

33 Available Options for Embryo Production in Cattle

Daniel Stein, Joao G. N. Moraes and Jazmin Markey

Objectives

- **Learn about the available options for embryo production in cattle.**
- **Discuss the history of embryo transfer.**
- **Learn about oocyte development.**
- **Discuss the selection of the donor cow.**
- **Discuss the benefits and drawbacks of utilizing MOET and IVF followed by embryo transfer.**
- **Learn about the evaluation, cryopreservation, labeling and transfer of embryos.**
- **Discuss the selection and preparation of the recipient females.**
- **Discuss the importance of a certificate of the procedure(s) performed.**

Available Options for Embryo Production

Embryo transfer is an assisted reproductive technology (ART) used by livestock producers to assist with the transfer of genetics to the next generation. In the beef and dairy cattle industries, there are currently two methods of producing embryos utilized in an Embryo Transfer (ET) program. Embryos can be either in vivo-derived (IVD) or in vitro-produced (IVP).

In vivo-derived embryos are generally produced using multiple ovulation embryo transfer (MOET) techniques. MOET is based on ovarian stimulation of donor cows or heifers with exogenous follicle-stimulating hormone (FSH) to promote multiple ovulations. Following estrus expression, multiple artificial inseminations (two to four) are performed to ensure the majority of the ovulated oocytes are fertilized. Approximately seven days post-insemination, trans-cervical uterine flush is conducted to recover the embryos from the uterine horns.

In vitro embryo production involves recovery of the oocytes directly from the follicle using ultrasound guided trans-vaginal follicular aspiration. The recovered oocytes are then matured, fertilized and cultured in vitro. In vivo-derived or in vitro-produced embryos may be transferred

fresh into synchronous recipients, or are cryopreserved (frozen) for transfer at a later time. Embryo transfer of both IVD and IVP embryos have gained a considerable level of popularity in the cattle industry. In this chapter, IVP embryos are referred to as IVF embryos.

A Brief History

Most of the current MOET technology was developed in the 1970s and 1980s; however, its history goes back much further. The first successful embryo transfer demonstrating that embryos of one breed could be transferred and develop in the uterus of another breed was first performed in rabbits by Walter Heape in 1890. Heape transferred two Angora rabbit embryos into an inseminated Belgian doe. The Belgian doe went on to produce a mixed litter of four Belgian and two Angora kits. In 1950, the first successful embryo transfer in the U.S. producing a live calf was reported by Elwyn Willett. Initially, embryos were recovered from donors and transferred into recipient animals using surgical procedures. The first report of a calf born from non-surgical embryo transfer was in 1964. It was not until the mid 1970s that non-surgical (transcervical) method for both the recovery and transfer of embryos eventually replaced the surgical methods completely and the utilization of MOET grew in popularity.

The first human birth utilizing IVF was in 1978. The first IVF calf was produced in 1981 by Dr. Benjamin Brackett at the University of Pennsylvania School of Veterinary Medicine. The procedure leading to the birth of the first IVF offspring in humans and cattle involved the recovery of oocytes obtained using laparotomy, a surgical incision into the abdominal cavity. In 1984, the first pregnancies in humans using the ultrasound to observe the ovary while aspirating the oocyte from the follicle termed transvaginal ultrasonography were reported. In 1988, a technique was described for the repeated collection of bovine oocytes using transvaginal ultrasound guided aspiration. Today, the ultrasound-guided transvaginal aspiration procedure has been adopted as a standard reproductive technology in humans and many farm animal species. Transvaginal ultrasound oocyte recovery is currently being performed in beef cattle, dairy cattle, goats, mares, pigs, as well as

All Web addresses given in this chapter are subject to change. The links to these websites will be updated regularly at the Master Cattleman website at extension.okstate.edu/programs/master-cattleman.html

other exotic species. There are currently many names for Ultrasound-guided Transvaginal Aspiration, and they all refer to the same procedure: Transvaginal Oocyte Recovery (TVOR), Transvaginal Aspiration (TVA), Follicular Aspiration (FA), Ovum Pick Up (OPU) and Ex Vivo Puncture (EP).

Oocyte Development in Bovine

The reproductive potential of every normal newborn calf, whether a male or female, is enormous. There are an estimated 150,000 potential eggs (ova) in the female and billions of sperm produced by each male. Through natural breeding, only a fraction of the reproductive potential of an outstanding individual, male or female, can be realized. The average herd bull sires 15 calves to 50 calves per year and the average cow will have one calf per year. With artificial insemination, it is possible to utilize the vast numbers of sperm produced by a genetically superior bull; however, the reproductive potential of the female has been largely underutilized. On average, the female will produce an average of eight calves to 10 calves in her lifetime with normal management programs. What artificial insemination has done for the genetically important bull in the transfer of genetics, the potential exists for MOET and IVF to do for the genetically important cow, which is to greatly increase the number of offspring she can produce in her lifetime than what is possible by natural service or artificial insemination.

The female is born with the number of oocytes she will ever have during her reproductive life. Oocytes (immature eggs or ova) in cattle are formed during embryogenesis and develop within individual follicles within each ovary. In cattle, there is an estimated 50,000 to 100,000 oocytes per ovary in the female at birth, each contained in their respective follicle. Within each female, the number of follicles in one ovary is comparable to the number of follicles in the other ovary; however, there can be substantial differences between females in the total number of follicles present in both ovaries. The follicles in the ovary are held in a state of dormancy until they are selected to grow and develop at regular intervals during the late fetal developmental stage and continuing throughout the reproductive life of the female. Once active, the follicles and oocytes either grow to maturity and ovulate or degenerate and die, ultimately depleting the ovary's pool of oocytes. This ovarian reserve of dormant follicles represents the entire supply of oocytes (or eggs) available to female throughout her reproductive life.

In follicles recruited to grow and develop, the follicles growth can be monitored by ultrasonography. Active follicles grow in cohorts, or groups, in what is referred to as a follicular wave. In bovine there can be two, three or four follicular waves which occur in each 21-day estrous cycle. Follicles, containing the oocytes, in a cohort either grow to maturation and ovulation or undergo atresia, or death. It takes approximately 100 days for a follicle and its oocyte to reach the mature ovulatory stage. The number of follicles detected in a follicular wave is related to the number of follicles populating the ovary. The number of follicles in

a follicular wave is highly repeatable and the estimated heritability of the number of follicles is approximately 0.35 in heifers.

Selection of the Donor Cow: MOET and IVF

Beef producers differ in their opinions as to the criteria for selecting a genetically outstanding or superior cow. Whether the criteria are performance records, show-ring appeal or both, consideration must be given to the potential dollar value of her calves as considerable expense can be incurred to achieve a successfully transferred pregnancy. Therefore, the sale value of the newborn calf should be high enough to warrant the expense of the MOET or IVF procedure. MOET or IVF is not a cure-all. The procedures do not make average cattle good or good cattle better. These procedures are suitable for a limited number of seedstock producers with cattle that can serve as breed or species improvers for one or more economically important traits.

Outside of genetics, nutrition is probably the greatest single factor that influences the response of a donor to stimulation by follicle stimulating hormone. This means the MOET or IVF donor should be maintained at the level of nutrition appropriate for her size and level of milk production. Both the very obese cow and the thin cow will have reduced fertility, so it is important the donor cow be in an appropriate body condition score (BCS 5 to BCS 6) at the time of embryo transfer. The potential MOET donor should be reproductively sound to produce maximal results. The MOET donor also should have a normal reproductive tract on rectal palpation and have a normal postpartum history, especially with regard to cycle lengths of 18 days to 24 days. Prospective donor cows in multiple ovulation embryo transfer (MOET) should be selected on the following criteria:

- Regular heat cycles commencing at a young age.
- A history of no more than two (2) breedings per conception.
- Previous calves born at approximately 365-day intervals.
- No parturition difficulties or reproductive irregularities.
- No conformational or detectable genetic defects.

Benefits and Drawbacks of Utilizing MOET vs IVF in an Embryo Transfer Program

MOET

There are benefits and drawbacks with the use of both MOET and IVF. The following is an abbreviated and somewhat simple explanation of a complex process to explain and discuss those benefits and drawbacks.

MOET donors are subject to a stimulatory protocol (superovulation) to increase the number of follicles that

are ovulated each estrous cycle. Factors that contribute to the success of the superovulation protocol are breed, age, parity, management practices, ovarian follicular reserve and superovulation history of the donor. FSH injections in a superovulation program are initiated at the onset of a follicular wave, before follicle dominance has occurred. An example (Figure 33.1) of a stimulatory protocol for *Bos taurus* cattle consists of the insertion of a CIDR (progesterone) on day “0” of the protocol followed by an injection of gonadotropin releasing hormone (GnRH) on day 2 followed by an FSH regime commencing on day 4 consisting of two injections per day during a 4-day period. The FSH regime consists of twice-daily injections (AM/PM) of decreasing doses of FSH. Individual animals might respond differently to the FSH treatment, so doses are normally adjusted as an animal re-enters a superovulation program. The purpose for the administration of FSH is to overcome the decrease in endogenous FSH that occurs during the estrous cycle as to either; A. stimulate follicular growth or B. inhibit follicular atresia (death) in order to initiate multiple ovulations from the available pool of follicles in the ovary. On day 7 the donor receives two injections of Prostaglandin $F_2\alpha$ ($PGF_{2\alpha}$) 12 hours apart (am/pm), with the CIDR being removed at the time of the second $PGF_{2\alpha}$ injection. The administration of $PGF_{2\alpha}$ causes the corpus luteum to regress and allow the animal to come into estrus or standing heat for insemination.

Because of the release of multiple ova from the multiple follicles on the ovary, there is a greater than normal need to be certain that viable sperm reach the oviducts of the superovulated female. Therefore, the donor is inseminated more than one time. Donors are usually inseminated with one unit of semen at the onset of standing estrus, 2 units of semen 12 hrs. after the onset of standing estrus, and 1 unit of semen 24 hrs. after the onset of estrus. Using high quality semen with a high percentage of normal, motile cells is a critical step in any embryo transfer program. The correct site for semen placement is in the uterine body.

Embryos are flushed approximately seven days after insemination. MOET of the donor is usually performed on a 60-day schedule, but there are some reports where MOET has been performed on a 35-day schedule for a short period of time. At times, there can be a lack of reliability or individual variation in the response when inducing superovulation

in MOET donor females with FSH. *Bos indicus* females have been shown to have a greater variability in the ovarian follicular response to FSH with studies indicating that *Bos indicus* donors may require less FSH than *Bos taurus* donors to achieve optimal responses therefore, lower doses of FSH are recommended for *Bos indicus* enrolled in a MOET program.

Embryo flushing and embryo transfer both are done after an epidural anesthetic has been given to block contractions of the rectum and aid in the ease of manipulation of the cervix and the uterine horns. To collect the embryos nonsurgically, a foley catheter is inserted through the cervix of the donor cow, and a special flush media is allowed to flow into and out of the uterus to flush, or collect, the embryos seven days after estrus (Figure 33.2). This collection procedure is relatively simple without harm to the cow. A pre-sterilized stylet is placed in the lumen of the foley catheter to offer rigidity for passage through the cervix into the body of the uterus. When the tip of the catheter is in the body of the uterus, the cuff is slowly inflated with air or flushing media. The catheter is then gently pulled so the cuff is seated into the internal os of the cervix. Additional air/flushing media is then added to the cuff to completely seal the internal os of the cervix. A Y-tubing with inflow and outflow tubes is attached to the

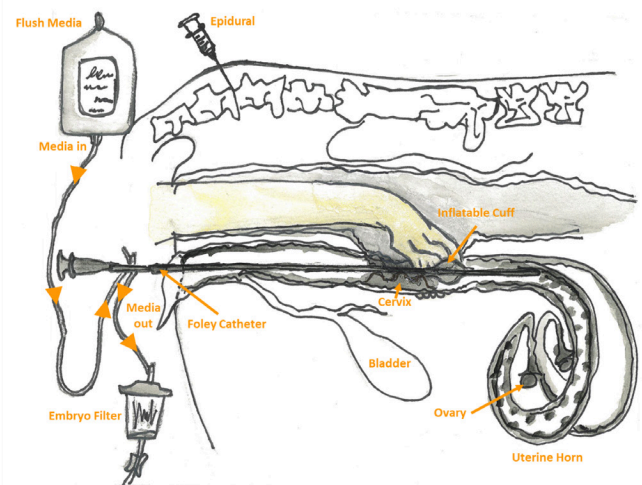


Figure 33.2. Placement of epidural anesthetic injection to block rectal contractions and placement of the foley catheter to flush and collect embryos.

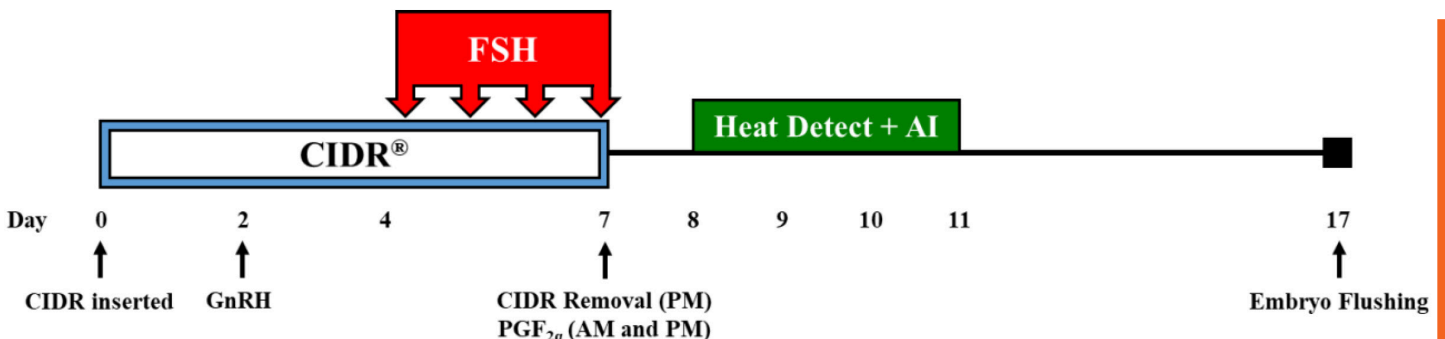


Figure 33.1. Protocol (or could use the term “timeline”) for superovulation of *Bos taurus* cattle.

catheter. A flow control valve on each inflow and outflow tube assists with the regulation in the flow of the flush media into and out of the uterus. The uterus is filled with the flush media to approximately the size of a 40-day pregnancy and the flush media in the uterus is agitated rectally, especially in the upper one-third of the uterine horn. The flush media is sequentially added and removed by gravity flow. Some technicians may choose to use a smaller volume and flush one uterine horn at a time using a syringe. Each uterine horn is filled and emptied with the flush media. The embryos are flushed out with this fluid into a filter designed to catch the embryos. The filter may include a search dish and is washed to recover the contents of the filter that can be transferred to a petri dish to search for the embryos under a stereomicroscope. Of the 31,803 MOET collections performed in the beef and dairy industry reported in the 2019 AETA survey (2018 data), there was an average of 11.5 ova collected per procedure resulting in an average of 6.5 viable embryos. Of these, 28% were transferred fresh with 72% being frozen.

IVF

In IVF donors, the predominant oocyte collection technique is ultrasound-guided transvaginal aspiration where unfertilized oocytes are harvested directly from the ovaries. IVF can often be used in donors that have been unable to achieve success in a traditional MOET program. Follicle aspiration in the literature has been reported as both a non-invasive and an invasive procedure that can cause injury to the reproductive tract if not performed by an experienced technician. Ultrasound-guided transvaginal aspiration may be performed two to three times during a cow's estrous cycle for a period as long as six months; however, there are reports of no long-term detrimental effects on the donor's fertility after two times per week aspirations for over a year when performed by an experienced technician. Usually, a greater number of transferrable embryos per donor can be generated through IVF than through MOET; thus, with the improvements in the in vitro-production systems, IVF seems to be gaining popularity over conventional MOET techniques because of this potential to produce more calves per year. Since ultrasound-guided transvaginal aspiration does not involve flushing the uterus as in MOET, pregnant donors can be collected through the first trimester of pregnancy (approximately to day 100 to day 120 of gestation). Heifers as young as 10 months of age also can enter an IVF program. In the case of the unexpected death or the slaughter of a valuable female, oocytes can be recovered from the ovaries at the time of death.

There are two major IVF protocols which includes a non-stimulation and pre-stimulation procedure. The difference between the protocols is whether the donor will be stimulated with FSH prior to aspiration. As *Bos indicus* cattle have high antral follicle population, large numbers of oocytes can be obtained by ultrasound-guided transvaginal aspiration without superstimulation. However, synchronization of follicular wave emergence and superstimulation is usually used in *Bos taurus* donors. The use of FSH generally increases embryo development rates

but does add an additional cost to the overall procedure. Of the 119,409 ultrasound-guided transvaginal aspirations performed in the beef and dairy industry reported in the 2019 AETA survey (2018 data), 49.7% were performed without FSH in the protocol while 50.3% did utilize FSH in the protocol. The average number of oocytes recovered per aspiration procedure with no FSH was 15.7, resulting in the average of 12.4 fertilized oocytes and a final number of 2.4 viable embryos. The average number of oocytes recovered per aspiration procedure with FSH was 18.7, resulting in the average of 17.7 fertilized oocytes and a final number of 6.1 viable embryos. Of the viable embryos (with FSH and without FSH), 55% were transferred fresh, with 45% being frozen.

The superovulation process begins by screening the donor and removing the dominant follicles on the ovary. This is done to synchronize a population, or a wave, of follicles to be stimulated by the administration of FSH if desired. The FSH regime commencing on day 4 consisting of two injections per day can occur over a two-day to a three-day period. Coasting, the time between the last FSH injection and the aspiration of the follicles using ultrasound-guided transvaginal aspiration can range from 20 hours to 40 hours or more. Follicles 7 mm to 10 mm seem to yield oocytes that are the most competent and have a higher chance of becoming an embryo.

The ultrasound-guided transvaginal aspiration system consists of an ultrasound with a transvaginal transducer probe to visualize the ovaries and a needle guide system through which a needle/tubing apparatus is guided to pierce the follicle and collect the oocyte complex along with follicular fluid (Figure 33.3). An aspiration pump, or syringe, is required to create suction, which causes the follicular fluid and oocytes to flow through the tubing and into a collection vial. Numerous heating apparatuses including a test tube warmer, filter warmer, warming table and stage warmer are necessary to keep the oocytes at a consistent temperature, between 95 F (35 C) and 98.6 F (37 C). Keeping the oocytes warm throughout the entire process is critical. The head of the ultrasound transducer is positioned to the left or right of the cervix, depending on which ovary is to be collected. The ovary is positioned against the transducer so the ovary

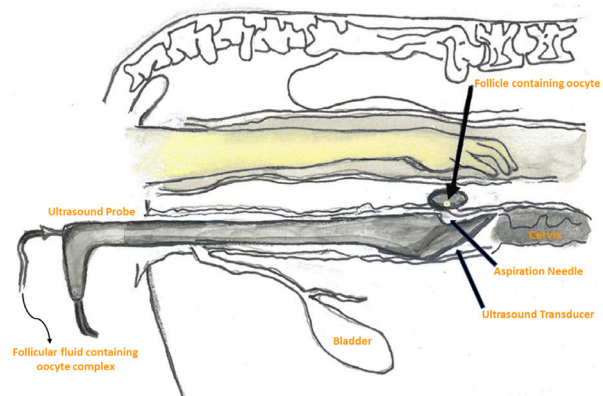


Figure 33.3. Ultrasound-guided transvaginal oocyte aspiration process for in vitro fertilization.

(follicles) can be visualized on the ultrasound screen. A “target line” programmed into the ultrasound’s software is displayed on the screen and indicates where the follicle needs to be positioned for successful aspiration. The needle is slowly advanced forward until the vaginal wall is pierced and the needle is visualized entering the ultrasound field. By monitoring the needle’s position and simultaneously manipulating the ovary per rectum, the needle can be directed into the follicle. Once the needle enters the follicle, the syringe or aspiration pump is activated and the follicular fluid and oocyte are collected into the embryo filter that contains some oocyte collection media. The filter contents are washed and transferred to a petri dish and the oocytes are evaluated using a stereomicroscope.

The aspirated oocytes are graded based on layers of cumulus cells and cytoplasm appearance (Figure 33.4). Oocytes with multiple layers of cumulus cells and a regular cytoplasm (grades 1 and 2) have a better chance of producing a viable embryo. The oocytes then go through an in vitro maturation (IVM) process for 18 hours to 24 hours to become ready for fertilization. The oocytes are fertilized with semen, then cultured in an incubator for seven days. The embryos are counted and graded, then frozen or transferred fresh into recipients.

Since the oocytes are matured and fertilized in the laboratory, one straw of semen can be used to fertilize the aspirated oocytes from several donors, allowing producers to more effectively and efficiently utilize scarce or expensive semen. The utilization of sexed semen is also more feasible using IVF. Additionally, multiple sires can be used on a donor’s oocytes collected during a single aspiration.

Evaluation of the Embryos

Using a stereomicroscope, the individual embryos are evaluated for their quality and classified numerically for the potential likelihood of success if transferred to a recipient female. Embryos are graded on their conformity to several physical characteristics including shape, color/density of cytoplasm, number and compactness of cells, area

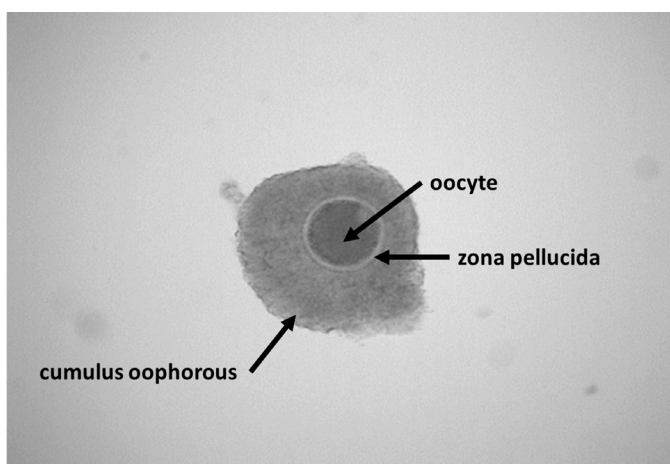


Figure 33.4. Aspirated Oocyte.

of perivitelline space, number of extruded or degenerate cells, frequency and size of cytoplasmic vesicles and stage of development relative to age. In vitro-produced embryos have morphologies that differ from in vivo-derived embryos. The best environment to develop bovine embryos is within the uterus of the cow, not in the laboratory. Embryo quality is of the utmost importance in the survival of the stress of freezing and thawing. Grade 1 embryos are generally considered the only ones to freeze. Grade 2 embryos can be frozen and thawed, yet pregnancy rates are typically reduced.

Embryos are classified as:

Grade 1: Excellent or good

Grade 2: Fair

Grade 3: Poor

Grade 4: Dead or degenerating

Embryos also are evaluated for their stage of development without regard to quality. These stages are numbered:

Stage 1: Unfertilized

Stage 2: Two-cell to 12-cell

Stage 3: Early Morula

Stage 4: Morula

Stage 5: Early Blastocyst

Stage 6: Blastocyst

Stage 7: Expanded Blastocyst

Stage 8: Hatched Blastocyst

Stage 9: Expanding Hatched Blastocyst

Cryopreservation of Embryos

The utilization of embryo cryopreservation has become a fundamental part of the technique of embryo transfer, particularly the commercial application in the bovine embryo transfer industry. There are generally two types of techniques used in the cryopreservation of bovine embryos; the conventional slow rate cryopreservation and vitrification. The most common slow-freeze cryoprotectants are glycerol and ethylene glycol. Vitrification is an ultra-rapid cryopreservation method where high concentrations of cryoprotectants are used in combination with a rapid cooling rate to avoid ice formation during freezing. Cryopreservation without the formation of damaging ice crystals is the primary advantage of vitrification over conventional slow rate freezing. Regardless of the method used, the selection of embryos to be cryopreserved is a key factor influencing the success of the procedure. It is important to realize that many of the differences in morphological characteristics that constitute the embryo grading criteria are magnified by the freeze-thaw process.

Embryo Labeling

- IETS stage and grade codes are not required but are strongly recommended to be printed on the label.
- Loading one embryo per straw is strongly recommended, regardless of cryoprotectant. However, it is allowable to

Straw No.	**Process Code	Practitioner Code	Donor Breed	Donor Reg. No.	Donor Mgmt. No./ Barn Name/Tattoo	Sire Breed	Sire Reg. No.	*Sire Abbreviated Name/Stud Code
2	DT	E515	HO	125796123	CORRY	HO	1879085	CLEITUS
	(5-1)		1 EMB	07JA14				
*Stage No.	*Quality Code	No. Embryo in Straw	YYMMDD Freeze Date					

**Use Appropriate Processing Code

DT	Direct Transfer in vivo
DT-IVF	Direct Transfer IVF
DTX	Direct Transfer Sexed Semen - Female
DTY	Direct Transfer Sexed Semen - Male
DTX-IVF	Direct Transfer Sexed Semen - Female-IVF
DTY-IVF	Direct Transfer Sexed Semen - Male-IVF
DTF-IVF	Direct Transfer Embryo Biopsied - Female-IVF
DTM-IVF	Direct Transfer Embryo Biopsied - Male-IVF
DTB	Direct Transfer Biopsied

freeze/vitrify more than one embryo per straw when they are not for direct transfer (DT).

The head of the cane or cane tab, top and bottom goblets and straw extension or plug should be the following colors as required in the IETS Manual.

- Yellow – Direct transfer (DT) embryos
- White – NON-direct transfer embryos
- Light Blue – Vitrified embryos (the use of this color indicates the method of cryopreservation, and thus, supersedes the use of Yellow, even if the embryo is vitrified for DT, but should be labeled “DT-VIT”).

Transfer of the Embryos

Recipient cows should be at least 60 days postpartum before ET. Just prior to transferring the embryo into a recipient animal, the ovaries of the recipient are palpated rectally to determine which ovary has ovulated. The use of an ultrasound machine to evaluate the corpus luteum (CL) is highly recommended. If a recipient does not have a CL at the time of transfer, she should not be used. The embryo will be transferred into the uterine horn on the side in which ovulation took place. This is determined by the presence of the CL on the ovary. The embryo is gently expelled in the forward tip of the uterine horn (Figure 33.5). Great care should be taken to prevent damage to the lining of the uterus. Because MOET embryos develop and grow in the ideal environment (the reproductive tract of the cow) they have greater viability than IVF embryos. Pregnancy rates are approximately 10% higher in MOET-derived embryos compared to IVF-derived embryos, and pregnancy losses throughout gestation are normally greater in IVF-derived embryos compared to MOET-derived embryos. Pregnancy rates depend on the quality of the embryos before freezing, the ability of the person freezing the embryos, the storage practices, the quality and preparation of the recipients and the skill of the person performing the transfers. So, if unacceptable pregnancy rates are experienced, don't forget

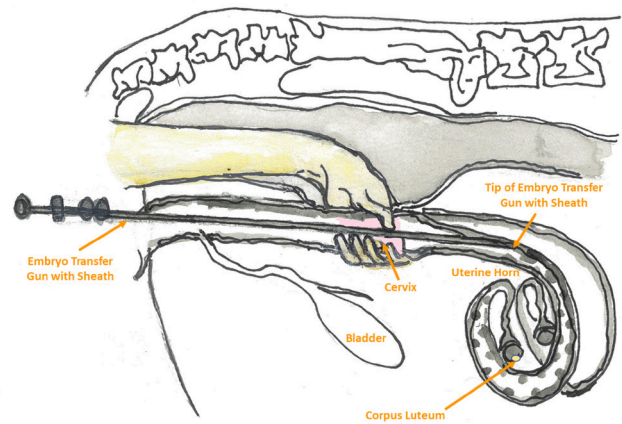


Figure 33.5. Drawing showing embryo transfer to a recipient cow.

it may not be the fault of the practitioner transferring the embryos (who usually receives the blame).

Selection and Preparation of Recipient Females

Proper recipient herd management is very critical to the success of a MOET or IVF program. Cows that are productively sound, exhibit calving ease, good temperament and good milking and mothering ability are good recipient prospects. They must be on a proper plane of nutrition (BCS 6 for beef cow recipients and BCS 3 to BCS 4 for dairy breed recipients) and on a sound herd health program. A recipient female can receive an embryo seven days after she is detected in standing estrus, or a group of recipients can be synchronized and multiple embryos transferred at one time. To maximize embryo survival in the recipient female following transfer, conditions in the recipient reproductive tract should closely resemble those of the donor. It is imperative the donor and the recipient have a very similar uterine environment when the transfer takes place. This requires synchronization of the estrus cycles between the donor and the recipients, optimally within 12 to 24 hours of each other. This is to ensure synchrony between the stage

of embryonic development in the donor cow with the stage of diestrus in the recipient cow. Embryo-uterine synchrony is very important to achieve good pregnancy rates. Many different estrous synchronization protocols are available that can be used to synchronize recipients for ET. Remember, synchronizing drugs are effective only on recipient females already cycling. Anestrus, or noncycling, cows that are too thin or too short in postpartum days will not make useful recipients.

Certificate of Procedure Performed

Following any of the aforementioned MOET or IVF procedures are performed; embryo recovery, oocyte recovery, IVF and transfers that are performed, a “Certificate” of the procedure should accompany each embryo. These documents signed by the technician, practitioner or team leader performing the procedure, provide pertinent information including the mating (sire and dam) for each embryo, the number of embryos in each straw, the stage and grade of each embryo, how the embryos were washed and handled, the time interval from collection until freezing and the cryoprotectant used to freeze the embryos. The freezing method and cryoprotectant used are of particular importance as to ensure the correct thawing technique is utilized to achieve optimal transfer rates.

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