Egg Transfer in Cattle

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[Editor's Note: The authors refer to both "egg" and "embryo" in this paper because they consider anything up to the blastocyst stage (Day 7) as the egg (ovum) and refer to embryos after that time.]

The current widespread interest in egg transfer has stemmed from the inflated value placed on some European (exotic) breeds of cattle in the recent past by cattlemen and entrepreneurs in the English speaking countries. The inflated value, whatever its cause, was the principal reason for the commercial viability of the surgical egg transfer technique. It was also very fortunate that at this time of interest in exotic breeds, Rowson and his colleagues (1) at Cambridge had achieved a major breakthrough and had for the first time reported a high success rate (91% pregnancy) after surgical egg transfer.

This field of study has been dominated by American and British workers since its early beginnings. It was at Cambridge in 1890 that Walter Heape (2) first used the technique of egg transfer in rabbits and demonstrated that the resulting progeny resembled their parents and not their foster parents. Shortly after the acceptance of artificial insemination in cattle, research attention was focussed on the possibilities of artificial inovulation. In a wave of enthusiasm, the National Egg Transfer Breeding Conference was held in 1949 at San Antonio, Texas. Two papers in that year described devices for the non-surgical removal of bovine eggs (3,4) one of which (4) was commercially available. In the same year a popular article suggested that an increased number of calves might be obtained from one's best cows using the technique (5). The first report of a calf being born following transplantation of an egg came from American workers in 1951 (6). Since then there was little real progress until 1969 (1). It had long been known that bovine serum had ovicidal properties (7) and it wasn't until a serum-free tissue culture medium (TCM 199) was used to transfer bovine eggs (1) to recipients that high pregnancy rates were achieved.

The methods of recovery used to collect eggs rely on suspending the eggs in a stream of tissue culture medium. Either the oviduct or the uterus may be flushed or both may be flushed simultaneously (8). Using a method which separates the oviduccal eggs from uterine eggs it has been shown that even when eggs are recovered after Day 4–the normal time of entry to the uterus–a proportion (Day 5 - 17.4 ± 2.0%, Day 7 - 7.9 ± 3.6%) remain within the oviduct (9). It also appears that fewer of these eggs are degenerate compared to eggs recovered from the uterus. It may be that the oviduct in some way protects the eggs within it from the usual environment which probably exists in superovulated donors as a consequence of the abnormally elevated levels of steroids (10). The proportion of degenerate eggs recovered progressively increases as the time interval between oestrus and egg recovery increases (9). Evidence that this is an effect of the environment of the superovulated donor has been provided by studies in which Day 3 eggs were transferred to the rabbit oviduct. The majority of eggs continued to develop normally and fewer degenerated (11).

Transfer of bovine eggs to the rabbit oviduct is a useful means of assessing their viability over a short period (up to four days). When eggs stored in this way are retransferred to cattle, normal pregnancy rates result (12). It has even been suggested that the rabbit oviduct may act as a means of screening eggs and thereby obtaining an even higher conception rate (13).

The problem of the non-surgical recovery of eggs appears to be basically one of technical inventiveness. Already there is an apparatus which is commercially available (14) and doubtless more will follow, but because it is possible to flush only the uterus non-surgically, it will always be less efficient than surgical methods and it can only be used to collect uterine stages of eggs.

In common with other species, oestrus in bovine donors and recipients must be closely synchronized (1,15,16,17). Transfers made even one day out of phase result in a significantly lower pregnancy rate (17). It is possible that synchronization requirements depend on the age of the egg transferred but this has yet to be established. It has however been shown clearly that the age of the egg transferred significantly affects the pregnancy rate following transfer (17). Transfer of Day 3 eggs (Day 0 = oestrus) to the uterus results in a very poor pregnancy rate. One reason for the poor success rate at Day 3 may well be that eggs are ejected. On Day 3 there is considerable myometrial activity which disappears by Day 4 or 5 (18). Ejection of transferred eggs cannot explain completely the low pregnancy rate with Day 3 transfers

because the transfer of Day 4 eggs to a three-day uterus, even though the transfer is out of phase by one day, results in a significantly higher conception rate than the transfer of Day 3 eggs (17). Early cleavage stages appear to be more susceptible to deleterious effects than later embryos. For example, medium 199 used as a transfer medium will give high pregnancy rates with later stage eggs (Days 5 and 6) but the medium usually has a weak bicarbonate buffer and has the disadvantage of changing pH, under conditions of bench storage, rather dramatically. A lower proportion of Day 3 eggs stored in medium 199 at ambient temperature continue to develop normally when transferred to the rabbit oviduct than Day 5 or 6 eggs (11) or Day 3 eggs stored in an enriched phosphate buffered saline. Similarly, it is not until Day 7 (blastocyst stage) that embryos are able to withstand cooling to 0°c (19). Embryos like this have been stored at 0°C for 48 hours and have given a pregnancy rate of 60% after bilateral transfers. The indications are that eggs of different ages vary in susceptibility to the vagaries of storage. The discovery that Day 7 blastocysts resist cooling to 0°C has made a notable contribution to the deep freeze preservation of embryos.

Pregnancy rates which would probably be acceptable to cattle exporters now follow the transfer of frozen bastocysts (20). In 1973 Wilmut and Rowson recorded the birth of the first calf (Frosty II) to be born following transfer of a hatched blastocyst which had been deep frozen (-196°C) for six days (21). The medium used was an enriched phosphate buffered saline (22) with the addition, for cryoprotection, of 2m Dimethyl sulphoxide (DMSO). Other cryoprotective agents studied such as sucrose and polyvinyl pyrrolidone (PVP) were unsatisfactory.

The surgical transfer of eggs to the bovine uterus is very simply achieved using a pasteur pipette, containing the eggs suspended in medium, to puncture the uterine wall. It is usual to transfer single eggs to the uterine horn adjacent (ipsilateral) to the ovary containing the corpus luteum and not to the opposite uterine horn (contralateral). This would appear to be vital because transfers to the ipsilateral horn resulted in pregnancies in 6 of 13 recipients but 0 of 13 recipients became pregnant when eggs were transferred only to the contralateral horn (23). It has also been demonstrated that after the transfer of one egg to each uterine horn and when only one subsequently survives, the surviving foetus is significantly more frequently present in the ipsilateral horn (16). All single egg transfers should therefore be made to the ipsilateral uterine horn. This constraint will demand a high degree of manual dexterity when egg transfer is to be accomplished non-surgically.

It is not necessary to transfer two eggs to the ipsilateral horn to establish twin pregnancy. In fact, fewer twin pregnancies are established by doing so (45%) than by transferring one egg to each uterine horn (72%) (24). A greater degree of control and success in establishing twin pregnancies can be achieved by using surgical egg transfer methods than by using hormonal methods to induce twin ovulation (25).

It is doubtful whether induction of twin pregnancy will have practical application and appeal unless methods of transcervical egg transfer can be perfected. Japanese workers have concentrated on a minor surgical method which bypasses the cervix (26) but because it requires a high degree of skill it is unlikely to have wide practical application. In any event, results achieved with this method are no better than those reported using the transcervical approach. In one experiment where eggs were transferred via the cervix to the right uterine horn by simply using a glass inseminating pipette (27), up to 40% of animals in some groups became pregnant. Had all transfers been made to the ipsilateral horn then an even higher conception rate might have been achieved.

The field of egg transfer is one which is full of conflicting influences. For example, a significantly higher proportion of eggs is recovered before Day 4 than later (25) and fewer of them are degenerate (9) but it is not until Day 5 that reasonable pregnancy rates may be expected after surgical transfer (17), and not until later (Day 7) when eggs may be cooled (19) and frozen (20), and even later before the highest pregnancy rate is obtained following non-surgical transfer (27).

So much basic information concerning egg transfer in cattle is still being discovered that it is difficult to foresee exactly what techniques will eventually be used. However, one can foresee that several different approaches may be used to recover eggs depending on whether they are to be directly retransferred surgically, non-surgically or deep frozen.

What may now be assumed with some certainty is that egg transfer methods are already sufficiently advanced to ensure their continued usefulness. Simply from an experimental viewpoint this technique is invaluable in fundamental and applied research. The interest in "exotic" breeds has demonstrated that, for animals of high value, surgical methods of egg transfer are commercially viable; and, for a much greater population of less expensive animals, improved non-surgical methods of egg transfer may prove equally attractive. Egg transfer could be used to induce twin pregnancy and so increase the output of beef without a corresponding increase in adult breeding stock.

An obstacle to induction of twin pregnancy using egg transfer is the large numbers of embryos required if the technique were to become popular. In the event of a superovulatory system being devised in which eggs are collected from heifers and cows going for slaughter, the clearance rate of PMSG or other gonadotrophins and the steroid tissue residues following superovulation would have to be taken into account to safeguard man as a consumer of the meat of such animals. The clearance rate of PMSG is

protracted (28) and the effects on endogenous steroid levels dramatic. Whereas a long interval between PMSG and slaughter might be desirable in order to ensure low carcass concentrations of this gonadotrophin, circulating endogenous steroids increase progressively until Day 6 when oestrogen falls and progesterone continues to rise (10).

As an alternative to a superovulatory system where tissue residues of hormone might be disadvantageous, the *in vitro* fertilization of ovarian oocytes collected from abattoir material, although as yet impracticable, might be considered more suitable. On an individual basis the ability to fertilize ovarian oocytes *in vitro* would be very attractive to the owner of a valuable cow which for physical reasons had become sterile. The deep freeze preservation of eggs is now sufficiently developed to consider intercontinental transport of embryos rather than cattle just in the same way that semen is now transported. Safeguarding the health of the national herd is one of the advantages of such methods of importation of new genetic material. Museum banks of the deep frozen eggs of rare breeds or valuable individuals could also be established. Of course, any egg transfer systems, particularly those involving abattoir material, would need very rigorous veterinary safeguards.

All manner of science fiction concepts in bioengineering may ultimately become reality. It may be possible for instance to effect nuclear transfers, to produce clones of calves or determine the sex of eggs economically, but these for the present remain only exciting ideas. At the moment, egg transfer techniques are sufficiently developed and reliable for inclusion in schemes of genetic improvement as well as for use in rapidly multiplying exotic stock. It is unlikely that techniques of artificial inovulation will ever be as cheap or as widely used as artificial insemination, but its impact nevertheless may be considerable. Egg transfer will provide a valuable addition to existing methods of cattle improvement; it could revolutionise cattle breeding.

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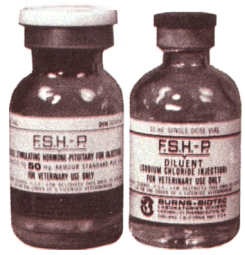

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