NON-SURGICAL EMBRYO TRANSPLANTATION FOR DAIRY CATTLE

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Introduction

Surgical methods for transferring supplemental embryos from a donor female to corresponding recipient females in swine, sheep, cattle and more recently in horses has been well documented in the scientific literature. During the last four years, non-surgical techniques have been developed for commercial use in cattle with recent emphasis on developing a non-surgical method for embryo transplantation in the mare.

Although non-surgical approaches for cattle have not reached the level of success achieved with standard surgical procedures for embryo transplantation, the non-surgical method is viewed as a major breakthrough for the commercial cattle industry. Experts predict that on-the-farm embryo transplants will become an important part of future cattle breeding programs in this and other countries of the world. Many progressive dairy cattle owners are now utilizing non-surgical embryo transplant procedures to attain an impressive number of high quality offspring from valuable donor cows. Furthermore, embryo culturing, embryo sexing and embryo freezing methodology is presently under intensive investigation by researchers and these procedures soon will likely become an important adjunct to the commercial embryo transplant scene.

Superovulation of Donor Cattle

Superovulation of the donor cow or heifer is accomplished by treating the animal with injectable hormones that stimulate growth of numerous follicles on each overy, most of which will mature and ovulate a single egg (ovum) potentially ready for fertilization. This is an important part of the overall procedure, since the cow usually ovulates only one ovum for fertilization during each 21-day estrous cycle. Superovulating donor animals allow the operator to collect many ova from each quality female.

Superovulation may be induced with either Pregnant Mare's Serum Gonadotropin (PMSG) or Follicle Stimulating Hormone (FSH) preparations given during the middle of the estrous cycle. The ovarian stimulatory substance (PMSG) found in pregnant mare's blood is found primarily in the serum of females between 40 and 150 days of gestation. In the past, commercial companies have harvested blood of pregnant mares and prepared

lyophilized PMSG for use by veterinarians and researchers. The FSH used for ovarian stimulation is extracted from commercial slaughterhouse pituitary specimens. It too has been made available for research and veterinary use in a crystalline, lyophilized form that is reconstituted prior to its use.

Proper use of FSH in a superovulation schedule for donor cattle has now been shown to be as effective in commercial transplant units as PMSG. In fact, many of the most successful embryo transplant stations in the United States and Canada now use FSH as the agent of choice for bovine superovulation in place of PMSG. Because of its chemical nature, FSH is short-acting, biologically. Since the half-life of exogenous FSH is believed to be 5 hours or less in the blood of donor females, twice daily injections are often administered (a.m. and p.m.) to maintain endogenous blood levels for efficient stimulation of follicular growth. These twice daily FSH injections are usually administered at 12-hour intervals for five consecutive days for a total of 10 individually given to each donor female. Most injection schedules use from 25 up to 50 milligrams (mg) of FSH-P® (Burns-Biotec) per donor animal during the superovulation procedure. Single doses given with various injection procedures are usually 5 mg or less per injection. In contrast, when PMSG is given as the stimulatory agent, it is administered all in one injection at the time of treatment.

In review of the information available, there appears to be as many dose level modifications of FSH injection schedules for donor cattle as there are active transplant units, with no one scheme proven conclusively to be superior over another. This modified FSH treatment scheme has, in many cases, become the "treatment" of individual embryo transplant units. At this institution, daily dose levels of FSH-P® are adjusted to individual donor cows following daily rectal ovarian palpation. This approach now has withstood the test of time and is used routinely on all donor females.

At 48 to 72 hours after the first FSH or the PMSG injection, a second agent is used in the treatment schedule to induce regression of the corpus luteum (luteolysis) on the surface of the cow's ovary. Inducing regression of this endocrine tissue allows the potential donor to exhibit heat (estrus) within 72 hours post-injection for subsequent artificial insemination (AI). The luteolytic agent used by embryo transplant units is the same drug made commercially available late in 1979 for estrus synchronization of beef and dairy cattle — prostaglandin $-F_2$ alpha (Lutalyse®:Upjohn Co.). Without the use of this luteolytic agent to regress functional luteal tissue in the donor animal, the follicular stimulating agent (FSH or PMSG) must be started on days 15, 16 or possibly 17 of the estrous cycle to take advantage of natural luteolysis of the cow (see Figure 1). However, with the use of a luteolytic agent the superovulation treatment schedule can begin on any time between days 6 and 15 of the estrous cycle (see Figure 2). Not only does the luteolytic agent increase the effectiveness of the superovulation procedure, but it also gives latitude in treating donors on different days of

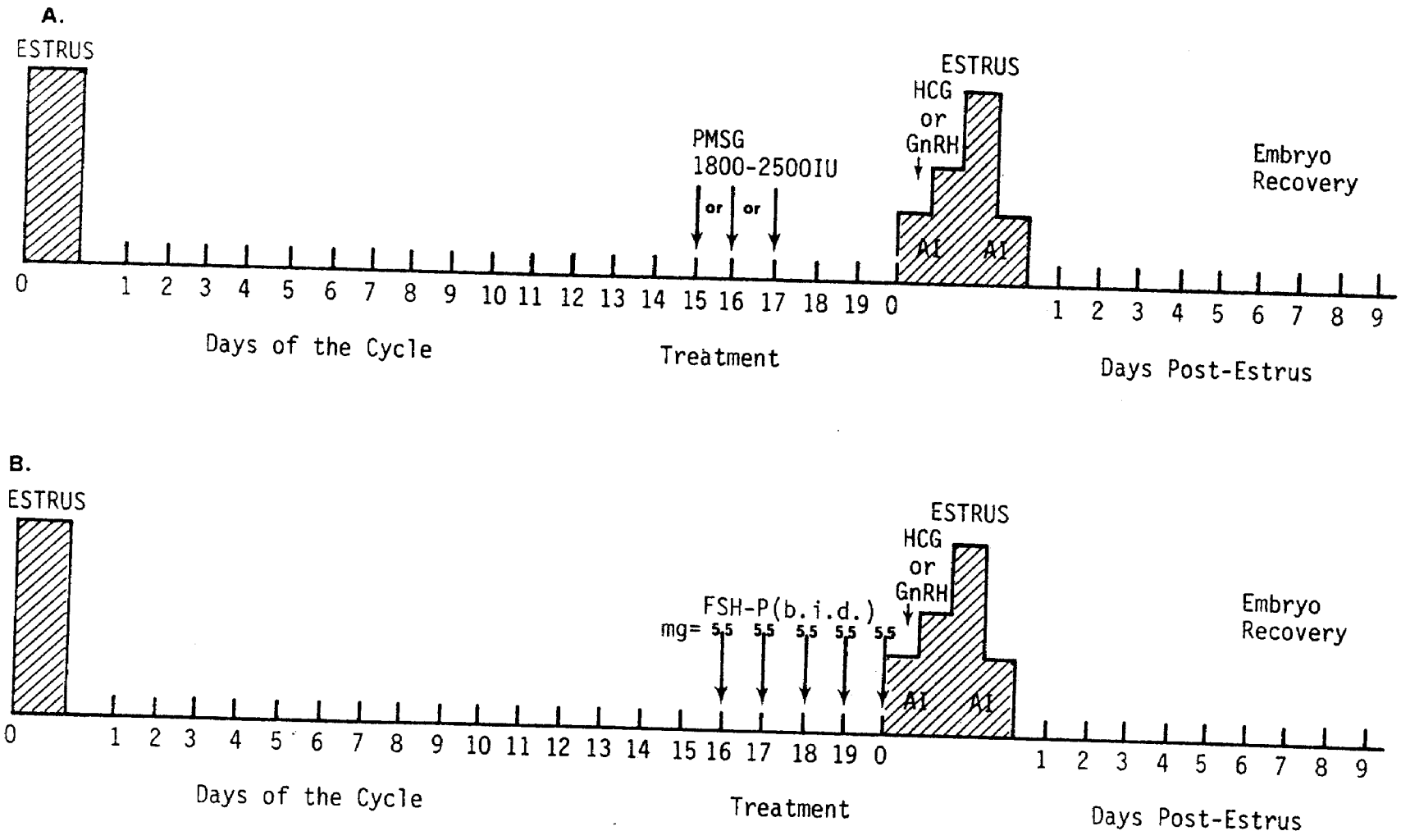


Figure 1. Examples of earlier injection schedules using either PMSG or FSH-P with the option of HCG or GnRH for superovulating donor cattle. Since prostaglandin agents were not used to induce luteolysis in these schemes, treatments were started on days 15, 16 or 17 of the estrous cycle (onset of estrus = day 0).

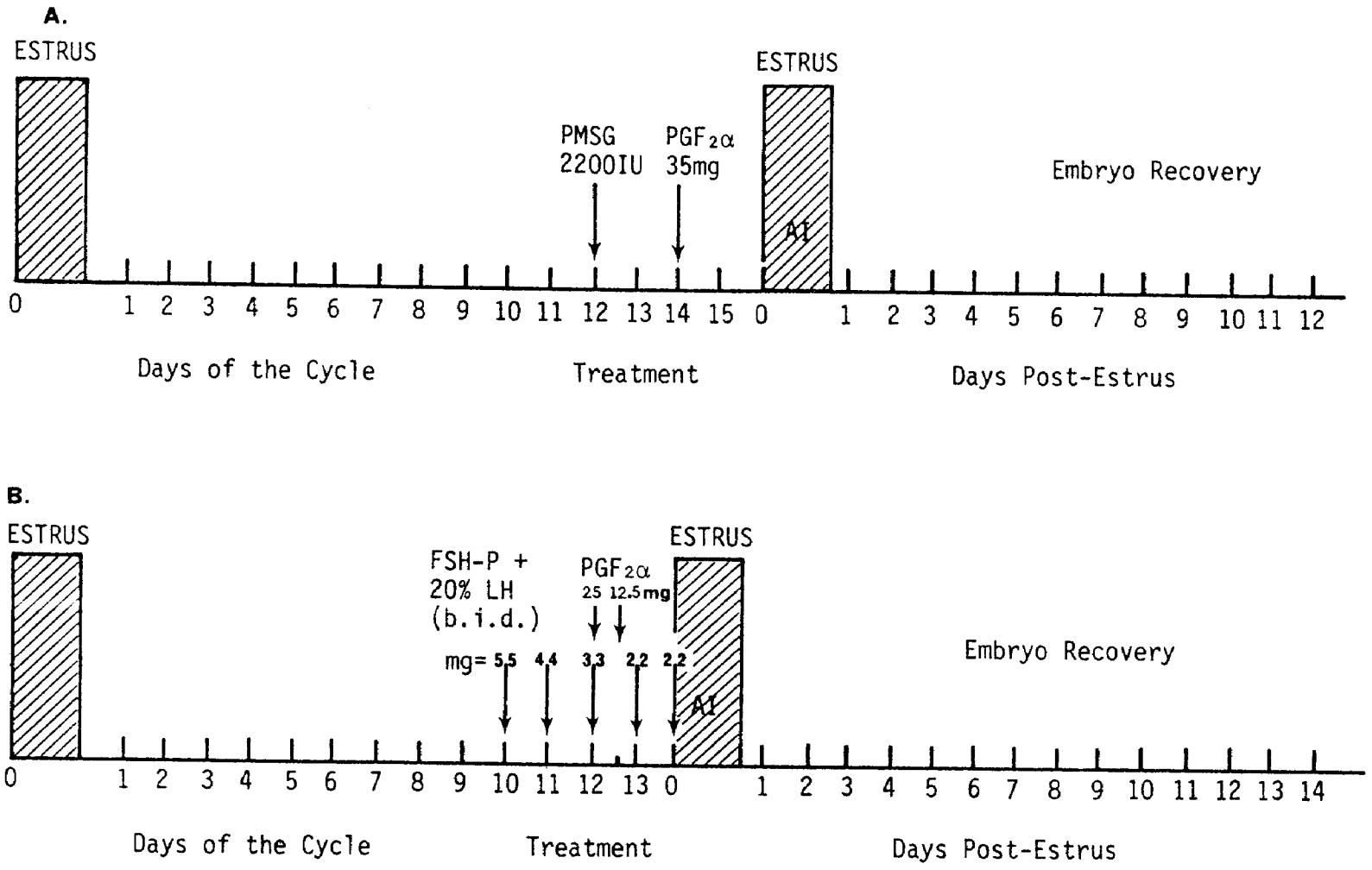


Figure 2. Examples of several more recent bovine superovulation schemes using either PMSG or FSH-P with $PGF_{2\alpha}$ as a luteolytic agent (onset of estrus = day 0). Donor animals usually ovulate without the use of HCG or GnRH with this schedule.

the estrous cycle (luteal phase) to better fit available recipient cyclicity and will allow dissemination of the daily work load of the embryo transplant team.

Although a single intramuscular dose of 25 mg of Lutalyse® is generally recommended for inducing luteolysis in cycling cattle, single doses of 25, 30, 33.5, 35, 37.5, 40 and 50 mg of this agent have been indicated as being effective for this purpose during superovulation procedures with donor cows and heifers. When administered as a single dose, prostaglandins are usually administered 48, 60 or 72 hours after the onset of the follicular stimulating treatment. Today many commercial transplant units administer a single intramuscular dose of 25 to 35 mg of Lutalyse® 48 hours after the initial treatment with the stimulatory agent. Several classical superovulation schemes used on donor cattle are presented for your evaluation in Figures 1 and 2.

Artificial Insemination of the Donor Females

Once the hormone-treated donor female exhibits estrus, several inseminations, usually with a commercial prepared semen, are used to provide ample spermatozoa (sperm cells) for fertilization of the increased number of superovulated ova. Since selected genetic matings are of primary interest in the embryo transplant business, frozen semen is used predominately at commercial bovine transplant stations.

Usually superovulated donor cattle are inseminated at least twice, and many times these donors are covered with a third and a fourth insemination. The job of estrus detection is probably one of the most important of those at an embryo transplant station. Since donor animals are inseminated at various time intervals in reference to the onset of standing estrus, it is of primary importance that this reference point be established for each donor and recipient female. This is essentially a full time job that requires near continuous observation by an observant team member during dusk, night-time and early morning hours. Although KaMar® heat detector patches, Gomer bulls and testosterone-treated heifers are useful aids in detecting estrus, there is no substitute for a heat checker riding frequently among donor and recipient herds to pinpoint the onset of standing estrus.

If the superovulated donor is inseminated three times, the first insemination is usually performed at the onset of standing estrus, followed by two subsequent inseminations at 12-hour intervals. One standard approach has been to inseminate with two units of semen at the onset of standing estrus followed 12 hours later with three units and a third inseminate with one or two units 24 hours after the first insemination. A more conservative approach would be to inseminate with two or three units 8 to 12 hours after the onset of standing estrus with two or three units 12 hours after the first insemination. Generally with an

intact, non-superovulated donor female slated for a single embryo collection less units are used per insemination. As with modifications of the superovulation treatment schedules, each embryo transplant unit seems to have their own modified procedure for time of insemination and number of units per donor animal.

Shortly after insemination, fertilization of multiple ovulated ova takes place in the upper one-third of each oviduct in treated donor cows. The developing embryos then remain in the oviducts of donor animals at least 3 days after fertilization. Approximately 4 or 5 days after estrus, the 4- to 16-cell embryos migrate from the oviducts through the uterotubal junction to the distal end of the uterine horns, where they remain grouped until the standard time of collection. It should be noted that following entry of the uterine horn greater than 75% of the fertilized ova remain in the anterior portion of the horn until day 8 of the subsequent estrous cycle.

Preparations for Non-Surgical Collection

A keen knowledge of endocrine control of the bovine estrous cycle, a highly developed skill in rectal ovarian palpation, and an abundance of patience are considered a necessity for efficient non-surgical embryo harvesting from cattle. A minimum of 12 months of intense practice is often required to attain the skills needed for optimal results in embryo collection.

The standard non-surgical collection procedure for cattle is accomplished while the donor is restrained in a squeeze chute. The tailhead region of the donor is clipped of excess hair, and external reproductive parts are washed thoroughly with surgical soap and water. Often injectable tranquilizing agents are used to calm the donor during the collection procedure.

The tailhead region is usually deadened temporarily with a local anesthetic to reduce straining and contractions by the donor during embryo flushing. Often a sling is placed under the heart girth and the flank region to keep the animal standing. Although difficult, maintaining cleanliness during embryo collection is of key importance.

Several types of non-surgical collection tubes, catheters, and similar devices have been used to collect cattle embryos. Two-way and three-way flexible Foley catheters (14 to 22 Fr.) have recently become the instruments of choice for this procedure in dairy cattle.

The donor's reproductive tract is manipulated rectally with one gloved hand, while the other hand is used to maneuver the Foley catheter (and stylet) through the cervical canal. The distal end of the catheter is directed into the uterine horn from which the operator wishes to collect first.

Many times the Foley catheter cannot be passed easily through the cervix (opening to the uterus) in this manner. In these cases, a stainless steel probe (expander) can be used to manipulate through the cervical canal. Cervical expanders are occasionally used to widen the canal for passage of the Foley catheter (and stylet) into the body of the uterus of young heifers. Once the catheter is in place in the uterine horn, the cuff on the catheter is inflated and the stylet is removed.

Embryo Flushing Procedures

Most embryo transplant units use a phosphate buffered saline or a tissue culture medium to flush free the developing embryos lodged in the upper end of the uterine horn. Donor cattle are most often flushed between days 5 and 8 after their induced estrus. The volume of fluid used by individual technicians to flush donor females usually ranges from 1/4 to 4 pints of medium.

It should be obvious that the flushing technique is very important to the success of this procedure. The flushing medium infused into the Foley catheter is usually controlled manually by a large syringe (40, 50 or 60 milliliter) or by a gravity flow system. The pressure of the medium in the gravity flow system is controlled by raising and lowering the receptacle containing the medium. After being filled with the desired volume of medium, the distended uterus is gently massaged to free the embryos (see Figure 3), which then flows into a collection vessel. After one uterine horn has been flushed two to four times, the Foley catheter is inserted into the opposite uterine horn and the flushing procedure is repeated. Some technicians have developed a uterine body flushing technique which is used in place of the uterine horn flushing procedure. Whichever one of these approaches is used to harvest embryos non-surgically, the collection procedure generally takes 15 to 30 minutes per donor cow. Although the non-surgical collection is done within a clean environment and generally only takes a short period of time, antibiotics are usually administered after flushing to protect the donor against the possibility of uterine infection.

Single Embryo Collections.

With the increased interest in non-surgical embryo transplants being generated by dairymen across the United States, the single embryo recovery approach has recently become the primary topic of discussion. Since most commercial transplant stations do not have adequate milking facilities to handle high producing dairy cows, donors are often transported to commercial units after they have completed their lactation record. Even when transplant stations are able to handle lactating donor cows, the producer often fears that the stress of travel and unfamiliar surroundings at the station will decrease daily milk production. For these reasons on-the-farm approach to embryo transplantation has

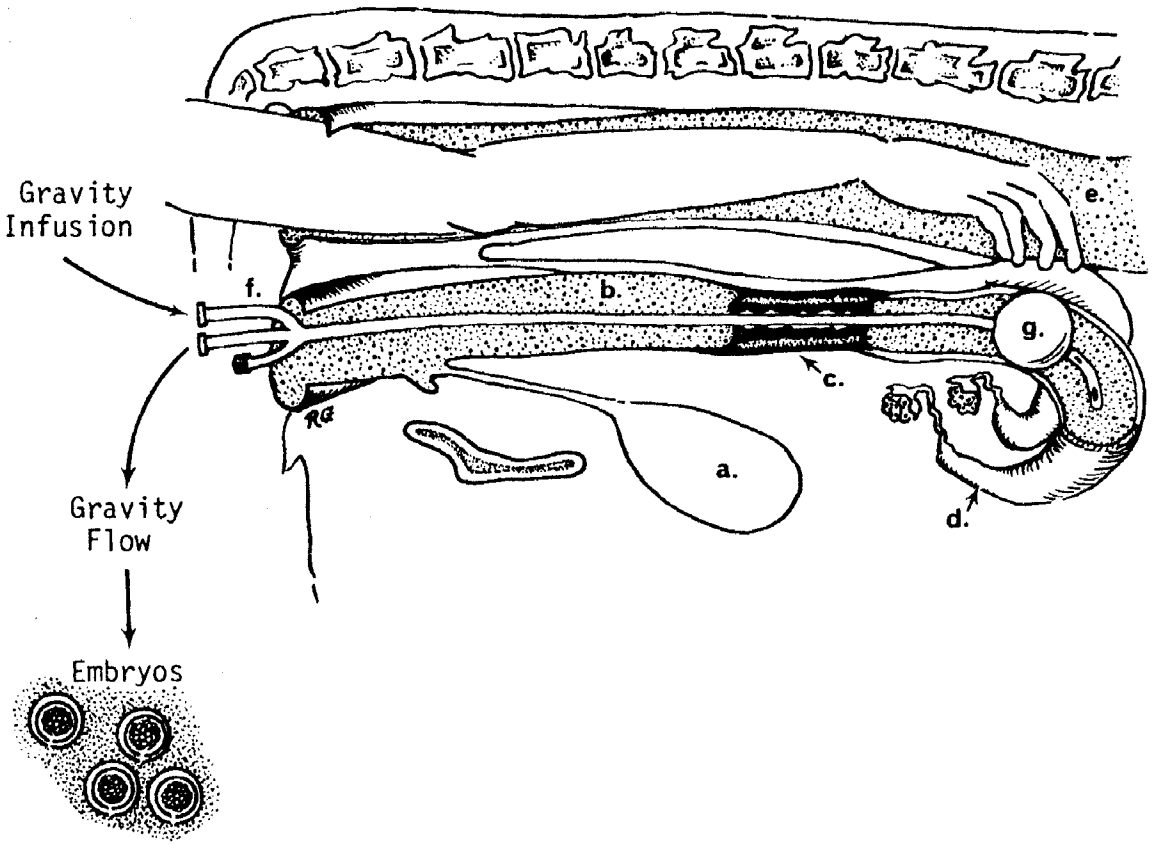


Figure 3. A gravity flow non-surgical embryo collection system for cattle: (a) bladder, (b) vagina, (c) cervix, (d) uterine horn, (e) rectum, (f) three-way Foley catheter and (g) inflated cuff.

become very popular in some areas of the United States during the last three years.

In addition, hormonal treatments used for stimulating follicular development are suspected of lowering the average daily milk production during the superovulating procedure, which has also shied many dairymen away from transplanting embryos from his high producing dairy cows. For this reason, on-the-farm embryo transplanting programs have recently been set up to collect single ovulated embryos from non-superovulated donors. Although only one embryo can be potentially collected from the donor per estrous cycle, the donor can be collected on several successive cycles, without the use of stimulatory hormone treatments. Furthermore, this procedure can be done without removing these high producing donor cows from the milking string. The flushing procedure is similar to that previously described for superovulated donors, however, only the uterine horn adjacent the ovary that ovulated is flushed at the time of collection. From our experience, single embryo recovery rates tend to be higher than those of superovulated donors. Dairymen are beginning to use this program to collect one, two or three extra embryos for transfer from their superior cows during early lactation, and then artificially inseminating the same donor to carry her own calf through the term. This approach to embryo transplantation appears to be very acceptable to the progressive dairyman.

Non-Surgical Recovery Rates.

During successful non-surgical flushing procedures, 85% or more of the flushing medium, containing 45 to 85% of the superovulated ova, is usually recovered by an experienced technician. On single embryo recoveries, some technicians are able to consistantly attain 65 to 98% of the embryos. Hours of practice, patience and perserverance appear to be the key factors associated to optimal non-surgical embryo recovery rates in cattle.

Once the flushing fluid has been allowed to settle for a short period of time, the medium is searched carefully for embryos with a dissecting steriomicroscope (40X). Being optimistic is helpful, but one must keep in mind that embryos apparently cannot be obtained from some donor cows even though they have been successfully superovulated and flushing went according to plan. Furthermore, the possibility always exists that some of the ova collected may not have been fertilized and therefore should not be transplanted. Records from commercial transplant units indicate that an average range of three to nine acceptable quality embryos should be obtained from a properly flushed, superovulated donor animal. One of the larger commercial embryo transplant stations has recently reported an average of 4.8 good quality embryos per donor cow from which embryos are attained. The true potential for embryo harvesting from a single donor cow has not been fully realized at this time.

Non-Surgical Embryo Transplantation

The first successful cervical transfer resulting in a live calf was reported in the literature in 1964. Since that time many dedicated researchers have attempted to perfect the technique so it could be used as a practical tool in the cattle industry. Until recently, it was doubtful that a cervical approach to embryo transfer could ever be mastered in cattle primarily because of uterine ejection of transferred embryos through the cervix and/or the problem of uterine infection following the transfer. Improvements were then made in the basic technique by using three concentric stainless steel tubes for cervical passage and a Rusch catheter for transferring the embryos to recipient females. Encouraging results were reported in 1975 and 1976, when individual bovine embryos were placed in either .25 or .50 milliliter straws and placed in the uterus of recipients using the Cassou AI gun. Today, embryo transplant technicians are routinely using a .25 milliliter French straw to house the embryo, and cervical transfers are with a French AI straw gun in a manner similar to that used in artificial insemination of cattle.

Selecting high-quality recipients is an important part of embryo transplantation, since these animals carry the developing embryo to term. Recipients must be free of disease, reproductively sound, and should have a large enough frame to carry a developing embryo from any breed type. It is also important that recipients produce enough milk to nurse their foster calves to weaning.

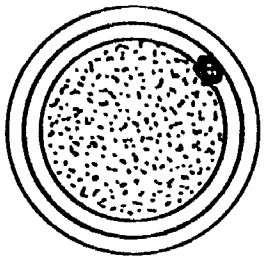
It is important to transplant embryos when donors and recipients are at the same stage of their reproductive cycles. A deviation of more than 1 day in synchronization of donors and recipients at the time of transfer may lower overall transplant pregnancy rates. Synchronization of both animals at transplantation is needed to insure that the uterine environment of the transplanted embryo is similar to that which was present in the donor cow.

The best success rate from embryo transplantation in cattle has been reported when embryos were recovered from donor animals on day 5, 6, 7 and 8 following estrus. During this interval, the majority of the embryos are at the 16-cell stage, morulae, early blastocysts or expanded blastocysts when transferred to the uterus of the surrogate (recipient) female (Figure 4). Pregnancy rates of recipient animals have been shown to be significantly less when embryos were collected from donors on days 3 and 4 following estrus. Occasionally, 2-cell and 4-cell embryos are found during collection procedures on days 5 and 6 post-estrus. These embryos will rarely survive the transfer to the uterus, nor will they develop if placed in the oviduct of recipient females. Field reports are now indicating that bovine embryos at a mature blastocyst or an expanded blastocyst stage of development at the time of transfer are giving the highest pregnancy rate per transfer.

Illustration number	Description of Embryo	Days after estrus normally found
1	1-cell	0-2
2	2-cell	1-3
3	4-cell	2-3
4	8-cell	3-5
5	16-cell	4-5
6	Morula	5-6
7	Tight morula	5-7
8	Early blastocyst	7-8
9	Blastocyst	7-9
10	Expanded blastocyst	8-10
11	Hatching blastocyst	9-11
12	Tight morula with oval zone	>5 Abnormal

Figure 4. Diagrams and reference descriptions of bovine embryos collected from donor animals at various intervals following a fertile estrus (Seidel et al., 1978).

Figure 4.



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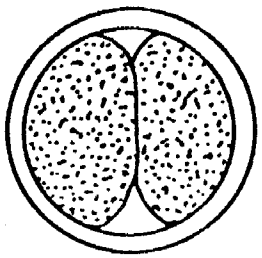

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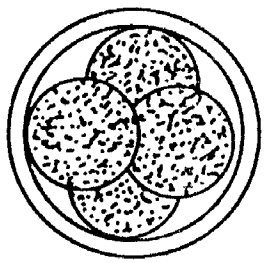

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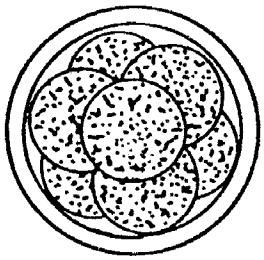

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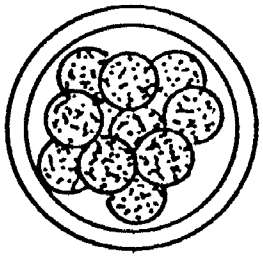

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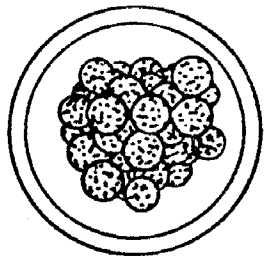

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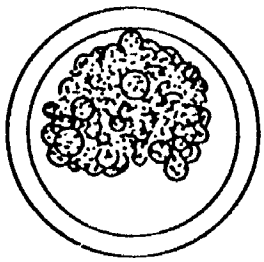

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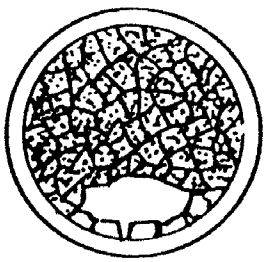

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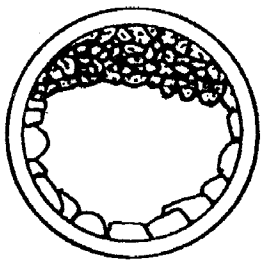

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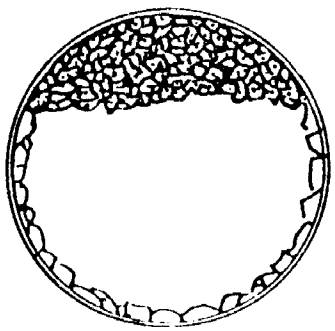

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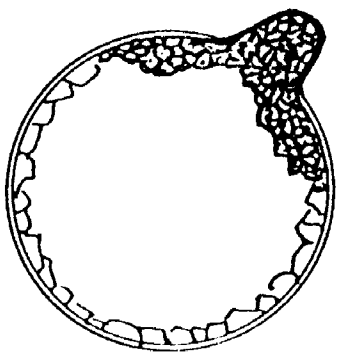

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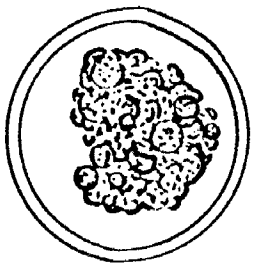

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After the embryo has been transplanted, the recipient female is returned to pasture and not disturbed until diagnosed for pregnancy, usually between 60 and 120 days after the transfer. A charge of $1,800 to $3,000 has not been unusual for each pregnant recipient resulting from a donor transplant at commercial embryo transplant stations. With the onset of on-the-farm transplantation, the charge for each pregnant recipient has dropped considerably over the past year. It is interesting to note that several donors have already produced more than 60 transplant calves with an 18-month interval.

Summary

Commercial embryo transplant units have begun to use non-surgical techniques in place of standard surgical approaches for donors and recipients. This allows transplants to be done routinely on farms or ranches, at reduced cost. Success rates for non-surgical transplantation of bovine embryos are reported to range from 40 to 70% in several transplant units, while success rates of 55 to 75% have resulted from the surgical approach in the same units. The single embryo collection approach on high producing dairy cows, without the use of follicular stimulating agents, appears to have a great deal of merit for the progressive dairyman.

Research efforts to improve non-surgical collection and transplantation methodology is in progress at several experiment stations. Techniques for sexing embryos before transplantation, and methods of freezing embryos, also are being developed. The capability of sexing embryos in the laboratory would give the producer the option of selecting heifer or bull calves. The potential for using frozen embryos in cattle breeding herds appears to be virtually unlimited. Frozen embryos could be purchased and stored by producers for transplantations on a year-round basis.

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