Embryo Transfer for the Practitioner

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Introduction

Although the technology for the successful collection and transfer of fertilized bovine ova has been known for almost 50 years (Betteridge, 1980), it was not until the early 1970's that great commercial interest in bovine embryo transfer occurred. By 1976 there were 20 registered embryo transfer units in North America (Elsden, 1977) and in the year 1979, there were approximately 20,000 pregnancies attributed to embryo transfer (Elsden, 1980).

In recent years, technology has improved with improved results, and decreased costs. In addition, the extrememly high overhead associated with the maintenance of a commercial embryo transfer unit and large recipient herd has resulted in increased "on-farm" work. "On-farm" embryo transfer, combined with the utilization of non-surgical techniques is now often preferred by breeders. It is less expensive and permits the utilization of a breeder's own recipients. It also permits a higher level of management, especially with milking cows, and above all it reduces the chance of disease introduction.

The advent of "on-farm" embryo transfer has provided the opportunity for practicing veterinarians to become involved. Embryo transfer, under the direct supervision of a practicing veterinarian, has become an important part of many breeders' programs.

Practitioners involved with embryo transfer have found it to be both financially and intellectually rewarding. It is a way in which their herd health programs can be broadened and services provided to clients can be improved. However, there are probably more failures than successes. A practitioner who fails to establish a successful embryo transfer service not only costs himself and his clients time and money, but also tarnishes his own reputation and that of the profession. In many cases, practicing veterinarians would have provided a much more valuable service by referring their clients to a specialized embryo transfer service. The practitioner, providing he is knowledgeable of embryo transfer procedures, can supervise donor and recipient cow selection and preparation. Others who are specially trained could conduct the actual embryo collection and transfer.

Practitioners who are anticipating the addition of embryo transfer to their services must be wary. They must be aware that the successful utilization of embryo transfer requires a great deal of time and practice. The program requires constant supervision and close attention to detail. Nothing can be left to chance. In addition, a great deal of dexterity in the manipulation of the reproductive tract of the cow is necessary. Seasonal rectal palpations are not adequate preparation for the skills required of this technology. Finally, a practitioner intending to become involved in embryo transfer must be prepared to become knowledgeable with all aspects of reproductive physiology in the cow and to keep abreast of research developments in reproductive physiology and embryo transfer technology. If a practitioner is prepared to make these committments, it is possible for him to successfully provide an embryo transfer service for his clients. His clients must also be patient in allowing this expertise to develop.

The following review is by no means exaustive but is intended to provide a practical approach to "on-farm" embryo transfer. It is intended that this review benefit practitioners whether they propose to simply supervise the program or to provide the service themselves. It must be remembered that embryo transfer is only a part of a breeder's total program and that the veterinary practitioner is best prepared to advise a breeder in this regard. Therefore, embryo transfer as a breeding tool must be considered in light of a total herd health program and applied accordingly.

Donor Selection

The most common use of embryo transfer is to increase numbers of offspring from planned matings of genetically superior parents. A client will base his decision on information from a variety of sources including that of his own veterinarian. Therefore, a herd health service includes genetic consultation in addition to attention to health needs. Practitioners providing such a service must be knowledgeable regarding genetic lines, AI bulls, their progeny test data and the specific regulations that each breed organization requires for embryo transfer.

The reader is referred to a more detailed discussion on the selection of a potential donor for embryo transfer (Church & Shea, 1977; Land, 1977; Elsden, 1980). Embryo transfer will provide its greatest benefit and costs will be markedly reduced if results are maximized. The practicing veterinarian can be of additional service in this regard. A highly fertile animal, in good body condition and with no underlying disease will superovulate best. Similarly, recipient animals must be healthy, cycling, a minimum of 50-60 days post partum and on a rising plane of nutrition. The veterinarian is in the best position to advise a client

regarding the fertility of his animals.

Superovulation

Most donor cows will respond most predictably and optimally if superovulation treatment is instituted between days 8 and 14 of the cycle (Betteridge, 1977). Normally, a luteolytic dose of prostaglandin $F_2 \alpha$ ($PGF_2 \alpha$) is administered 48 hours after initiation of treatment. Estrus is expected 48 hours later. Recipients are usually injected with $PGF_2 \alpha$ 12-18 hours earlier than the donor cow. Three methods of superovulation are outlined in Table 1.

TABLE 1

SUPEROVULATION TREATMENTS IN THE COW

TREATMENTS[a]		Tx I	Tx II	Tx III
D.10	A.M.	2500 IU PMSG	7 mg FSH-P	5 mg FSH-P
	P.M.		7 mg FSH-P	5 mg FSH-P
D.11	A.M.		6 mg FSH-P	5 mg FSH-P
	P.M.		6 mg FSH-P	5 mg FSH-P
		----------Recipients[b] $PGF_2\alpha$ ----------		
D.12	A.M.	----------Donors $PGF_2\alpha$ ----------		
			5 mg FSH-P	5 mg FSH-P
	P.M.		5 mg FSH-P	5 mg FSH-P
D.13	A.M.		4 mg FSH-P	5 mg FSH-P
	P.M.		4 mg FSH-P	5 mg FSH-P
D.14	A.M.		3 mg FSH-P	5 mg FSH-P
	P.M.	A.I.[c]	A.I.	A.I.
D.15	A.M.	A.I.	A.I.	A.I.
	P.M.	A.I.	A.I.	A.I.

[a] Superovulation treatments commence on day 8-14 of the cycle. Estrus = day 0.

[b] Recipients receive $PGF_2\alpha$ 12-18 hours before donors.

[c] Breeding commence 8-12 hours after onset of estrus and is repeated twice at 12-hour intervals.

A single injection of 1500-3000 I.U. of pregnant mare's serum gonadotropin (PMSG) has been most traditionally used to superovulate cows (Betteridge, 1977; Newcomb *et al,* 1979; Newcomb, 1980). However, Elsden *et al* (1978) reported more ovulations, embryos recovered and pregnancies after a superovulatory regimen with follicle stimulating hormone (FSH)[a] and luteinizing hormone (LH)[b] in a 5:1 ratio, administered twice daily in decreasing doses for five days. A recent study (Miller *et al,* 1981) has indicated that an improved response could be obtained when LH was not used. Consequently, two superovulatory regimens with only FSH are in favour today (Table 1).

Estrus normally occurs on the fifth day and treatments cease once estrus occurs. If estrus does not occur, treatments are continued until it does. Estrus will occasionally occur 12-24 hours early. These cows usually have a good superovulatory response.

Although ovulations in superovulated cows have been shown to be spread over 24-48 hours, the time from onset of estrus to first ovulation is essentially unchanged (Maxwell *et al,* 1978). Therefore, superovulated cows are normally inseminated three to four times at 12-hour intervals beginning 8-12 hours after the onset of estrus. Some groups use two doses of semen at each insemination time (Elsden, 1980). Fresh semen or natural service also provides good fertilization rates. Insemination schedule and the inherent fertility of the bull used have been shown to be the most limiting factors in numbers of fertilized ova recovered (Newcomb, 1980). Therefore, good heat detection, sound insemination techniques and high quality semen are necessary to ensure success.

Embryo Recovery

Until 1975 most ova were recovered by surgical methods. However, surgical techniques often resulted in adhesions of the reproductive tract which in turn reduced the fertility of valuable donor cows. In 1976, two methods of non-surgical embryo recovery were published in the same journal (Rowe *et al,* 1976; Elsden *et al,* 1976). Most embryo collection techniques today are variations of those described. Non-surgical techniques are preferred as they are not damaging to the reproductive tract, are repeatable and can be performed on the farm. However, non-surgical techniques can only be performed when embryos enter the uterus and on cows in which the cervix can be entered during diestrum. In addition, considerable manipulative skill is necessary to non-surgically flush ova from the uterus of the cow.

Normally, embryos are collected on days 6 to 8 after the onset of estrus. Before this time embryos may still be in the oviduct and after this time ova begin to hatch from the zona pellucida and are extremely difficult to find. We do not starve our donor cows, as a matter fact, we often prefer that they have a full rumen at the time of collection. The donor is restrained in a squeeze chute, preferably with front feet elevated. After the rectum is evacuated of feces and air, an estimation of corpora lutea (CL) numbers is made. Collection is not attempted until a satisfactory epidural is completed. Then the perineal region and vulvar lips are thoroughly washed and the tail is tied out of the way.

A thorough discussion of embryo collection by way of a foley catheter and a continuous flow closed circuit system has been presented by Elsden (1980). We prefer to use the "Modell Neustadt/Aisch" two-way catheter[c] (Fig. 1) as described by Schneider and Hahn (1979). It is 67 cm long,

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[a] *FSH-P - Burns-Biotec, Laboratories Division, Chromalloy Pharmaceuticals Inc., Oakland, California 94621.*

[b] *PLH - Burns-Biotec, Laboratories Division, Chromalloy Pharmaceuticals Inc., Oakland, California 94621.*

[c] *Embryo Transfer Katheter - Walter Worrlein, Ruglander Str. 3 - 88 Ansbach, West Germany.*

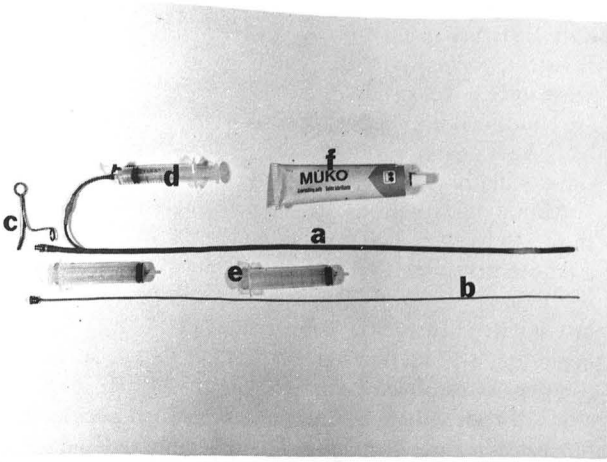

FIGURE 1 — Non-surgical embryo collection equipment.
a) "Modell Neustadt/Aisch" embryo collection catheter
b) Stainless steel stilette
c) Tube clamp
d) Twenty-ml syringe to inflate cuff
e) Thirty-ml syringes for flushing
f) Sterile lubricant

18-gauge outside diameter and has a Luer-Lock fitting. The tip in front of the cuff measures 5.5 cm and has four holes. The catheter is stiffened by a stainless steel stilette which locks into the Luer-Lock fitting.

The catheter with the stilette in place is coated with a sterile lubricant and is passed through the vagina and the cervix. It seldom takes more than a few seconds to thread the cervix. However, in some donors, it is difficult to thread the cervix. In these cases, a cervical dilator (Fig. 2) is used to dilate the cervical canal before the catheter is passed. The catheter is directed into the right uterine horn and the stilette is gradually removed as the catheter is threaded down the horn. The catheter is placed so that the inflated cuff is approximately half-way down the horn. The cuff is inflated with 10 to 20 ml of sterile saline. Over-inflation or too rapid inflation of the cuff may split the endometrium causing hemorrhage and loss of flushing medium into the broad ligament. The stilette is then removed and a clamp is placed on the catheter at its exterior end.

We prefer to collect with syringes of 25-35 ml of media per flush. This is particularly useful when one is collecting a single embryo as flushes are placed singly into petri dishes and searched. The embryo is usually found in one of the first four flushes. In the superovulated donor, each uterine horn is flushed with eight individual flushes which are placed in a sterile 500-ml graduated cyliner (Fig. 3).

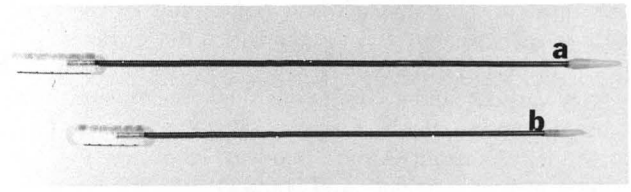

FIGURE 2 — Cervical dilaters.
a) Large size
b) Small size

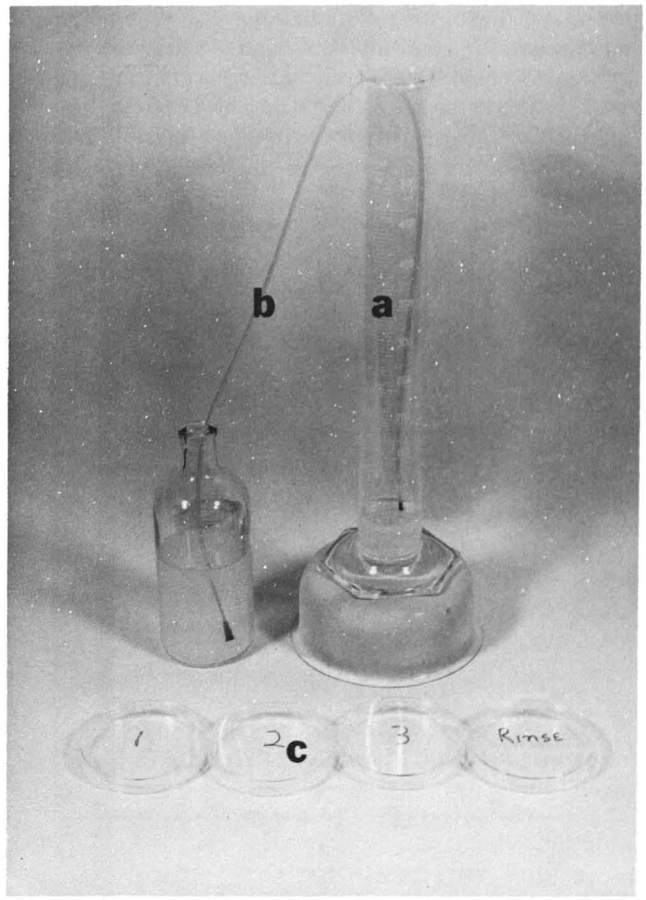

FIGURE 3 — Equipment for collection of flushing media.
a) 500 ml cylinder
b) Siphon rubing
c) Large petri dishes for searching

The technique for collection of embryos is one of the most difficult steps to master. One must be gentle and yet ensure that media reaches all areas of the endometrium. It is important that media is not forced into the uterus under too much pressure as the endometrium will split. It is also important to prevent kinking of the uterine horn as a small portion may become over-inflated, again resulting in a split in the endometrium. Two or more flushes are often placed in the horn together before agitation and aspiration. In our experience, 85% of embryos appear in the first four flushes. As we have found embryos up to the seventh flush, we normally flush each horn eight times.

When the collection of the right horn is completed, the stilette is inserted until it enters the cervix. Then the cuff is deflated and the catheter is withdrawn while the stilette is

gently advanced to the body of the uterus. In this way the stilette is reinserted without withdrawing the catheter from the uterus. The catheter with the stilette in place is then inserted into the left horn and the cuff is placed as previously described. When the flush is completed, the cuff is deflated and the catheter is removed. Then each uterine horn is infused with 30 ml of a 0.5% aqueous iodine solution using a syringe and an uterine infusion catheter. $PGF_2 \alpha$ may be given at this time or slightly later. However, return to estrus cannot be expected for one to two weeks.

Flushing media is prepared prior to preparation of the cow. Phosphate buffered saline (PBS)[d] in 500-ml bottles is refrigerated ready for use. In addition, 10 ml of heat inactivated fetal calf serum (FCS)[e], 5 ml of an antibiotic/antimycotic solution [f] and a solution of glucose and pyruvate (Whittingham, 1971) are kept frozen so that a single quantity is required for each 500 ml of PBS. Media will then contain 100 I.U. penicillin, 100 ug streptomycin, 0.25 ug fungizone and 1 mg glucose per ml of PBS and .33 mM of sodium pyruvate. Eight ml of FCS is added to the flushing media and eight ml of the flushing media is in turn added to the remaining two ml of FCS to produce a 20% solution with FCS for culture. Culture media is passed through a disposable 0.22 u millipore filter[g] prior to use (Fig. 5). It would seem that the glucose and pyruvate are only necessary for longer terms of culture of embryos. Therefore, media for "on-farm" work need only contain PBS, antibiotics and approximately 2% FCS for collection and 20% FCS for culture prior to transfer.

Embryo Handling

There is no question that one of the most difficult tasks facing a practitioner who is learning embryo transfer techniques is searching for, identifying and evaluating ova. An absolute necessity is a good dissecting microscope (Fig. 4). All microscopes do not work. Select the microscope you intend to work with, use it and establish confidence in it.

Media is allowed to settle in the 500-ml cylinder for a minimum of 35 minutes after the collection is completed. Then a sterilized silastic tubing[h] of 0.062 in inside diameter is used to siphon the media down to the 50-ml mark. The remaining media is swirled, aspirated into a syringe with a uterine infusion pipette and placed in sterile disposable petri

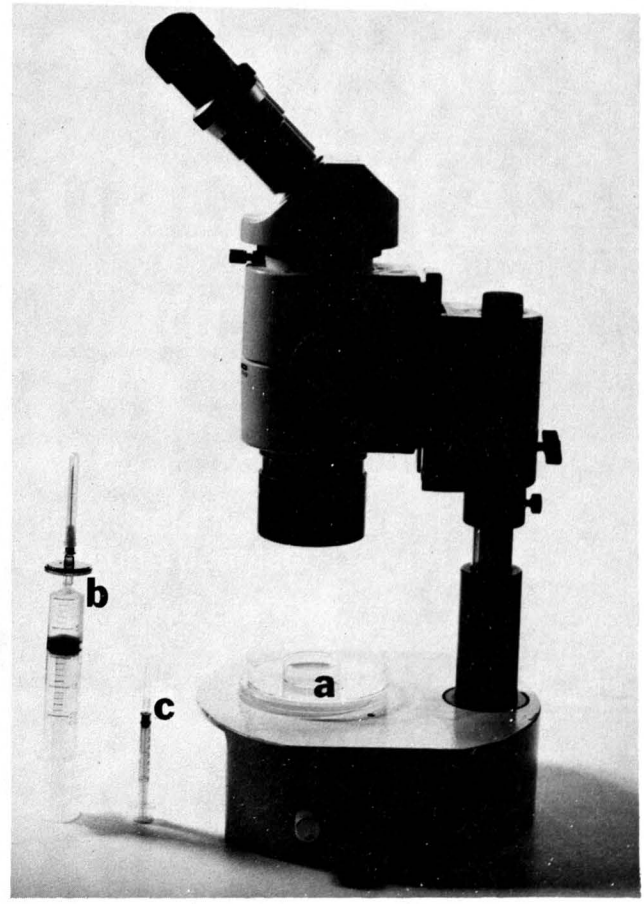

FIGURE 4 — Dissecting microscope with:
a) Grid and embryo culture dish
b) Culture media in a 20-ml syringe and disposable 0.22 u millipore filter
c) Medicut catheter and one-ml syringe

dishes[i] (100 x 15 mm in size) and searched at 10X magnification. A lined petri dish is used as a grid beneath the dish containing the media to assist in searching. Once the dish is searched, it is swirled to move ova away from the outer perimeter and searched again. Each dish is searched twice after the last embryo is found repeating the swirling motion between each search.

Culture media containing 20% FCS is filtered into a smaller sterile disposable petri dish (35 x 10 mm) which is in turn placed in a large petri dish to provide stability (Fig. 5). Ova, when they are located, are picked up with a sterile disposable 20-gauge intravenous plastic catheter[j] (Fig. 5) attached to a one-ml syringe and deposited in the culture media. When searching is completed ova are placed in fresh media, evaluated, illustrated and described. Culture dishes

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[d] Dulbeccos phosphate buffered saline - Gibco Laboratories, 3175 Staley Rd., Grand Island, New York 14072.

[e] Fetal bovine serum HI - Gibco Laboratories, 3175 Staley Rd, Grand Island, New York, 14072.

[f] Antibiotic/antimycotic solution - Gibco Laboratories, 3175 Staley Rd., Grand Island, New York 14072.

[g] Millex - GS - Millipore Corp., Ashby Rd. Bedford, Mass. 01730.

[h] Silastic Medical-Grade Tubing - Dow Corning Corp., Medical Products, Midland, MI 46640.

[i] Fisher Scientific Co. - 711 Forbes Ave., Pittsburgh, PA 15219.

[j] Medicut - Sherwood Medical Ind., St. Louis, MO 63103.

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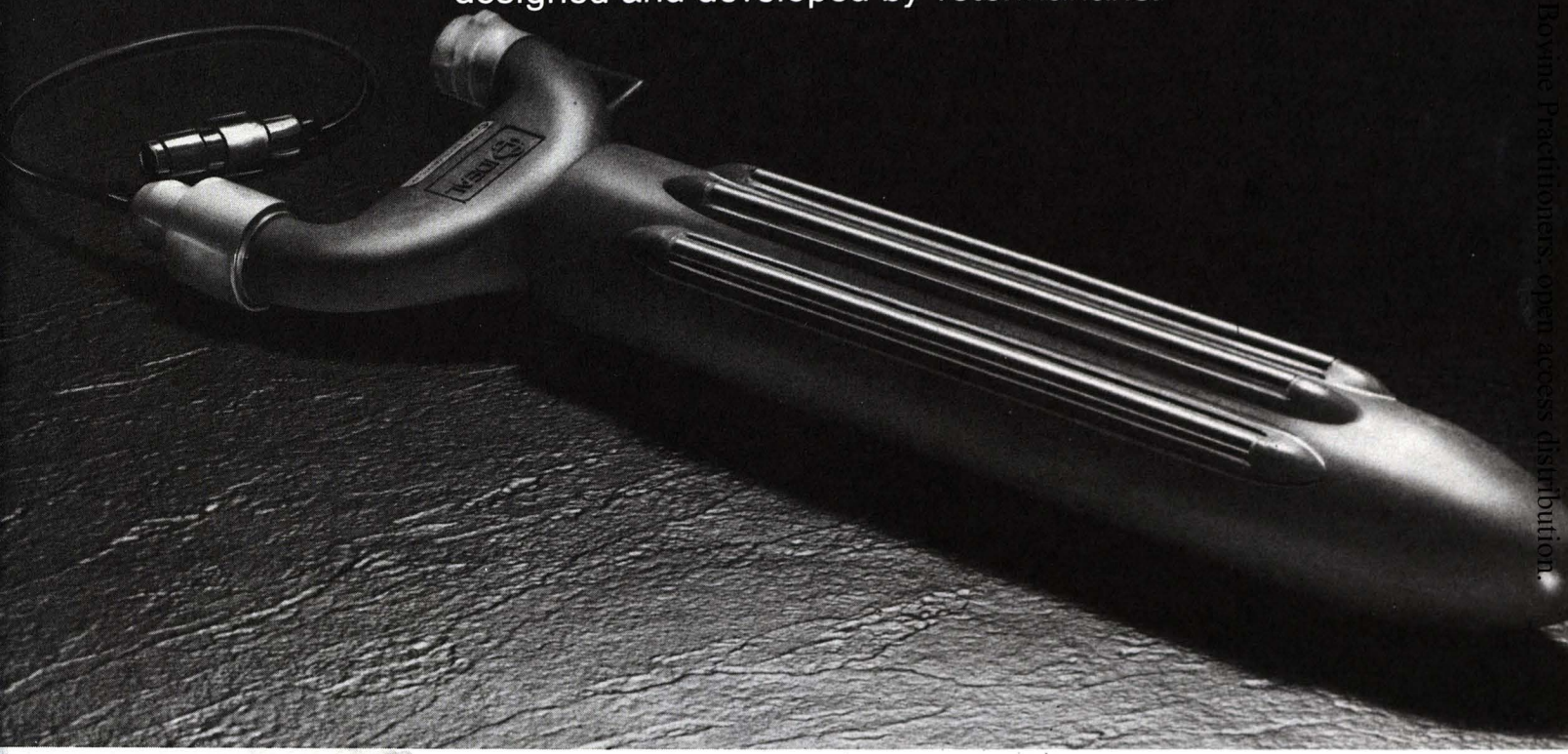

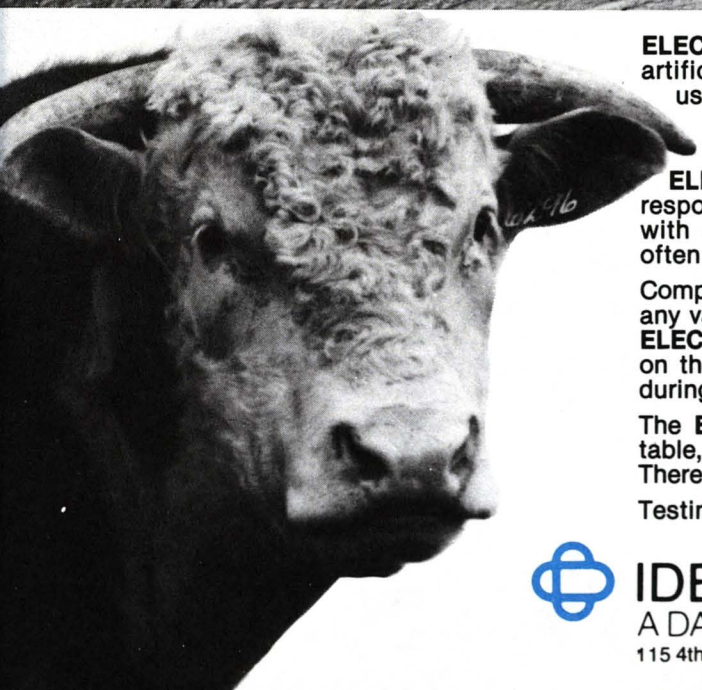

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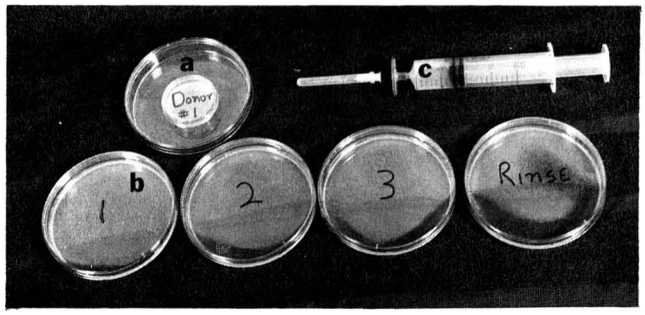

FIGURE 5 — Media maintained at room temperature between two layers of terry towels.

a) Embyro culture media in a small petri dish placed in a large petri dish.
b) Aspirated collection media in large petri dishes for searching
c) Culture media in 20-ml syringe and a disposable 0.22 u millipore filter

are then placed between two layers of terry towels at room temperature or on a warm stage or in an incubator at 35-37°C. Just prior to transfer embryos are again placed in fresh culture media.

Readers are referred to the numerous detailed descriptions, with photographs, of the different stages of development of the normal and abnormal bovine embryo (Church & Shea, 1976; Betteridge, 1977; Linares & King, 1980; Elsden, 1980). Evaluation is normally done at 50-100X magnification with the embryo in the fresh clear media of the small culture dish. It is important to be able to recognize the various stages of development and to compare this with the developmental stage that the embryo should be at this time after fertilization. Embryos that are of doubtful quality can be cultured for a few hours. An improvement in appearance will indicate that the embryo is of transferrable quality. Often a decision as to whether an embryo is worthy of transfer will depend on the availability of a recipient. There are many reports which confirm that embryos classified as good or excellent result in high pregnancy rates (Schneider *et al,* 1980; Elsden, 1980). However, fair and poor quality embryos may also result in pregnancies and consideration should be given to their use if recipients are available (Table 2).

TABLE 2

THE EFFECT OF EMBRYO QUALITY ON PREGNANCY RATE

Embryo Quality	No. of Embryos	No. of Pregnancies	Pregnancy Rate (%)
Good & Excellent	2236	1533	69
Fair & Poor	778	398	51

From: Elsden et al, 1978 and Schneider et al, 1980. (combined data)

Embryo Transfer

There are basically two alternatives available for transplanting embryos on-farm, either surgically by flank approach or non-surgically. Non-surgical embryo transfer is certainly the method of choice. However, there is a great variation in success rates, depending on the experience and dexterity of the technician (Schneider *et al,* 1980; Rowe *et al,* 1980; Elsden, 1980). Surgical techniques tend to produce higher pregnancy rates in most veterinarians' hands but do require more trained personnel and more specialized facilities.

Both methods of transfer have been described in detail (Elsden, 1980) so only a brief review will be covered herein. The flank method (Evans *et al,* 1979) of surgical transfer is not difficult. Running water, protection from the weather, a squeeze chute and other minimal handling facilities are necessary. One veterinarian, one technician and two farm hands with good facilities could transfer 6-8 embryos per hour. In our hands, flank surgical transfers, on-farm, have resulted in a mean pregnancy rate of 65% (n = 308) during 1980.

A skin incision is made in the flank ipsilateral to the CL as far posteriorly as possible. The abdominal muscle layers are separated and the peritoneal cavity is entered by blunt dissection. The CL is identified and the uterine horn on that side is exteriorized by placing tension on the broad ligament over the distal one-third of the horn. The uterine horn is fixed at the incision site by holding the broad ligament with a 4 x 4 gauze sponge over the uterine horn and between fingertips. The serosal surface of the uterine horn is punctured with a blunted 18-gauge needle. The needle characteristically "pops" through the endometrium. Care is taken to not pass through the uterine lumen into the submucosa on the opposite side. The embryo is aspirated into a 20-gauge sterile plastic intravenous catheter (Fig. 4) between two pockets of air and media. The blunted needle is withdrawn and the embryo is deposited in the uterine lumen by carefully passing the intravenous catheter through the puncture site and by slowly depressing the plunger of the one-ml syringe. The catheter is slowly withdrawn and the uterus is returned to abdominal cavity. Twenty ml of penicillin-streptomycin combination[k] is sprayed into the abdominal cavity and the abdominal wall is routinely closed.

The method of non-surgical embryo transfer has also been thoroughly described (Newcomb & Rowson, 1980; Tervit *et al,* 1980; Rowe *et al,* 1980; Elsden, 1980). Most methods utilize the one-quarter ml French straw and the Cassou AI gun (Fig. 6). The recipient's rectum is evacuated of feces and air and the side of the CL is identified. An epidural injection with four ml of 2% xylocaine is done. The perineal region is thoroughly washed and sprayed with alcohol. The embryo is aspirated into a one-quarter ml French straw between two air pockets and two columns of culture media. The is

[k] *Cillimycin - Austin Laboratories*

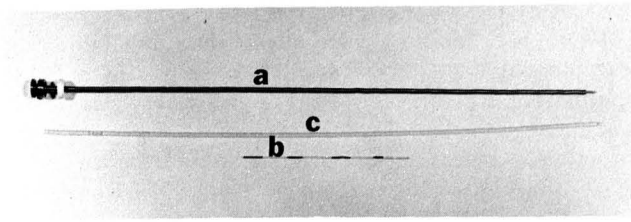

FIGURE 6 — Non-surgical embryo transfer equipment.
a) Cassou AI gun
b) One-quarter ml French straw
c) Plastic sheath

inserted into the Cassou gun and shortened to fit even with the end of the gun. A previously gas sterilized one-quarter ml sheath is fitted over the Cassou gun and fixed in place. The sheath is covered with a sterile lubricant and passed as aseptically as possible through the vagina. The cervix is threaded and the uterine horn on the side of the CL is entered as gently as possible. The embryo is deposited at a point approximately one-third of the way down the uterine horn and the Cassou gun is removed slowly. This procedure can be done easily and quickly. A negative correlation between time spent in the uterine horn and pregnancy rate has been demonstrated (Rowe *et al,* 1980). This may indicate the potential detrimental effect of endometrial trauma. Although the introduction of bacterial contaminants is always of concern, reduced pregnancy rates associated with non-surgical transfer do not seem to be associated with infection or stimulation of the cervix (Rowe *et al,* 1979). Generally, it is considered that success is related to dexterity and practice and that pregnancy rates achieved by most operators will improve with time.

Recipient animals must be healthy, cycling and on an increasing plane of nutrition. Body condition is as important for embryo transfer as it is for artificial insemination. Recipients must be sufficiently well grown to produce a live, healthy calf and provide a sufficient quantity of high quality milk to enable a transplant calf to realize his genetic potential. Synchronization of estrus in recipients with $PGF_2\alpha$ does not seem to affect pregnancy rates. However, the onset of estrus in recipients should be as synchronous as possible with that of the donor. In addition, more efficient use of recipients can be made if two or three donors are superovulated at the same time. Under these circumstances, six to eight recipients can be synchronized for each donor cow.

Embryo Transfer Success Rates

Superovulation with FSH is likely to result in 8-12 ovulations per donor cow (Elsden *et al,* 1978; Schneider *et al,* 1980). Non-surgical embryo collections will yield 7-10 ova of which reports of in excess of 80% fertilization rate have occurred (Shea *et al,* 1976; Schneider *et al,* 1980; Elsden, 1980). We have difficulty achieving 80% fertilization rate. Four to seven embryos will usually be worthy of transfer (Brand *et al,* 1978; Newcomb, 1980; Schneider *et al,* 1980; Elsden, 1980). Surgical embryo transfer results in pregnancy rates of 60-70% while non-surgical rates are likely to range from 35-55%. Single egg collections tend to result in a slightly higher pregnancy rate (Schneider *et al,* 1980; Elsden, 1980).

Results of embryo transfer can be extremely gratifying or extremely disappointing. As there are some animals that fail to superovulate or superovulate poorly, disappointment will occur if one of these animals is done by herself. However, if several animals are superovulated together, responses tend to average. On the average superovulation and embryo transfer will result in two to four pregnancies per donor cow. However, the range of results varies a great deal from cow to cow.

Cows can be superovulated every 50-60 days for three treatments without any appreciable decrease in response (Elsden, 1980). Between these collections the occasional single egg collection can be made. There does not seem to be any appreciable differences in numbers of pregnancies obtained between seasons, breeds, lactating vs non-lactating or heifers vs cows (Elsden, 1980).

Embryo Transfer & Herd Health Programs

The role that embryo transfer can play in cattle improvement programs has been thoroughly reviewed (Krausslich, 1976; Cunningham, 1976; Hill & Land, 1976; Hansen, 1976; Church & Shea, 1977; Land, 1977, Bradford & Kennedy, 1980). Genetic improvement with embryo transfer will be relatively slow compared to that of AI (Bradford & Kennedy, 1980). However, one must also take into consideration the benefits of the production of increased numbers of offspring from selected matings, the increased selection pressure for heifers and the reduced generation interval. Combined with AI, embryo transfer can make a great contribution to genetic improvement.

The potential value of the embryo transfer procedure in the problem or terminally ill cow has also been documented (Bowen *et al,* 1978; Elsden *et al,* 1979; Mapletoft *et al,* 1980a). Embryo transfer procedures provide an alternative in the diagnosis, treatment and finally salvage of reproductive function in valuable but infertile cows (Mapletoft *et al,* 1980a). Furthermore, cows with abnormal ovarian function and cyclicity can also be superovulated successfully using progestational compounds to control the cycle (Mapletoft *et al,* 1980b). Embryo transfer does provide an acceptable alternative in the problem cow. A large number of potential donors that a practitioner will encounter will be cows which have failed to become pregnant.

Embryo transfer can be applied in a routine herd health program. Single egg collection and transfer could very easily be done during routine herd visits. In this way cows could be made to produce up to two off-spring per year. A veterinarian and a technician could collect a single embryo and transfer it either surgically or non-surgically and be off the farm in one to two hours. This could easily be worked into a herd health visit. Other clients may desire to

superovulate all top cows once before rebreeding. Animals of lesser genetic value could serve as recipients. Cows could be superovulated, collected and transferred just prior to rebreeding. In this way a 12-14-month calving interval could be maintained.

Still other clients may decide to leave an old but valuable donor cow open to be superovulated and transferred a number of times during the year. Single egg collections could be done between superovulation procedures. In this way a client could increase the numbers of offspring from a given animal in a very short period of time. Remember, most efficient use of recipients can be made if a number of donor animals are superovulated at the same time.

Finally, embryo transfer can be used to establish new lines or herds (Newcomb, 1978) and herds which may be considered disease free (Eaglesome *et al* 1980). Although specialized assistance may be required for such an undertaking, the practitioner is in a position to co-ordinate the program.

There are also new developments on the horizon. With the advent of embryo freezing and embryo sexing, the AI industry is becoming involved with embryo transfer services. There is also the question of transportation of bovine embryos. There is little question that this will be the method of choice for importation and exportation of genetic material (Bedirian *et al,* 1979). Familiarize yourself with the regulations and work closely with the various organizations involved. Your client will be better served because of the involvement of someone he knows and trusts and you as a practitioner will find it interesting and rewarding.

No discussion would be complete without reference to cost. You must be in a position to sit down with your clients in order to discuss costs and benefits to him. Elsden (1980) reviewed some very pertinent considerations in this regard. From your point of view, fees may be charged on an hourly basis or on a per pregnancy basis. Generally fees on a per pregnancy basis range from $400-$1000 per pregnancy plus mileage and drugs. Once your clients gain confidence in your results they may prefer to pay on an hourly basis plus mileage and drugs. Remember, embryo transfer is a specialized procedure. Two to four times regular hourly charge is not out of order. You must also cover the overhead cost of your training and equipment.

In summary, it is possible for a practicing veterinarian to provide a successful embryo transfer service in his herd health programs. However, it requires extensive training and practice to develop these skills to an acceptable level. In addition, constant attention must be paid to detail. It must be a major part of the herd health service. A practitioner who does not have the time to develop and maintain skills in the area of embryo transfer would be well advised to supervise the program while a specialist applies the technology.

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