Bovine superovulation and embryo transfer – how to make babies!

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Abstract

Bovine embryo transfer technology is the pursuit of managing genetics for a more preferred calving outcome. This technology has allowed for great genetic advancements by increasing selection pressure or decreasing generation interval. Embryos can be derived by 2 methods. In-vivo embryo collection allows the donor dam to grow and develop the embryo. In-vitro produced embryos require fertilization and development of the embryo in a lab setting. This is a discussion of the concepts of embryo production and transfer. These methods have not changed drastically over the years; however, their application continues to evolve. Embryo transfer provides the opportunity to make more of the desired bovine babies.

Key words: embryo transfer, in vivo, in vitro

Bovine embryo transfer

The goal of bovine embryo transfer is to produce the most calves from the most from the most valuable genetics. This holds true across all aspects of the cattle industry. This could be the production of the next great sire, the elite show heifer or the selection of great replacement females. Typically, this technology has been adopted as a more value-added service and reserved for elite genetics. This dogma is changing as embryo production technology is changing. Embryo transfer is being applied to produce more valuable commercial beef genetics from dairy cows and opportunities to select commercial replacements by female sexed embryos. All these business model applications have led to an increase in embryos produced. The bottleneck of the embryo transfer industry is the recipients and opportunities to transfer the embryos produced. This changing embryo production model has led to a more segmented industry with a need for veterinarians to be involved.

The purpose of this presentation is to introduce the concepts of embryo transfer. There are a vast number of variables that lead to success in embryo transfer. These variables can be in-depth and require more discussion than is available in this presentation. Each year the AABP and American Embryo Transfer Association (AETA) offer an in-depth training in embryo transfer through the Edwin Roberston Beginning Embryo Transfer Seminar. This 3-day course covers all the topics of embryo collection, evaluation and transfer.

Embryo production

Embryos can be produced through 2 major pathways. In vivo-produced embryos are commonly referred to as conventional embryos. These are collected from the donor dam 7 days after estrus, where the fertilization and development have all occurred within the donor. In vitro-produced embryos are often referred to as IVF (invitro-fertilized) or IVP (in vitro-produced). The IVP embryo production system begins with an ovum pick-up (OPU) procedure to aspirate the oocyte from the follicle. These embryos are fertilized and developed outside of the donor in a lab setting. In the last decade, IVP has become the most prominent production method. The AETA data from 2021 is shown below on the trend of in vivo-and in vitro-embryo transfers. In 2016 IVP became the most common form of embryo transferred for dairy embryos. The same trend occurred in 2020 for the beef industry (Figure 1).

Conventional embryo production requires administering follicle stimulating hormone (FSH) at the emergence of the follicular wave to achieve superovulation. The donor undergoes a series of FSH injections prior to breeding. The timing of FSH injections needs to coincide with the emergence of a follicular wave. This process requires either synchronization of the donor dam, or the use of chemical or mechanical treatments to initiate a new follicular wave. The donor is bred, and embryos are then collected 7 days post estrus. The embryos are collected with a transcervical catheter placed in the uterus to allow for uterine lavage. A conventional collection can be complete by placing the catheter in the uterine body or the uterine horn. Embryos are suspended in the lavage media and then collected in a filter. AETA data from 2021 indicates that the average embryo production per collection was 4.9 viable embryos/7.7 total ova for dairy and 6.0 viable/10.8 total for beef. The process of conventional embryo production requires a more labor-intensive set-up process but produces a more robust embryo.

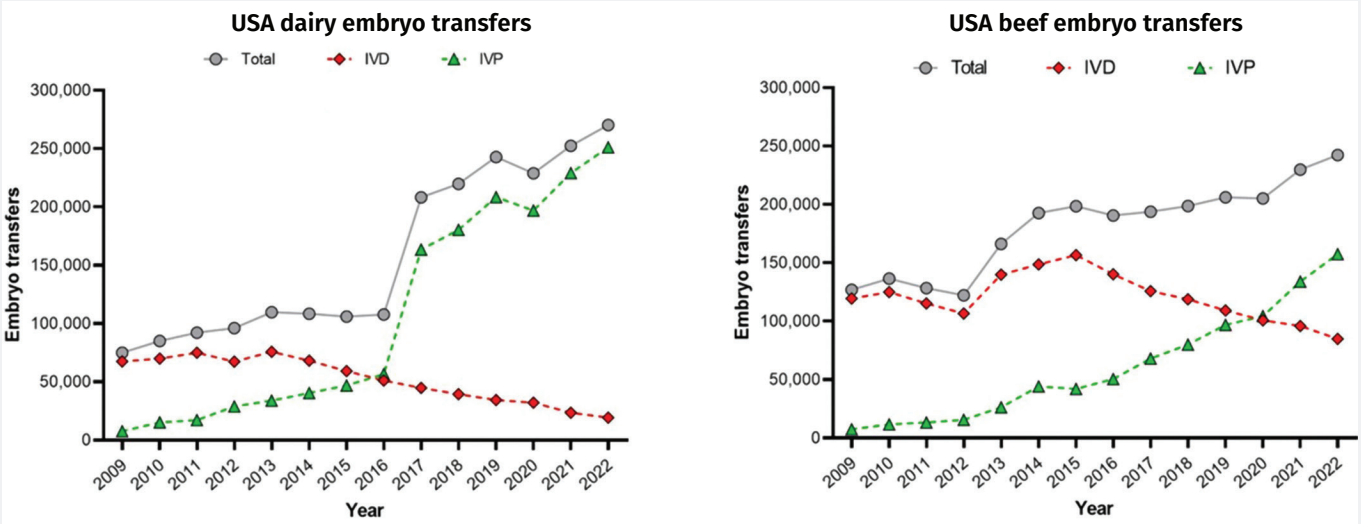
IVP embryo production is more of an a la carte system. Donors can be stimulated with FSH or not, can be pregnant or not, and can be done with a set-up schedule or not. IVP begins with an OPU. The OPU requires a transvaginal ultrasound guided needle aspiration. The oocytes are collected directly from the follicles of the donor. Oocytes are then fertilized and developed in an incubator and lab setting. Oocytes can be fertilized with less semen, sexed semen and even reverse sorted semen. OPU can be performed as often as every 14 days. IVP allows producers to work with donors with less time between collections. AETA data from 2021 indicates average production per OPU was 3.8 viable/18.7 oocytes for dairy and 6.8 viable/23.8 oocytes for beef. IVP requires a more labor-intensive collection and embryo development process but creates options for embryo production that conventional does not.

The selection process for which type of embryo production system requires client communication. The pros and cons of each system must be evaluated to select the appropriate procedure for the individual client.

Embryo evaluation

Once embryos are collected, they must be evaluated for cryopreservation or transfer. Embryos are evaluated according to the International Embryo Transfer Society (IETS) standards. This system, while subjective, does guide the practitioner to select viable embryos to lead to pregnancy production. The

Figure 1: AETA embryo transfer data



embryos are assigned a stage and grade. The grade is equivalent to the quality of the embryo. Viability is included in the embryo grading system. The quality grading system utilizes a 1-4 scale. The stage of an embryo reflects its maturity of development. This system utilizes a 1-9 scale. The stage can provide guidance on transfer day to match recipient synchrony. The corresponding stages and grades of an embryo based on development are found in Table 1. Quality grade embryos 1-2 are suitable for cryopreservation or transfer. Rarely, grade 3 embryos will be transferred or frozen, but are not common in marketable embryos.

Embryo cryopreservation

Embryos can be cryopreserved and stored in liquid nitrogen. The frozen embryo allows for the storage of embryos outside of breeding seasons and allows for the movement of genetics without having to ship live animals. Embryo freezing can be completed with a variety of methods. The most common is the use of ethylene glycol and slow-freeze curve. This procedure allows for the direct transfer (DT) of embryos. Embryos are frozen with 1 embryo per straw and then stored in canes similar to semen. All DT embryos are designated by yellow cane goblets, yellow straws and yellow labels. Embryo and cane labeling can be found at AETA.org or IETS.org. There is a minimum standard of information to be included with a frozen embryo product. This information is critical to allow the transferring practitioner and producer to maintain accurate records.

Embryo transfer

Embryo transfer is the end goal of this entire process. The bottleneck for the embryo transfer industry is the recipient. In 2021, the AETA data reflects the production of 801,699 total viable embryos. The total transfers reported was 482,153. Transferring embryos is the greatest opportunity in the embryo transfer business.

Recipient selection is an in-depth topic but can be summarized as a healthy bovine that is 6-8 days post-estrus. Recipient synchrony can be managed with estrus information. Recipients can be managed with a timed transfer schedule that mimics fixed-time AI. If utilizing heat information, the goal is to work

Table 1: Embryo stage and grade

Stage	Description of development	Grade	Description of quality
1	Unfertilized	1	Excellent or Good
2	2- to 12-cell	2	Fair
3	Early morula	3	Poor
4	Morula	4	Dead or Degenerating
5	Early blastocyst		
6	Blastocyst		
7	Expanded blastocyst		
8	Hatched blastocyst		
9	Expanded hatched blastocyst		

Table 2: Estrus synchrony and embryo selection

Recipient days since estrus	Synchrony	Embryo stage
5 Day	-48 hours	
6 Day	-24 hours	3
6.5 day	-12 hours	4
7 day	0 hours	5
7.5 day	+12 hours	6
8 day	+24 hours	7
9 day	+48 hours	

with minimal asynchrony. Table 2 below is an example of estrus synchrony and embryo selection.

Embryos can be transferred fresh or frozen. Embryo performance can be thought of in relative standings. The fresh conventional embryos are often the best performing. Fresh IVP and frozen conventional have similar expectations. On average, the poorest performing embryo is the frozen IVP. Fresh embryos are transferred without undergoing cryopreservation. Coordinating logistics are required to transfer fresh embryos. Fresh embryos will provide the greatest opportunity for pregnancy production. Frozen embryos are convenient to eliminate the logistics required for fresh transfers. The frozen embryo allows for the storage and shipment of genetics as well. The steps for the completion of a transfer can be found in Table 3.

Conclusion

The clinical skill of adding embryo transfer to veterinary practice requires attention to detail and repetition. The goal of this presentation was to introduce the concepts. This material is not extensive enough to complete the process. Please consider attending the AABP/AETA Edwin Roberston Beginning Embryo Transfer Seminar to get a more complete training in embryo transfer.

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Table 3: Embryo transfer

Identify the embryos prior to recipient evaluation – either fresh or frozen
Identify the recipient and record management number
Palpate the recipient and identify the side of the corpus luteum (CL)
A lidocaine epidural may be used to help facilitate transfer – if fresh embryo, skip to step 7
Frozen embryo – the embryo should be removed from liquid nitrogen and each end of the straw should be touched to dissipate any nitrogen
Thaw the embryo in a water bath at a temperature range of 85-95 °F
Load the embryo in a transfer gun, place a sheath
Pass the gun through the cervix and deposit the embryo in the horn ipsilateral to the CL in an atraumatic manner

