Embryo Transfer in Cattle

Maarten Drost, *D.V.M.*
Department of Reproduction
School of Veterinary Medicine
University of California
Davis, California 95616

Introduction

The topic of embryo transfer in cattle has received a great deal of publicity in all major cattle-producing countries in the world. This has come about, as has often happened in science, by a fortunate combination of major economic changes in the cattle industry, causing increased demand and higher prices for valuable livestock, and by improvements in the state of the art, especially the reports from Rowson and his colleagues (16-19) of high fertility after transfer of either one or two fertilized eggs to suitable recipients. Cattle ovum-transfer teams have sprung up in many countries and include veterinarians operating out of private clinics, as well as commercial enterprises devoted to techniques of improving cattle production. Substantial numbers of transfers have been performed commercially in Canada and Britain but have not yet been fully reported. Limited numbers of transfers have been reported from research laboratories in Japan, France, Canada and the United States (4,8,20,23-27). There is widespread interest by organizations associated with artificial insemination; however, before the commercial use of the technique of embryo transfer can be considered a success, it seems obvious that many problems have to be overcome. It is the purpose of this presentation to assess the present position and, particularly, to draw attention to the gaps in knowledge which are evident in this field. It is not my purpose to be unduly cautious; on the contrary, I believe the technique will find an important place in increasing efficiency of cattle production.

History

As early as 1890, Heape (9) at Cambridge University in England demonstrated that fertilized ova could be isolated from one rabbit and transferred to a host rabbit, which served as a foster mother, for the production of living young. Since the first work in ova transfer by Heape, many successful transfers were reported on laboratory animals such as guinea pigs, mice, rats and rabbits. Warwick, Berry and Horlacher (29), working in the United States, reported in 1934 the first successful ova transfers from the domestic goat and sheep. Then, in 1951, Willett, Black, Casida, Stone and Buckner in Wisconsin (31) reported the first successful ovum transfer in cattle using surgical techniques; and a little over a year ago, on June 11, 1973, the first calf was born after the 11-day blastocyst had been frozen prior to transfer by Rowson and his colleagues at Cambridge, England.

It is humbling to read the proceedings of the First National Egg Transfer Breeding Conference, which was held in San Antonio, Texas, in 1949, twenty-five years ago, and to realize how slow the progress has been during that quarter of a century. Chang cited among the major problems the lack of a suitable superovulation technique to yield a fairly large number of eggs, and the limited knowledge of physiology of bovine eggs *in vitro* and *in vivo*—problems which are still very much with us today.

There is considerable speculation as to the future of embryo transfer in cattle. The ultimate goal may well be a combination of the harvesting of (follicular) oocytes from slaughterhouse material, from prepuberal calves or from ovarian tissue culture, with *in vitro* fertilization and subsequent controlled development, plus long-term storage by freezing. It would be nice to be able to alert the artificial inovulator when the recipient is in estrus for delivery of such an embryo by nonsurgical means at a pre-determined time 4-7 days hence. But we have not reached that stage yet; in fact, we are a long ways from it. We are not even as close as the ads from several commercial egg transfer organizations would like us to believe.

Commercial Application

A wide variety of applications are possible, if we assume that it is possible to superovulate appropriate donor animals, recover adequate numbers of fertilized eggs, transfer them rapidly and at moderate cost to suitable recipients and obtain

pregnancy rates both after single and twin egg transfer, which are at least comparable with accepted values of normal fertility.

Widespread Use of Valuable Females

A. *The new-breed syndrome.* Due to a scarcity in the United States and other countries of bulls and cows of many breeds which are desired by farmers for cross-breeding purposes, there is a great demand for introduction and multiplication of valuable stock. This demand is expected to continue. In the United States and Canada, for example, there are approximately 60 million breeding cows and indications are that farmers wish to replace many of the straight-breds with cross-bred animals. Present interest is exemplified by the use of yearling heifers of scarce Simmental, Limousin, Chianina, Maine-Anjou and other so-called exotic breeds in Canada. A great deal of interest is shown also in the use of prepuberal calves with the obvious advantage of reducing the generation interval. However, there are, as yet, no published reports of *reliable* methods of producing large numbers of fertilized eggs from calves.

B. *Use of cows with proven records of performance.* Such cows may or may not have an infertility record, but it is likely that difficult breeders will be candidates for superovulations and egg transfer. It is imperative, however, that an accurate diagnosis of the cause of infertility be made, especially in relation to gamete transport. It would also seem appropriate in valuable cows to carry out a hormonal profile to aid in selecting the correct stimulatory treatment.

Multiple Births

Transfer of two or more eggs to recipients will not increase the number of fertile female progeny born because of the free-martin condition, but will greatly increase the number of male calves and the total weight of calves. When the economic value of extra calves is weighed against the added costs, it is found that calf crops in the range of 120% to 150% would be profitable. The two technical questions which are highlighted by an economic analysis are the availability of fertilized eggs (i.e., yield per donor) and the proportion of fertilized eggs which implant and develop (i.e., fertility rate per recipient). These two aspects of the technique will be discussed in detail later. Sequelae of multiple births, such as calving difficulties, calf losses, retained placenta, and temporary infertility, can be overcome by improved management, especially in relation to housing, nutrition and supervision. This emphasizes the point that multiple births cannot be disassociated from intensive management systems. Decreased milk and butterfat production during subsequent lactation in dairy cows (7,28) is less avoidable, but of minor significance in most recipients.

The continuation of commercial use of embryo transfer depends on continued scarcity of high-priced cattle. It is well established that there is a clear relationship between the numbers of valuable cattle and the price, but it is not obvious at what point the prices begin to decrease rapidly. Clearly, there is a risk factor involved.

Another factor is the considerable variability between donors in production of fertilized eggs. In terms of the economics of the program, the wide variability introduces an additional cost factor which may, in the long run, be one of the most important limiting factors in the whole field of egg transfer. It is obvious that there are major organizational problems concerned in the handling of donors and synchronizing recipients, in organizing the teams for surgical transfer, etc. Under these conditions the prices presently being paid for transfer are not considered excessive.

Research Application

Large numbers of fertilized eggs are required for study of storage and shipment of embryos—the latter as it pertains to the importation and exportation of embryos perhaps contained in a heterologous species such as the rabbit. The opportunity exists to study alleged and proven transmission of viruses through semen. The development of instrumentation for collection and transfer will provide a spinoff to bovine reproductive physiology. Hormonal monitoring, which may be necessary to determine optimum conditions for egg production, will also be a spinoff for gynecology and reproductive endocrinology. For example, we stand to gain an insight into the causes of early embryonic death through the process of reciprocal transfer.

The Production of Fertilized Ova

The objective is to collect many embryos with morphological characteristics acceptable for transfer. Such characteristics are 4-cell to 16-cell eggs with even-sized blastomeres of similar density and filling the cavity inside the zona. At the present time, it is not practicable to carry out metabolic studies. It is possible, however, that developments in the future will include holding the eggs in a suitable environment for specific short-term metabolic evaluation.

Cows do not have very large numbers of normal primordial and growing follicles. Hence, it would seem prudent for someone to carefully investigate

the effect of a high level of stimulation in prepuberal heifers on subsequent follicular development and fertility.

The steps in obtaining fertilized ova include hormonal stimulation of multiple ovulation, insemination, and collection of fertilized eggs.

Multiple ovulation

A. *Pregnant mare serum gonadotropin (PMSG) during the estrous cycle.* PMSG may be injected subcutaneously or intramuscularly on Days 16 or 17 of an estrous cycle; doses range from 1,500 to 3,000 i.u., depending on factors such as age, lactational status, breed, nutrition and season. Sub-threshold doses (range 1,000 to 1,500 i.u.) may block ovulation. A double injection procedure consisting of 1,500 i.u. of PMSG on Day 5 of the estrous cycle, in addition to that given on Day 16, may improve ovulation rate. Human chorionic gonadotropin (HCG; 2,000 i.u.) intravenously given at the beginning of estrus following PMSG administration may increase the yield of fertilized eggs by producing ovulations over a smaller range of time than is believed to occur when it is brought about by an endogenous surge of gonadotropin. It is unlikely, however, that the total ovulation rate is influenced by HCG in normal cycling cows.

The ovulation rates and yields of fertilized eggs with these treatments are extremely variable as indicated in the Table 1 (10). It can be seen that it is not unusual for animals receiving the same treatment to have no ovulations and others to have large numbers.

PMSG has several inherent problems. As a placental hormone it is perhaps fortuitous that PMSG acts on the ovary. The half-life of PMSG is 24-30 hours, which makes it difficult to use; one cannot turn it off. Add to this the facts that the cow is very sensitive to PMSG and that the dose response curve is atypical, i.e., not bell-shaped, making its effects somewhat unpredictable. Furthermore, mare serum is difficult to standardize; as a result there are variations in ovarian response to different batch numbers (5). The ratio of FSH vs. LH activity of PMSG is 5:1. If PMSG is used too late when follicles are already present, it then speeds up growth and luteinization.

B. *PMSG and prostaglandin (PGF_{2a}).* PGF_{2a} will cause luteolysis in the cow. The administration of PMSG between Days 10 and 14 of an estrous cycle followed by a luteolytic dose of PGF_{2a} one or two days later is believed to give adequate control of multiple ovulation without the wide variability in ovulation rate attributed to PMSG on its own. Experience under local conditions with a given breed should be used to determine an optimum dose of PMSG and whether it should be given one or two days before regression of the corpus luteum. However, there are as yet no published data supporting the claim of greater uniformity in response compared with PMSG alone.

C. *Progestins and PMSG.* A good deal of work has been done on the combination of progestins and PMSG. PMSG should be injected before the end of the progestin regime (13). A fast of about three days during the period when follicular growth occurs is reported to reduce variability in ovulation rate and possibly number of fertilized eggs (13). Progestins which have been used include: MGA, CAP, Norethandrolone, and progesterone. The doses of PMSG are comparable with those given during the normal estrous cycle; however, because of the feedback of progestins on the hypothalamo-pituitary system, it would be expected that the optimum dose of PMSG will be influenced by the same factors which affec' hypothalamo-pituitary-ovarian functions, both o exogenous and endogenous sources. Thus, in the presence of CAP, Herefords are more sensitive to PMSG than Angus.

D. *FSH Preparations.* Numerous pituitary FSH

Table 1

The Effect of PMSG on Ovarian Morphology and Fertilization Rate in Heifers

	Treatment		
	Control	1600 i.u. PMSG on Day 16 of cycle	3200 i.u. PMSG on Day 16 of cycle
Interval from Day 16 until oestrus	4.9 ± 1.4*	4.0 ± 1.3	4.0 ± 1.6
No. of ovulations	1.0 ± 0.0	6.4 ± 5.0	12.6 ± 4.7
No. of fertile ova	1.0 ± 0.0	4.4 ± 4.6	4.8 ± 5.7
No. of non-fertile ova	0.0	0.0	3.0 ± 2.1
No. of ova not received	0.0	2.0 ± 0.5	4.7 ± 2.9
No. of follicles 10 mm diameter	0.5 ± 0.5	6.4 ± 11.0	22.0 ± 13.0
Total ovarian wt (g)	10.1 ± 2.8	29.3 ± 12.7	103.0 ± 53.5

*Mean ± S. E.
Henricks, et al., 1973 (Ref. 10)

preparations have been used. Most are crude preparations; there is no pure FSH. As with PMSG, standardization of crude products is problematic. An advantage of the pituitary preparations is their short biological half-life, which enables us to be more specific in terms of timing. The usual approach is to give a number of injections beginning on or about Day 14 of the estrous cycle and continuing for four or five days. This may be followed by HCG or pituitary LH preparations at the beginning of estrus. For commercial application, only in the prepuberal animal are LH preparations necessary. It is not difficult to stimulate follicle growth, causing large numbers of ovulations (more than 50) by the use of pituitary FSH followed by LH. However, the yield of fertilized ova is not generally high. Foote and Onuma (8) listed various treatments, kinds of doses used, routes of administration and the results obtained in the superovulation of cattle by several investigators, in Table 2 (8).

E. *Availability of compounds.* In the United States, progestins or prostaglandins are not available for general use, and this restricts an optimum approach to the use of PMSG on Day 16 of the estrous cycle. In many European countries progestins are available. Prostaglandins can also already be obtained in some countries. It is expected that widespread interest and activity by drug firms will result in availability of prostaglandins in due course in this country as well.

Insemination

In the presence of hormonally-stimulated ovulations, fresh semen gives higher fertilization rates than frozen semen (8,15). It is believed that conditions created in the genital tract by the hormonal regime used for superovulation will reduce viability of semen, especially of frozen semen. Insemination should be carried out intrauterine and should be associated with palpation of the follicle to insure it is being deposited during a period when ovulation occurs. It is imperative to place the semen into the uterus in prepuberal animals, because of difficulties of transport through the cervix.

Recovery of Fertilized Ova

Surgical recovery

The surgical techniques involved in embryo

Table 2

Superovulation in Cattle

Animals		Gonadotropin Treatment	Follicles	Ovulations	Ova	
Age	No.				Recovered	Cleaved
			(no.)	(no.)	(%)	(%)
Sexually mature	32	Days 1-4[a]: 20,10,10,10mg FSH + 5mg LH each day Day 5: 100mg LH		21 (4-55)[b]	53	61
"	14	Day 16: 3,000 IU PMS Days 19, 20: 20 mg 17 β-estradiol Day 21: 2,000 IU HCG	59	33 (14-104)	50	54
"		Same as in Ref. 50 but 4,000-7,000 IU PMS on 16th or 17th day		≃12	≃47	
"	89	Day 16: 3,000 IU PMS Day 21 or 1st day in estrus: 2,000 IU HCG	15	9 (0-55)	71	68
"	7	Synchronized (see text) and 2.5mg FSH 2X daily, days 8-12	27	15 (1-32)	63	84
65-173 days	43	20,10,5,0 and 10mg FSH on Days 1-5; Day 6: 100mg LH		34 (1-88)	33	24[d]
4-24 weeks	13	Day 1: 2,000 IU PMS Day 6: 10mg NIH-LH	28	16 (0-55)	27-60	6
8-17 weeks	55	Day 1: 1,500-2,000 IU PMS Day 6: 50mg PLH	53	43	8-75	72[c]

[a]Day 1 was the 16th day of the cycle for cows not receiving progesterone.

[b]Numbers in parentheses are ranges.

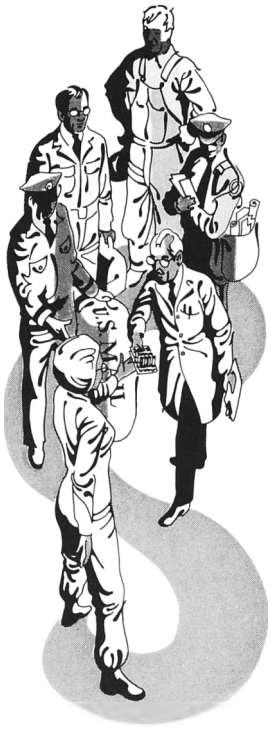
[c]Method currently employed. High pregnant mares' serum dosage possibly represents a difference in potency. Number of ovulation points was estimated by rectal palpation and ova collected nonsurgically.

[d]Included only older calves where rectal examination was possible.

[e]When inseminated 2-3 times with liquid semen.

Foote & Onuma, 1970 (Ref. 8)

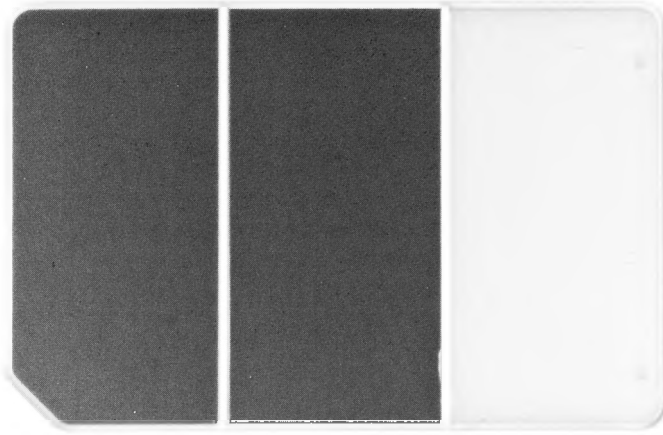
IS MASTITIS DIAGNOSIS GETTING OUT OF HAND?



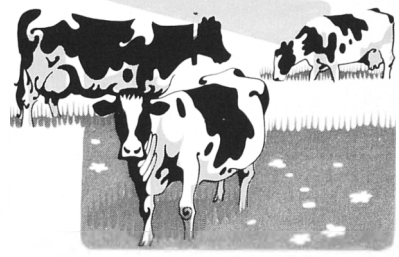
A lot can happen to milk specimens before a diagnosis can be made. The more people who handle those samples, the greater the chances for breaks, spills and delays.

That's why Pitman-Moore developed MASTASSAY* D and MASTASSAY* S Mastitis Diagnostic Media—to put the control of mastitis testing back into the hands where it belongs... yours.

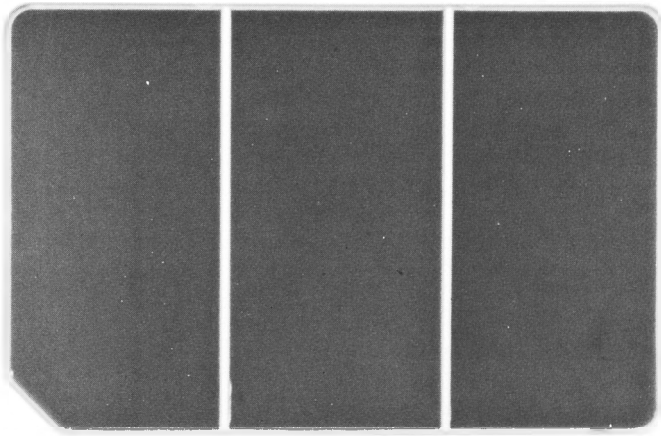
MASTASSAY D and MASTASSAY S give you direct control over all phases of mastitis sampling, testing and treatment and enables you to make an accurate diagnosis within 24 hours.



MASTASSAY D Mastitis Diagnostic Media are three-chambered culture plates that detect streptococcal pathogens in one chamber, common bacterial agents in another and coliform bacteria in the third.



MASTASSAY S Mastitis Diagnostic Media are three-chambered culture plates for the detection of quarters infected with *Streptococcus agalactiae* and allow you to test three cows on each plate.



Together, they give you the tools to establish a rapid, convenient and complete mastitis control program right in your own office.

Contact your Pitman-Moore sales representative for more information about MASTASSAY D and MASTASSAY S Mastitis Diagnostic Media.

*Trademark

MASTASSAY*

Mastitis Diagnostic Media

PITMAN·MOORE
Where better ideas are put into practice

transfer are not complicated. Anyone with large animal surgical experience and familiar with sterile procedures can learn the manipulations involved rather quickly.

There are several factors in the selection of donors which have a direct bearing on the facility with which the egg recovery procedure can be carried out—factors which are directly related to exposure of the uterine horns and the oviducts. Adequate exposure can be a real problem in large cows, e.g., Brown Swiss, in fat cows, cows with large (pendulous) udders, cows with a small genital tract (particularly with short broad-ligaments). These are hardly criteria in the selection of the genetically superior dam, however, the 15-year-old Holstein cow with the consistent annual record milk production in excess of 30,000 lbs. is a potential candidate with such attributes.

Starving the animal 36-48 hours prior to surgery and using the proper restraint and anesthesia facilitates the procedure. General anesthesia may be induced with Surital to effect, the animal intubated and maintained under Halothane inhalation anesthesia.

The preparation of the surgical site is routine for a sterile surgical procedure and includes draping. The hindquarters are slightly elevated to allow the viscera to fall forward in the abdominal cavity, which aids in the locating and positioning of the genital tract. A mid-line incision is made, about 30 cm long, as far posteriorly as possible, even to the point of undermining the udder, depending on the size of the latter. After locating the uterus, umbilical tape is placed through the broad-ligament around the body of the uterus. This provides a "handle" on the tract. It is anchored to a crossbar which is located transversely at the posterior end of the incision. An ovary is now exposed and the number of corpora hemorrhagica and anovulatory follicles are recorded.

The infundibulum and oviduct are canulated. A small glass canula can be used or a variety of other canulae such as silastic, teflon, polyethylene tubing (PE 240) works well. The canula may be held in place by hand with slight digital pressure, with a vasectomy forceps, or with a baby doll clothespin. Trauma is to be avoided—not only is blood undesirable in the collection fluid but adhesions could form.

Embryos are found in the oviduct until about the fourth day after ovulation. The oviducts may be flushed by inserting a blunt needle into the tip of the uterine horn and injecting 10-15 ml. flushing medium through the tubo-uterine junction into the collecting dish. The horn itself is then flushed by clamping it off with a rubber-shod Doyen intestinal forceps, or an Allis tissue forceps across the base, or simply by closing it off with digital pressure and then inserting a needle near the base and injecting an additional 30-50 ml. of medium. Most ova are found in the first flushings, particularly from the oviduct. The technique described so far is most commonly used with possible minor modifications.

The simplest procedure is to remove the organs at slaughter, clamp the horns of the uterus near the body, ligate the oviducts and aseptically flush the oviducts and uterine horns. However, Rowson, Moor and Lawson (19) obtained a 91% pregnant rate for surgically recovered eggs, and 33% for eggs recovered at slaughter and then transferred surgically.

Non-surgical recovery

The non-surgical approach of embryo recovery offers the possibility of repeated collections of embryos from the same donor with minimum trauma. Non-surgical procedures and their results in cattle have been reported by several workers (1,24,25). Tubes are passed through the cervix as in artificial insemination. Most procedures have involved irrigation of the uterus with varying numbers of canulae to inject fluid and wash out any embryos. The approach is only applicable to embryos which have advanced in development, i.e., they must be in the uterus vs. the oviduct. Several apparatuses described have an inflatable cuff to hold the instrument in place and to prevent loss of flushing medium. In general, the results have not been encouraging, varying degrees of success were reported, but none of the procedures gave as consistent a recovery as surgical methods. Sugie (24) in Japan has reported the results from superovulated cattle. He used a two-canula device and 2000-3000 ml. of flushing medium. Out of 32 cows, 1-4 ova were collected from 10 cows, 5-8 ova from 11 cows, 10-16 ova from 5 cows, and none from the remaining six cows. The entire uterine horn was flushed with this equipment rather than just the anterior portion; this may have contributed to the high recovery rate. If this recovery rate can be attained regularly and embryos obtained undamaged, the procedure could provide an acceptable means for routine recovery of embryos in cattle.

Handling and Storage of Embryos

As the fertilized ova are flushed from the protective environment of the uterus, it becomes of the utmost importance that the medium used is not detrimental to the embryo and preferably that it enhances its viability.

The same kind of meticulous care in preparation of glassware for tissue culture must be given to the preparation of glassware, syringes and tubing used in the collection of embryos; a tedious dishwashing protocol is required. The temperature of the medium is important as is the gaseous environment.

Chang (6) showed that rabbit embryos could be stored in homologous serum at 10°C with some embryos remaining viable 6-7 days. Further studies showed that serum of several species, including cattle and sheep, contained a thermolabile factor which was lethal to rabbit ova after 10 minutes of exposure. Heating serum to 55°C for 30 minutes destroyed the factor.

Little is known about the requirements for culturing cattle ova. When ova were placed in homologous serum, usually not more than one cleavage occurred. Sreeman, Scanlon and Gordon (21,22) obtained better results with follicular fluid than with serum. Rowson, et al., (19) obtained no pregnancies when 33 ova in homologous serum were transferred to recipients, but when a synthetic medium TCM 199 was used 9 out of 11 heifers were pregnant following surgical recovery and transfer of ova.

TCM 199 is now widely used to flush the fertilized ova into a warm collecting dish, usually a watch glass or a similarly concave recepticle which can be placed directly under the dissecting microscope. Ova are located in the nadir of the dish with a magnification of 10-12 power, which is then increased to 40 power to determine if the ovum is suitable for transfer. The fertilized ova are carefully removed from the flushing fluid with a special small pipette and placed in fresh medium. In a sense the embryos are rinsed this way, removing any undesirable debris that might inadvertently have been picked up during flushing.

There is considerable interest in bovine embryo culture in order to determine growth requirements by manipulation of the culture medium as well as the gaseous environment. It would further aid in the development of methods of maturing an embryo to a stage of optimal compatibility with the uterus of the recipient.

Inovulation of Fertilized Ova

Management and synchrony of recipients

The focus thus far has been on the donor animal and rightfully so; however, the process of embryo transfer is a factorial one. Hence, selection of recipients warrants greater attention than it generally receives. Besides careful synchrony with the cycle of the donor, factors such as nutrition and after-care are important. The animal should be protected against diseases which may cause abortion, viz. IBR, BVD, Brucellosis and Leptospirosis.

Recipients may be selected from a large pool of normally cycling cows and heifers, or they may be brought into synchrony with the donor by the use of PGF_{2a}. As mentioned earlier the latter compound is not commercially available in the United States, nor will it be, apparently, in the near future. With either natural or induced synchronization, the timing of ovulation should be monitored by rectal examination.

Inovulation

The final step in embryo transfer in cattle is inovulation. Inovulation is a term comparable to insemination and refers to the introduction of fertilized ova into the uterus of the synchronized recipient, or foster dam. Thus far the best results have been obtained by surgical means. The recipient is held off feed, anesthesized and prepped in a manner identical to the one described for the donor. The midline or paramedian approach provides good exposure of both horns.

Transfer should be made from oviduct to oviduct or from uterus to uterus. Generally, the latter is the case. A fine stab wound is made with an atraumatic object, such as the eye-end of a suture needle, inasmuch as bleeding is to be avoided. The fertilized egg is then introduced via this opening with a modified Pasteur pipette, and deposited in the tip of the uterine horn about 5 cm away from the point of entrance. For optimal results the horn ipsilateral to the corpus luteum is used. It is possible to inovulate two embryos per recipient. Rowson, Lawson and Moor (17,16) transferred two eggs to a single horn of a group of heifers (11) and obtained a pregnancy rate of 73%, while only 45% had twins at slaughter or calving. Of a second group of heifers (11) receiving only one egg in *each* uterine horn, the pregnancy rate was the same (73%) but 73% had twins. A much greater degree of embryonic loss was found in the slaughtered group which had received two eggs to a single uterine horn. The number of placentomes which developed was much greater in heifers with a bilateral twin pregnancy.

Alternately, a standing laparotomy via the paralumbar fossa can be used if only one egg is transferred to the horn ipsilateral to the corpus luteum. Adequate exposure of the tip of the uterine horn can be obtained with a diagonal incision starting at the tuber coxae in an anteroventral direction. This is parallel to the fibers of

the internal abdominal oblique muscle. The advantage is that several recipients can be prepared in stocks or stanchions, making the procedure faster and cheaper.

Non-surgical transfer

Despite repeated attempts by a number of workers to transfer ova to the cow by non-surgical methods, only very limited success has been obtained. Two basic reasons have emerged: 1. uterine infection (12), due to the progestational influence of the cycle, and 2. expulsion of the eggs via the cervix (3). These factors may work either singly or in conjunction with each other.

Sugie (23,25) reported success by using a technique by-passing the cervix and involving puncture of the fornix of the vagina and inflation of the uterus with CO_2. The mechanism by which CO_2 assists in the establishment of pregnancy remains as yet unresolved. It has been thought to depend on a relaxing or anesthesizing effect on the uterine musculature, which in turn prevents the expulsion of the transferred egg from the uterus of the recipient. Methods to introduce the egg directly through the cervical canal have, with rare exceptions, resulted in failure even when a variety of drugs were used to minimize stimulation of the cervix.

Conclusions

It is obvious that many problems will have to be overcome before the commercial use of the technique of embryo transfer can be considered a success. Betteridge and Mitchell (4) present an honest appraisal of 24 completed cases of embryo transfer in cattle. Their experiences are candidly summarized in Table 3.

It is appropriate that an early scientific evaluation of recognized problems be attempted, while for the commercial organizations the importance of records and their analysis cannot be overemphasized. No data are available as yet on the subsequent fertility of donors. However, personal experience suggests that adhesions may occur between the ovaries, oviduct and uterine horn in a proportion of cases. Data on the response following repeated superovulation are limited.

Lest the optimist become complacent, at least three obstacles will need to be cleared before embryo transfer in cattle will be as common as artificial insemination today: 1. non-surgical recovery of ova; 2. long-term storage; and 3. non-surgical inovulation.

Table 3

Wastage in Embryo Transfer Between Donors (D) and Recipients (R)

Potential Transfers 39 D 47 R			
Completed 20 D 24 R		Not Completed 19 D 23 R	
Pregnant	Not Pregnant	Loss of Synchrony	Donor Failure
11 R	13 R	2 D 4 R	15 D* (16 R)
			Recipient Failure (2 D) 3 R**

*No estrus (3); Anovulatory estrus (3); 0/1 ova recovered (5); no fertilization (4).
**Pregnant (1); Anovulatory estrus (2).
Betteridge & Mitchell, 1974 (Ref. 4)
Donor failure accounted for the majority of unsuccessful procedures.

Acknowledgement

The author gratefully acknowledges Dr. D. R. Lamond for his encouragement and advice in the preparation of the manuscript.

References

1. Bedirian, K. N. and Baker, R. D. An Intravaginal Method for the Recovery and Transfer of Bovine Eggs. Can. J. Anim. Sci. 53:67-69, 1973. – 2. Bellows, R. A. and Short, R. E. Superovulation and Multiple Births in Beef Cattle. J. An. Sci. 34: Suppl. I: 67-69, 1972. – 3. Bennett, J. P. and Rowson, L. E. A. The Use of Radioactive Artificial Eggs in Studies of Egg Transfer and Transport in the Female Reproductive Tract. Proc. IVth Intl. Congr. Anim. Reprod., The Hague, 1961. – 4. Betteridge, K. J. and Mitchell, D. Embryo Transfer in Cattle–Experience of Twenty-four Completed Cases. Theriogenol. 1: 69-82, 1974. – 5. Bowen, J. M. Problems Associated with Ovum Transplants. Vet. Rec. 92(1): 17, 1973. – 6. Chang, M. C. Effects of Heterologous Sera of Fertilized Rabbit Ova. J. Gen. Physiol. 32: 291-300, 1949. – 7. Cloninger, W. H. and Thoele, N. W. Some Relationships Between Twin Births and Milk Production and Breeding Efficiency. J. Dairy Sci. 40: 630, 1957. – 8. Foote, R. H. and H. Onuma. Superovulation, Ovum Collection, Culture and Transfer. A Review. J. Dairy Sci. 53: 1681-1692, 1970. – 9. Heape, W. Preliminary Note on the Transplantation and Growth of Mammalian Ova Within a Uterine Foster-mother. Proc. Roy. Soc. (London), 48: 457, 1890. – 10. Henricks, D. M., Hill, Jr., J. R., Dickey, J. F. and Lamond, D. R. Plasma Hormone Levels in Beef Cows with Induced Multiple Ovulations. J. Reprod. Fert. 35: 225-233, 1973. – 11. Henricks, D. M. and Lamond, D. R. Hormonal Interrelations in Beef Cows with Induced Multiple Ovulations. Nature, Lond. 235: 222-223, 1972. – 12. Lamming, G. E. and Rowson, L. E. A. Ovarian Hormones and Uterine Infection. Proc. Roy. Soc. Med. 46: 387-392, 1953. – 13. Lamond, D. R. The Effect of Pregnant Mare Serum Gonadotropin (PMS) on Ovarian Function of Beef Heifers, as Influenced by Progestins, Plane of Nutrition, and Fasting. Aust. J. Agric. Res. 21: 153-161, 1970. – 14. Laster, D. B. Ovulation, Fertility and Prenatal Mortality in Heifers Treated with PMSG or Porcine FSH. J. Reprod. Fert. 33: 275-282, 1973. – 15. McKenzie, Basil E. and Kenney, R. M. In Vitro Culture of Bovine Embryos. Am. J. Vet. Res. 34(10): 1271-1275, 1973. – 16. Rowson, L. E. A., Lamming, G. E. and Fry, R. M. Influence of Ovarian Hormones on Uterine Infection. Nature, Lond. 171: 749, 1953. – 17. Rowson, L. E. A., Lawson, R. A. S. and Moor, R. M. Production of Twins in Cattle by Egg Transfer. J. Reprod. Fert. 25:261-268, 1971. – 18. Rowson, L. E. A. and Moor, R. M. Non-surgical Transfer of Cow Eggs. J. Reprod. Fert. 11: 311-312, 1966. – 19. Rowson, L. E. A., Moor, R. M., and Lawson, L. A. S. Fertility Following Egg Transfer in the Cow: Effect of Method, Medium and Synchronization of Oestrus. J. Reprod. Fert. 18: 517-523, 1969. – 20. Seidel, Jr., G. E., Larson, L. L., Spilman, C. H., Hahn, J. and Foote, R. H. Culture and Transfer of Calf Ova. J. Dairy Sci. 54(6): 923-926, 1971. – 21. Sreenan, J., Scanlon, P.

(Continued on page 56)

accessibility to cattle. Insecticides, herbicides, and fungicides are routinely and haphazardly applied to animal and environmental surfaces alike. Drugs are considered to have therapeutic effects; but disregard for recommended dosages can result in poisonings.

Failure to provide satisfactory storage facilities for animal feeds and the improper preservation and handling of feedstuffs allow the development of a variety of mycotoxins.

The dependence of animals upon their owners for the total environment makes these animals susceptible to environmental pollutants. Exposure to noxious gases, irritating and hazardous industrial materials and wastes, water contaminants, and casually discarded compounds of man's own use can result in illnesses and death. As long as such potentially toxic materials exist and are utilized, the hazards for cattle will be a prominent concern of the bovine practitioner.

Selected References

Buck, W. B., G. D. Osweiler, and G. A. Van Gelder: Clinical and Diagnostic Veterinary Toxicology. Kendall/Hunt Publishing Co., Dubuque, Iowa, 1973. – Clarke, E. G. C. and J. L. Clarke: Garner's Veterinary Toxicology, 3rd Ed. Williams & Wilkins Co., Baltimore, 1967. – Hulbert, L. C. and F. W. Oehme: Plants Poisonous to Livestock, 3rd Ed. Kansas State University Press, Manhattan, 1968. – Oehme, F. W.: Copper Toxicity in Ruminant Animals. Southwestern Vet., XIX, (Summer, 1966): 295-301. – Oehme, F. W.: Tissue Residues in Meat. Kansas Veterinarian, 25, (Oct., 1971): 14-20. – Oehme, F. W., ed: Symposium on Veterinary Toxicology. Clinical Toxicology, 5, (Summer, 1972): 141-302. – Oehme, F. W.: Significance of Chemical Residues in United States Food-Producing Animals. Toxicology, 1, (1973): 205-215. – Olson, J. R., F. W. Oehme, and D. L. Carnahan: Relationship of Nitrate Levels in Water and Livestock Feeds to Herd Health Problems on 25 Kansas Farms. Veterinary Medicine/Small Animal Clinician, 67, (1972): 257-260. – Radeleff, R. D.: Veterinary Toxicology, 2nd Ed. Lea & Febiger, Philadelphia, 1970. – Ridder, W. E. and F. W. Oehme: Nitrates as an Environmental, Animal and Human Hazard. Clinical Toxicology, 7, (1974): 145-159.

The Dairy Herd Health Program Method

(Continued from page 14)

enables us to find out exactly what the farmer is doing in his management, or conversely, to convey to the farmer exactly what we suggest he do in his management.

At an annual meeting with all the farmers in the group, the average performance of all herds is discussed, and an anonymous list of individual herd performances. Each farmer can see where he is in the efficiency order, learn what the potential is, and in the discussion, what are the techniques which are best used to achieve it. These are good meetings for us to measure consumer resistance to new procedures we would like to introduce, like a rise in fees.

Conclusions

Well, that is the system and I hope you were not too confused. It is a difficult subject to describe in detail in a few minutes, but I could see no point in discussing the subject only in generalities. Consideration of the detailed workings of a program such as this is one of the two important ways of conveying whether or not it is practicable. The other important way of demonstrating practicability is by demonstrating that the desired results can be achieved. I think I have done that in the mastitis paper and I hope to add to that in the talk on fertility tomorrow (see other paper). Those results should convey the impression that in our hands it is a practicable program. However, in spite of anything I may have said or may still say about its virtues, and I am inclined to exaggerate to make a point, the cold fact is that it is a provisional program and very much on trial in a full commercial situation.

We have every confidence in it in the rather narrow limits of a high-priced liquid milk production system.

Although we think it can be readily adapted to dairy herds producing milk for processing into other dairy products, especially butter, and to beef herds, we have not had enough experience in these areas to say how the adaptation should be done.

Editor's Note: For an extensive discussion of this and other programs, please refer to pages 13-26, Proceedings of the 1973 AABP Convention.

Embryo Transfer in Cattle

(Continued from page 26)

and Gordon, I. Culture of Fertilized Cattle Eggs. J. Agric. Sci. Camb. 70– 183-185. 1968. – 22. Sreenan, J., Scanlon, P. and Gordon, I. Storage of Fertilized Cattle Ova In Vitro. J. Agric. Sci. Camb. 70: 593-594, 1970. – 23. Sugie, T. Successful Transfer of a Fertilized Bovine Egg by Non-Surgical Techniques. J. Reprod. Fert. 10: 197-201, 1965. – 24. Sugie, T. Non-Surgical Ova Collection in Cattle. (Jap.) Ann. Report Nat. Inst. An. Ind., Chiba-shi, Japan 1968, 8: 55, 1970. – 25. Sugie, T., Soma, T., Fukumitsu, S., Otsuki, K. and H. Onuma. Studies on Egg Transfer in the Cattle: On Non-Surgical Recovery and Transfer. (Abstract). Jap. J. Zootech. Sci. 42: Suppl. 1, 48, 1971. – 26. Testart, J. and LeGlise, P. C. Transplantation d'oeufs divises, chez la vache, par voie transvaginale. C. r. Acad. Sci. (Paris) Series D, 2591-2592, 1971. – 27. Vincent, C. K., Mills, A. C. and Rundell, J. W. Non-Surgical Transfer of Embryos in Beef Cattle. (Abstract). J. An. Sci. 28: 147, 1969. – 28. Ward, A. H. Annual Report. Rep. N. Z. Dairy Bd. 26: 53, 1950. – 29. Warwick, B. L., Berry, R. O. and Horlacher, W. R. Results of Mating Rams to Angora Female Goats. Proc. Am. Soc. An. Prod. 225-227, 1934. – 30. Whittingham, D. G., Leibo, S. P. and Mazur, P. Survival of Mouse Embryos Frozen to -196° and -269° C. Science 178: 411-414, 1972. – 31. Willett, E. L., Black, W. G., Casida, L. E., Stone, W. H. and Buckner, P. J. Successful Transplantation of a Fertilized Bovine Ovum. Science 113: 247, 1951. – 32. Wilmut, I. and Rowson, L. E. A. The Successful Low-Temperature Preservation of Mouse and Cow Embryos. J. Reprod. Fert. 33: 352-353, 1973.