most acceptable technique at our disposal. The expense incurred in surgery can be offset by the tremendous genetic gain which can be readily and rapidly attained, especially in the exchange of "exotic" beef breeds or superior dairy breeds between different countries.

Twinning in Cattle

In a commercial cow-calf enterprise the profit margin is greatly influenced by the number of pounds of calf weaned per cow. Efficiency of production is primarily influenced by calving percentages. The calving percentage is subject to a variety of influences such as management, nutrition and breeding. However, while some of these influences are difficult to correct overnight, it would be possible to increase significantly the number of pounds of calf weaned per cow if each cow could produce twins.

Gonadotrophin induced twinning in cattle

Production of twins by this method has obvious advantages but the success rate using PMSG in conjunction with HCG has been limited. The primary reasons for this is the variability of response to PMSG (Gordon *et al.*, 1962 and Vincent, 1970).

A further difficulty with the hormonal approach is that even when the number of ovulations is successfully restricted to two, twins are usually not conceived if the two eggs are released from the same ovary. With the advent of PGF 2 α for precise timing of ovulation, it is possible that a more physiological oestrus will occur as a result of control. There is also some hope for a more consistent dose response from LH and FSH releasing factors.

Egg transfer – Induced twinning

Induction of twinning by egg transfer is one of the major practical benefits to be derived from such a program-

me. However, until the recent work of Rowson *et al.* (1971) in which it was clearly demonstrated that a much greater percentage of viable embryos were obtained (73%) in heifers that conceived following transfer of a single egg to each uterine horn rather than if two eggs were transferred to a single horn (45%), it seemed that egg transfer to the horn contralateral to the corpus luteum might not be possible due to the lack of a unilateral ovarian embryo relationship. It was suggested that failure to obtain satisfactory twinning rates in cattle when two eggs are transferred to a single horn may be due to the fact that transuterine migration rarely occurs with the result that embryonic death ensues due to overcrowding. It was demonstrated that the number of cotyledons to which embryos were attached was much greater for bilateral as compared to unilateral pregnancies (Rowson *et al.*, 1971).

From the foregoing it is evident that development of twins in each uterine horn provides a more favourable environment for growth and development of the embryo. No evidence for an increase in dystocia or retained placentae has been recorded where twins develop in each uterine horn.

The possible disadvantages of twinning are smaller calves at birth, high incidence of freemartinism and reduced butterfat content of milk. However, Gordon *et al.* (1962) clearly demonstrated that if careful attention was given to the nutritional status of the cow during the last two months of gestation, then the resultant calves were not significantly different in weight from single calves. The implication is that if twins are diagnosed during the latter trimester of gestation then attention can be given to the cow to offset some of the apparent stresses of twinning.

It must be recognised that freemartins will occur in twin pregnancies where the calves are of the male and female genotype. Nevertheless, where the major emphasis will be on twinning in beef cattle, this disadvantage becomes inconsequential. In the cow where the foetal membranes fuse in the majority of twin pregnancies, the resultant offspring will develop as chimaeras. Rowson *et al.* (1971) suggested that such chimaeras might be used to study production and development from say two different breeds. Ward (1950) and Cloninger & Thoele (1957) reported that a fall of 11 to 12 lb of fat per lacation occurred in animals which carried twins. This finding loses significance today where the emphasis is on low fat milk due to the link between fat intake and arterioschlerosis in humans. Cognizant of the fact that there are some disadvantages involved in a twinning programme, the advantages from the standpoint of an immediate increase in the production of red meat far outweigh the drawbacks.

While it appears that twins can be induced satisfactorily by means of bilateral egg transfer,a number of technical difficulties must be overcome before such a programme can be launched on a commercial scale. Factors such as oestrous control, superovulation, recovery, culture and transfer procedures will have to be streamlined before egg transfer can be applied at farm level. Despite the apparent difficulties a number of groups in the U.S. and Canada are promoting egg transfer as a means of introducing "exotic" breeds to the North American Continent. The expense incurred in transferring eggs from superior donors is dwarfed by the rapid genetic progress which can be achieved by using such a programme if and when technological difficulties have been overcome. At this juncture there is justification for egg transfer in nucleus cattle herds for introduction of new blood lines. The factor most likely to play a role in stimulating any egg transfer programme is supply and demand for red meat. If the demand is great enough, then priorities will be given to overcoming the technical difficulties and egg transfer as an adjunct to twinning in cattle will become a reality similar to A.I. in cattle with frozen semen. Egg transfer as a means of inducing twinning can find immediate application if the fertilized egg can be frozen and then inseminated via the cervix into the horn contralateral to the corpus luteum. Another aspect which would find immediate application would be the induction of twins by hormone administration, if the twins could be produced in each horn without the present variation in individual response to gonadotrophins.

Potential Applications of Egg Transfer

1. Increased production of offspring from selected individuals of superior genetic merit in order to increase meat and/or milk production.

2. Increased calf production by twin transfers especially in beef breeds using inferior recipients.

3. International import/export trade, particularly to introduce new breeds or blood lines.

4. Repopulation of herds after epidemic diseases such as foot and mouth disease, where a slaughter policy is imposed.

5. Salvage of genetic material where the superior donor cow can produce fertilized eggs, but cannot produce live calves.

6. Research applications such as production of artificial chimaeras by interchange of cells *in vitro* between developing blastocysts.

7. Production of twins, triplets or even quadruplets by using individual blastomeres.

8. Sexing of embryos prior to transfer by removing a few cells for examination of sex chromatin.

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