University of Veterinary Medicine Budapest
Department of Obstetrics and Food Animal Medicine Clinic

# Embryo Technology in Dairy Cattle

**By:**
**Emer O' Roarke**

Supervisor: Dr. Somoskői Bence

Budapest, Hungary
2024

**Abstract**

In this literature review I intend to discuss the embryo technology used in the dairy cattle industry. Embryo technology is a broad topic. Nowadays, embryo technology is a combination of many things, such as cellular and molecular biology, assisted reproduction and genomic techniques. The main goal of embryo technology is to increase the number of superior genotypes, but in recent years it has also assisted in transgenesis and genotyping. During this literature review, my aim is to explore and examine the most popular and most common embryo technology available in the ever-growing dairy industry.

Absztrakt

Ebben a szakirodalmi áttekintésben a tejelő szarvasmarháknál alkalmazott embriótechnológiát kívánom tárgyalni. Az embriótechnológia széleskörű téma, amely napjainkban már nem csak az embriótranszfert jelenti, hanem számos metodika kombinációja, mint például a sejt- és molekuláris biológia, az asszisztált reprodukció, az embriótermelés és in vitro tenyésztés. Az embriótechnológia fő célja a kiváló genotípusú állatok számának növelése, amelyben az utóbbi években már szerepet játszik a genotipizálás,
valamint transzgenikus módszerek is elérhetőek.
Szakdolgozatom célja áttekinteni a szakterület alapjait, a napi rutin részét képező módszereket, valamint betekintést nyújtani a legutóbbi trendekbe

1. Introduction……..5
2. Literature Review……6
2.1. History and relevance of Embryo Transfer…….6
2.2. Conditions and steps of Embryo Transfer…….7
2.2.1. The Recipient……...7
2.2.2. The Donor…….8
2.2.3. Oestrus Synchronisation……9
2.2.4. Artificial Insemination…….11
2.2.4.1. Methods of Semen Collection…… 13
2.2.4.2. Semen Evaluation……14
2.2.5. Flushing……15
2.2.6. In vitro produced embryos……16
2.2.7. Embryo Transfer……16
2.2.7.1 In vivo fertilization…….17
2.3. Embryo Recovery……17
2.3.1. Embryo Handling……..18
2.3.2. Embryo Evaluation……18
2.3.3. Embryo Storage…….19
2.4. Embryo Technologies…….20
2.4.1. Embryo Splitting……20
2.4.2. Embryo Sexing……..21
2.4.2.1. Cytological methods or karyotyping..22
2.4.2.2. Polymerase Chain Reaction……23
2.4.2.3. Detection of X linked enzymes……24
2.4.2.4. Detection H-Y antigens……25
2.4.3. Cryopreservation…….25
2.5. Factors Affecting the success of Embryo Transfer…27
2.5.1. Superovulation……27
2.5.2. Embryo Recovery……27

2.5.3. Embryo Transfer…….28
2.6. Factors Affecting Offspring…….29
2.6.1. Pregnancy Rates…….29
3. Conclusion……31
4. Acknowledgments……32
5. References……33

1. Introduction

Dairy farming is a type of farming used for long term production of milk, which then in turn leads to the production of dairy products. Beginning back in the Neolithic era, dairy farming has been around for a very long time. Years ago, dairy farming was performed on farms by hand, but advances in the last number of years has led to milking being performed in rotary parlours and automatic milking parlours. Holstein Friesian and Jersey cattle are two of the most popular breeds that are used for the production of milk. Both breeds are renowned for their butterfat and protein production. Dairy cattle are lean animals, with very little body fat. They have a light frame and are not particularly tall animals. They should have good udder conformation, feet and leg conformation, thoracic and abdominal body conformation and rump and loin structure. They usually are placid animals with a docile temperament.

The main goal of reproduction of a dairy heifer or cow is the production of an offspring for future use. Naturally, dairy farmers desire female calves for future use in milking and breeding. With natural breeding, there is no control on the number of female calves born. However, nowadays, we can achieve this with the technology available. With embryo transfer, we can collect multiple embryos off dams of superior quality and place them in dams of a lesser quality, in turn, we will have more off spring produced of a superior quality.

“Embryo technologies are a combination of assisted reproduction, cellular and molecular biology and genomic techniques. Their classical use in animal breeding has been to increase the number of superior genotypes but with advancement in biotechnology and genomics they have become a tool for transgenesis and genotyping”. [1]

## 2. Literature Review

### 2.1. History and relevance of Embryo Transfer

Embryo transfer in cattle has become increasingly popular among seedstock dairy and beef producers. While most of the relevant embryo transfer technology was developed in the 1970s and 1980s, the idea itself has a much older history. Embryo Transfer was performed first by Walter Heape in 1890, when he transferred two Angora rabbit embryos into a gestating Belgium doe [2].

In 1922 in Vienna, they used rabbits as the experimental animals for embryo transfer. However, the success of this experiment was questionable, as the success rate was quite low but also the following morning the young had been eaten during the night [3]. They continued on with research into other areas also, such as superovulation, synchronisation of oestrus, artificial insemination and early transfers which were mainly carried out on farm animals [2]. They began embryo transfer on food animals in the 1930s on sheep and goats, it was in the 1950s when the embryo transfers were reported successful in cattle and pigs by Jim Rowson at Cambridge, England [2]. In Cambridge, they developed surgical methods of both embryo transfer and recovery in both cattle and horses [4].

Adaptions of embryo transfer and the various related technologies have generally been more rapid and progressive in equine than in cattle, with the exceptions of superovulation, in vitro fertilisation, and cryopreservation [5].

Today, widely accepted and dependable techniques such as superovulation, embryo recovery and transfer, cryopreservation, and IVF are used globally by hundreds, if not thousands, of commercial operations across numerous countries. While the average number of embryos

produced through superovulation has remained relatively consistent over the past 40 years, there have been advancements in synchronisation and hormonal protocols [6].

## 2.2. Conditions and steps of Embryo Transfer

What is Embryo Transfer? “The embryo transfer process begins with cows receiving a hormone treatment to produce more than one ovulation (egg) at a time. The cows are then artificially inseminated with bulls also possessing desirable genetics.” [7]

Embryo transfer involves multiple steps. In simple terms, it is when a dam of a superior quality is injected with hormones to cause multiple ovulations, the dam is then inseminated with semen of a superior quality and desirable characteristics, the embryos are then collected before implantation and can be placed in several females for implantation or can be cryopreserved. [7]

### 2.2.1. The Recipient

The cow who receives the embryo is called the ‘recipient’. When the embryos are collected and if they are not placed into a recipient cow straight away they can be frozen and kept safely for future use.

There are many different factors to be considered when selecting a heifer or a cow to be a recipient. The first decision is if the farmer is going to choose a heifer or a cow. There are advantages to using cows as there is likely to be less difficulty with calving. However, on the other hand, heifers may be easier to manage as they are not lactating. Another factor, is that heifers generally have a higher fertility than

cows, particularly in dairy cattle. The decision must be made on both the advantages and disadvantages of both the heifers and the cows. [8]

On-the-farm recipient programs for embryo transfer should be customized to fit available resources and must include several key components: ensuring cycling animals are not exposed to bulls, maintaining good nutrition and weight gain, implementing herd health programs (especially for diseases like brucellosis), having reliable identification for each animal, and providing adequate facilities for synchronization and embryo transfer. Effective estrus detection and conscientious staff are also crucial for the success of these programs. [8]

Timed ovulation induction and fixed-time artificial insemination (FTAI) in superstimulated donors, as well as synchronization protocols for fixed-time embryo transfer (FTET) in recipients, can be achieved using GnRH or estradiol with progesterone/progestin-releasing devices and prostaglandin F2α (PGF2α). These methods enable the control of follicular wave emergence and ovulation at specific times, eliminating the need for estrus detection and simplifying the management of both donors and recipients. [9]

### 2.2.2. The Donor

Selecting donors for embryo transfer programs typically involves two main criteria: genetic superiority and the potential to produce a high number of viable embryos. Genetic superiority means choosing animals that align with the program's breeding goals, often influenced by market demand. For instance, it is practical to choose donors whose offspring are profitable enough to offset embryo transfer costs. It’s important to avoid producing animals that farmers are unlikely to accept, ensuring that the selected donors meet both genetic and economic criteria. [8]

Objective measures of genetic superiority can include various factors such as milk production, milk composition, growth rates, ease of calving, and resistance to diseases. These metrics help in assessing the quality and suitability of donor animals for embryo transfer programs. “Healthy, cycling cattle with a history of high fertility make the most successful donors.” Overweight cows are not suitable donors for embryo transfer due to their poor response to superovulation and the increased difficulty in manipulating their reproductive tracts. Additionally, sick animals tend to produce fewer viable embryos, further reducing their effectiveness as donors. [8]

Apart from the criteria mentioned above, there are also several other important factors for the selection of donor females. Suitable donors for embryo transfer should have regular estrous cycles starting at an early age, require no more than two inseminations for conception, maintain a calving interval of 14 months or less, experience trouble-free calvings, and exhibit no reproductive abnormalities, diseases, or known genetic defects. Correct and adequate nutrition is also essential. After the female reaches peak lactation and has reached a positive energy balance, she should then be flushed. [10]

### 2.2.3. Oestrus Synchronisation

Oestrus Synchronisation plays a big role in a successful embryo transfer. The main aim of oestrus synchronisation is to get the recipient and the donor cow to ovulate at the same time or as close as possible. This is essential as it ensures that the uterus of the recipient cow will be at a similar stage to the uterus of the donor cow. To ensure this process runs smoothly hormones can be used on both the recipient and the donor cows.

“Classical synchronization protocols attempted to synchronize corpus luteum (CL) function to synchronize expression of estrus. More recent protocols

are focused on synchronizing time of ovulation using different reproductive hormones that synchronize both CL function and follicular waves to allow AI at a fixed time at the end of the protocol (FTAI).” [11] Farmers usually breed all the cows at the time of Fixed Time Artifical Insemination, FTAI, independent of if they display signs of oestrus or not, however, if the cow has displayed signs of oestrus it is associated with a greater fertility. [11]

Various strategies have been employed to boost circulating E2, estradiol levels and enhance estrus expression in FTAI, Fixed Time Artifical Insemination programs. These programs include adding E2 at the end of the Ovsynch program, using treatments such as ECP, which triggers ovulation, extending the time between the luteolytic PGF2α injection and AI, Artifical Insemination, and lengthening the overall protocol. All of these methods help to optimise ovulation timing and improve the reproductive outcomes in cattle. [11]

There have been several hormonal treatments developed in order to synchronise the time of ovulation, which allows TAI, Timed Artifical Insemination without the detection of oestrus. Many methods have been evaluated to increase the fertility during synchronisation, including increasing the concentration of P4, progesterone, during the ovulatory follicle development. [12]

FTET, Fixed Time Embryo Transfer programs can be used. They are based on the use of GnRH, Gonadotrophin- Releasing Hormone and PG, Prostaglandin or P4, progesterone- releasing devices and oestradiol. By using these protocols, it can increase the proportion of the recipients transferred. By adding additional hormones such as eCG, equine chorionic gonadotrophin to the P4 and oestradiol to the FTET programs, it can result in a larger proportion of recipients transferred and therefore leads to higher pregnancy rates. [13]

The hormone eCG, has many benefits to it. It is associated with the increased growth of the dominant follicle and it is also associated with the increased plasma P4 concentration during the luteal phase. FTET programs have decreased the need for detection of oestrus and therefore resulted in a greater proportion of transferred recipients and greater pregnancy rates. [13]

“In addition to TAI, timed embryo transfer (TET) is becoming an important tool to increase reproductive efficiency  in lactating dairy cows”. [12] These programs help to combat the negative effects such as heat stress or extended follicle growth on the oocyte and early embryo development. They can do this by ensuring that all cows receive a high quality embryo around one week after the synchronised ovulation. [12]

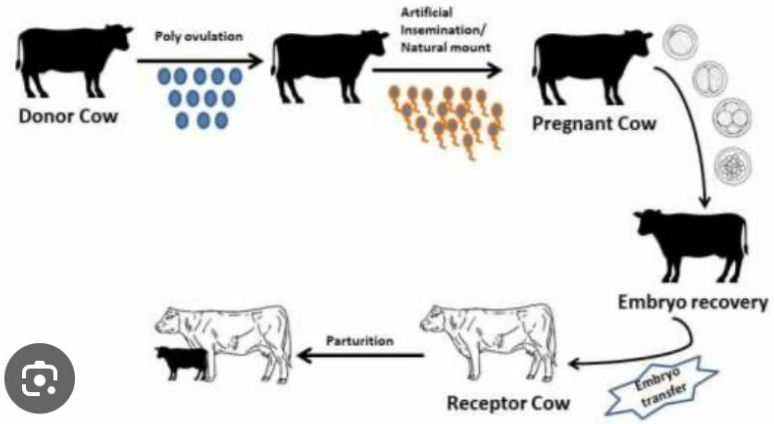


Figure 1: The beginning to end of the transfer process. [32]

### 2.2.4. Artificial Insemination

AI, known as Artificial Insemination, was originally introduced in the United States during the 1930s and 1940s. There are many different factors which can influence the fertility potential of an AI dose such as the function of the quality, the quantity and also the health status of the semen. Another key factor is the management of sire health and testing protocols of associated diseases. [14]

“Artificial Insemination (AI) is the act of collecting semen from the male, preserving it until use, and depositing it in the reproductive tract of the female when she expresses estrus.”[15]

The process involves collecting sperm from the male, processing and storing it and then artificially introducing it into the female reproductive tract to achieve conception. The semen collected from the bull is kept in containers, deep frozen and stored in liquid nitrogen at a temperature of minus 196 degrees Celsius. [15]

Identification of semen quality factors associated with fertility can provide us with more accurate information in order to predict, manage and then select the sire for AI. Evaluations such as checking semen quality, adjustments to the cell numbers per dose and minimising the variation of fertility among ejaculates are paramount in selecting the sire for AI. [14]

In order to minimise and prevent disease the bull should undergo a BSE, Breedness Soundness Examination by a qualified veterinarian for infectious agents and disease prior to introduction into a new herd. It is advised to purchase bulls only from reputable stock produces with herd health programs, including vaccination for infectious diseases such as leptospirosis and campylobacteriosis. Additionally, bulls should undergo annual brucellosis testing but should not be vaccinated against this disease. In selective cases, vaccinations for BVD, Bovine Viral Diarrhoea, IBR, Infectious Bovine Rhinotracheitis and Trichomoniasis are also highly recommended. [15]

There are both advantages and disadvantages of Artificial Insemination. Some of the advantages include reducing the safety risks associated with the keeping and breeding of male animals, better utilisation of females and it also allows for the utilisation of several sires. AI has significantly contributed to improving animal

productivity, particularly milk production, in developing countries that have established breeding strategies and a solid technical foundation to effectively implement and adapt this technology to their specific needs. [15]

The disadvantages include a high cost, it requires skilled personnel and trained management. The primary reasons cited for preferring herd bulls over artificial insemination(AI) include the higher costs associated with AI compared to maintaining bulls and the additional expenses incurred from prolonged calving intervals due to low heat detection rates with AI. Costs associated with AI include labour, equipment, liquid nitrogen, semen and the ratio of services required per successful conception. [15]

#### 2.2.4.1. Methods of Semen Collection

Semen can be collected by artificial vaginas or by electroejaculators. Artifical vaginas are widely used and a rubber-barrel with an inner rubber liner which is not spermiotoxic is recommended. It is also essential to check before use that there are no leakages or breakage in the rubber. A lubricant, which is sterile and non-spermiotoxic should also be applied before collection. [15]

The second option is electroejaculators. Semen which is collected through electroejaculation is of comparable quality to that obtained via an artificial vagina, with similar processing, storage and usage protocols. Electroejaculation is particularly advantageous for dairy bulls that are unable to use the artificial vagina due to issues like age-related sexual inactivity or physical disabilities. However, it is not advisable to collect semen from males that have not exhibited normal sexual behaviour or the ability to ejaculate, as this may indicate a genetic issue that could be passed on to their offspring. [15]

#### 2.2.4.2. Semen Evaluation

There are various different factors which must be evaluated from the semen. Factors such as, the volume, the mass activity, the spermatozoa motility and the sperm morphology.

The volume of ejaculate can be measured by collecting the sample in a graduated vial or by weighing the collection tube on a balance and converting the weight to millimeters using a computer program. Ejaculate volume can decrease in young bulls with frequent or incomplete ejaculations and in the case of bilateral seminal vasculitis. Seasonal variations, collection methods and the bull's sexual preparation also influence semen volume. Generally, the acceptable semen volume is at least 2ml per ejaculate with variability among bulls, breeds and the age. [15]

Mass activity can be determined by placing a drop of semen onto a slide with no cover slip under a low magnification. A good quality of semen can be associated with a rapid wave motion with the formation of eddies at the end of the waves. [15]

Spermatozoa motility refers to the percentage of sperm cells that can move independently, while progressive motility specifically describes those sperm that travel in a relatively straight line from one point to another. Sperm cells are considered motile when they move in a straight line. For bull semen to be deemed acceptable, it should exhibit at least 70% motility at the time of collection and it should maintain a 40% motility after freezing. [15]
The typical structure of spermatozoa includes a head and a tail, which is then further divided into three sections: the mid-piece, main-piece and the end-

piece. Common abnormalities include tailless sperm, sperms with a looped tail and the most common would be the detachment of the sperm head and the bending of the middle piece and the tail around and over the sperm head. [15]

### 2.2.5. Flushing

Flushing involves the removal of fertilised embryos from the uterus of a donor cow.

Prior to beginning the process of flushing, an epidural containing lidocaine is routinely given to the cow. During flushing, the uterus is filled with flush fluid repeatedly in order to flush the embryos from the uterus and into the filter. Typically, 1-2 L of flush media is required to flush a cows uterus. Many flush solutions are made up primarily of Dulbecco phosphate-buffered solution (PBS) alongside antimicrobials and 1% fetal bovine serum (FBS) or alternatively 0.1% bovine serum albumin. The uterine horn may be flushed by repeating small amounts of the flush fluid, 20-25ml, that allow it to drain into an embryo filter or alternatively, the uterine horn may be flushed continuously, with 1-2L of flush media. When the flush is complete, the contents are allowed to flow through into the embryo filter my deflating the cuff. [16]

The filter is then taken to a laboratory where the contents are examined under a microscope. Embryos are transferred over to clean dishes. The dishes contain media very similar in composition to the flush media except it will contain a higher concentration of FBS or bovine calf serum (10-20%) or bovine serum albumin (0.4%).

The embryos are thoroughly examined at a high magnification under the microscope. They are then classified according to their morphology, developmental stage and embryo quality. The embryo should be classified as fair,

good or excellent to be transferred. A key step in the safekeeping of the embryos is embryo washing. It is carried out by transferring embryos into clean wells containing holding media. The International Embryo Transfer Society highly recommend carrying out this step as it is essential in the removal of cellular debris and potential pathogens which can be adhered to the zona pellucida. [16]

After this, embryos are either transferred to donor cows or they may be prepped for freezing, splitting, also known as bisection or embryo sexing. Embryos can be stored in a transport medium in a refrigerator for up to 24 hours without any negative consequences.

### 2.2.6. In vitro produced embryos

At birth, each ovary contains hundreds of thousands of oocytes, but most are lost through atresia. This significant loss of genetic material can be mitigated by harvesting oocytes and employing in vitro production (IVP) techniques. Bovine IVP is now a well-established and efficient method. Frequent ovum pickup (OPU) combined with in vitro fertilisation (IVF) has been effective in increasing embryo yields from selected donors. The IVF process consists of four main steps: oocyte maturation, sperm capacitation, fertilisation and embryo culture until freezing or transfer. [17]

### 2.2.7. Embryo Transfer

The embryo transfer process is quite similar to the process involved with artificial insemination, except in this case the transfer gun is inserted deep into the uterine horn on the same side as the CL, corpus luteum. The donor can be inseminated either naturally or artificially, with embryos collected by non-surgical means six to eight days post-breeding. After collection, embryos must be identified, assessed

and kept in an appropriate medium before transfer. They may also undergo procedures like splitting and sexing, and can be cooled or frozen for extended storage. [17]

There are two types of fertilisation, in vivo and in vitro fertilisation.

#### 2.2.7.1. In vivo fertilisation

During normal embryonic development in vivo, blastomeres undergo a series of cleavage divisions following fertilisation. The ovum divides into a 2-cell embryo, which then progresses to a 4-cell and subsequently to an 8-cell stage. When the blastomeres cluster together, resembling a bunch of grapes, this phases is referred to as the morula stage. As development continues, the embryo prepares for its first differentiation stage, known as blastulation. Before this occurs, the morula cells compact and differentiate into ‘inside’ and ‘outside’ components of the embryos. [17]

## 2.3. Embryo Recovery

Typically, embryos are retrieved six to eight days after the onset of oestrus(day 0). While some cows can have embryos collected non-surgically as early as four days post-oestrus, the recovery rates are generally lower before day 6 compared to days 6-8. Embryos can also be harvested from days 9-14, however, they begin to hatch from the zona pellucida around days 9 or 10, complicating their identification and making them more prone to infection. By day 13, embryos may elongate significantly, risking damage during retrieval or entanglement with one another. Procedures for cryopreservation and bisection are optimised for embryos collected during days 6-8, which is another reason for this timing. [17]

### 2.3.1. Embryo Handling

Embryos are typically maintained in the same or a comparable medium used during their collection. It is crucial to handle embryos carefully between collection and transfer to avoid the spread of pathogens. Employing aseptic techniques, along with sterile solutions and equipment, is vital to ensure their safety and integrity. [17]

### 2.3.2. Embryo Evaluation

Embryos are assessed and classified through morphological examination at 50-100 X magnification, following the guidelines of the IETS, International Embryo Transfer Society. Bovine embryos typically have an overall diameter of 150-190 μm, with the zona pellucida measuring 12-15 mm thick. This diameter remains stable from the one-cell stage to the blastocyst stage. Key quality assessment criteria include the embryo's shape, blastomere compactness, cell size variation, cytoplasmic characteristics, overall diameter, zona pellucida attributes and any extruded cells. [17]

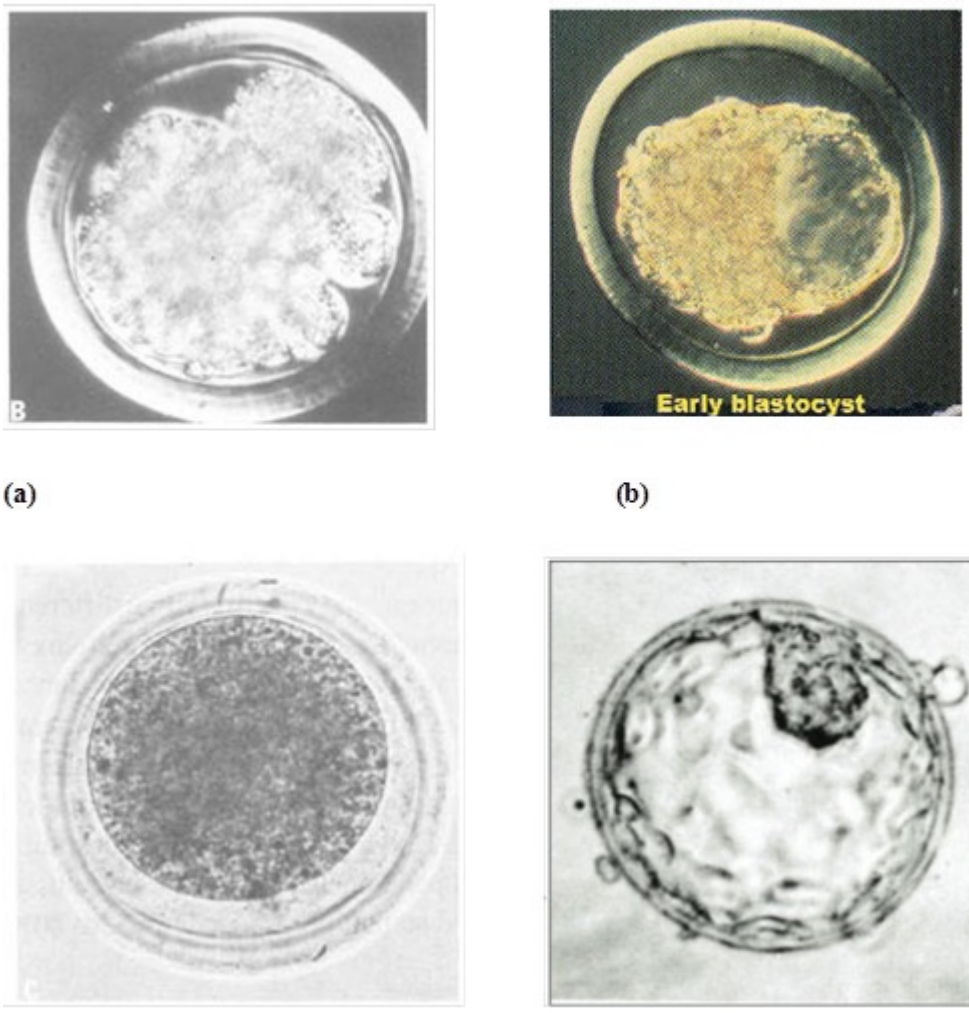


Figure2 : (a)Morula,(b)EarlyBlastocyst,(c)FullBlastocyst,and(d)ExpandedBlastocyst. [29]

### 2.3.3. Embryo Storage

Techniques like embryo transfer (ET), in vitro fertilisation (IVF), sex determination, and cloning rely on keeping embryos viable for several hours or even days outside the reproductive tract. For many uses, the storage system must not only preserve the embryos' viability but also facilitate their continued development. [17]

## 2.4. Embryo Technologies

Beside the common techniques used for breeding purposes, there are some advanced or special method which were developed to improve breeding efficiency and refine genetics of dairy cattles.

### 2.4.1. Embryo Splitting

Embryo splitting is carried by bisecting from the two-cell stage through the hatched blastocyst stage. This process is carried out for two main reasons, to achieve the goal of identical twins or to increase productivity. There are various procedures which can be used to carry this process out. Ultimately, most procedures consist of two main stages, which involve immobilising the embryo with a holding pipette and bisecting it. Immobilisation is carried out by the application of suction to the zona pellucida, which makes a depression in the container and constructs a device which in turn traps the embryo. The second step, the bisection, is usually carried out using a broken fragment of a razor blade or fine glass needle. It is essential that the procedure is carried out without damaging the embryo and that the process does not damage too many cells. Another key step is to ensure the embryos are bisected reasonably symmetrically.

Embryo splitting is a technique used in cattle in vitro production (IVP) to enhance the availability of embryos and increase the intensity of genetic selection. “Embryo splitting has the potential to offer significant benefits to cattle in vitro production (IVP) by increasing the number of embryos available for transfer, thereby increasing embryo transfer success.”[18]

Embryo splitting enables the production of more offspring from highly valuable gamete donors, which enhances genetic progress by allowing for greater selection

intensity. It was first developed in the 1980s and since then has been applied to both cleavage and blastocyst stage bovine embryos. [18]

According to G. Silvestri, K.J. Turner, J.L. Silcock, K.D. Sinclair and D.K. Griffin, published in 2022, the strategy most likely to produce the greatest number of viable embryos is the splitting of the day 3 embryos into four parts, irrespective of whether embryos with exactly eight cells or an atypical number of blastomeres were used. [18]

Another method used in embryo splitting is described as quick-splitting. It is a simplified method for bisecting bovine embryos suitable for on-farm transfers. It involves using a microblade controlled by a handheld micromanipulator to split day 7 embryos while still enclosed in the zona pellucida. The embryo is cut by pressing it against the bottom of a petri dish using a vertical motion. The resulting half-embryos are then transferred non surgically into a synchronised recipient animals, without retaining the original zona pellucida. [19]

### 2.4.2. Embryo Sexing

Embryo sexing is a hugely valuable tool. In the beef industry, there are more benefits to the production of male animals whereas in the dairy industry there are many more benefits to the production of female animals, primarily for milk production. The ability to predetermine the sex of an embryo at conception can have significant economic benefits, especially in regions such as India, where restrictions on cow slaughter and economic challenges related to calves exist. Preimplantation sex determination can aid in better livestock management, breeding and genetic disease detection. Techniques such as sex-sorted semen and chromosome examination in embryos are available, though sex- sorting can be costly and less efficient than traditional methods. Pre-transfer embryo sexing

enhances the efficiency of embryo transfer, accelerating the production of desired genetically superior animals. [20]

The genetic sex of a zygote is determined at fertilisation, depending on whether the ovum is fertilised by a sperm carrying an X chromosome, which results in a female or a Y chromosome, which results in a male. Several methods have been used for the sexing of embryos by means of invasive or by non-invasive methods. By using the non-invasive methods, the integrity of the embryo is not harmed. This can be beneficial as it ensures the normal embryo development. However, a negative of non-invasive is that it can be less accurate. Some of the invasive methods include cytological methods or karyotyping, identification of sex chromatin, Y chromosome specific DNA probes, Polymerase chain reaction (PCR), Loop mediated isothermal amplifications (LAMP) and Fluorescence in situ hybridisation (FISH). While some of the non-invasive methods include the detection of X-linked enzymes, detection H-Y antigens, sexing based on cleavage and development. [20]

#### 2.4.2.1. Cytological methods or karyotyping

Cytogenic sexing, or karyotyping, involves analysing a cell's genomic structure to determine the sex of an embryo based on the presence of the X or the Y chromosomes during the metaphase stage of mitosis. This technique uses blastomere cells from day 6-8 old embryos, which are cultured with agents like colchicine or colcemid to halt mitosis. Following cell lysis in a hypotonic solution, chromosomes are stained with Giemsa for microscopic analysis. While karyotyping can reveal chromosomal abnormalities and determine sex with high accuracy, it is labour-intensive, time-consuming and may also reduce embryo viability. [20]

According to one study, the blastomeres were incubated in TCM 199 medium with 0.08 μg/ml colchicine for 8-10 hours to arrest mitosis at the metaphase stage. They were then treated with a 1% sodium citrate hypotonic solution for 10 minutes and placed on clean, grease-free glass slides. A fixative solution, methanol and acetic acid, at a 1:1 ratio was applied, followed by staining with 5% Giemsa at pH 6.8 for 10 minutes. The chromosomes were identified under a microscope, counting at 200x magnification and examining sex chromosomes at 1000x to determines the embryo's sex. [21]

Karyotyping offers advantages such as lower cost, ease of use with high accuracy and no need for expensive equipment. However, it can process fewer animals, limiting the number of metaphase plates for analysis. The downsides include being time-consuming, labour-intensive and has a low success rate due to challenges in metaphase chromosome dispersion. Also, it can reduce embryo viability, success rates of pregnancies and requires skilled technicians. [22]

#### 2.4.2.2. Polymerase Chain Reaction

The isolation of sex in domestic animals is essential for breeders to manage their breeding programs effectively. Currently, determining fetal sex is commonly done using DNA fragments from maternal plasma. PCR can be used for sex determination by analysing embryos from super ovulated donors, aiding in farm-level sex ratio control. This process requires a blastomere biopsy and amplification of Y-chromosome DNA, offering high accuracy and reliable pregnancy rates, though it does require skilled personnel and it can be time-consuming. [22]

Embryo biopsy: Embryos are collected on day 6.5 after the first artificial insemination, with only high-quality compact morula to early blastocyst embryos being used for biopsy via a micromanipulator. [22]

Amplification: This process involves cycles of denaturation (94-97 degrees Celsius), primer annealing (50-72 degrees Celsius) and DNA extension (72 degrees Celsius) using Taq DNA polymerase. [22]

PCR-based sexing offers faster results compared to FISH, allowing embryo transfer without cryopreservation. However, precise thermal regulation and potential DNA contamination make PCR challenging for field applications. [22]

According to the same study as mentioned above, the process can be completed by washing the blastomeres in PBS and placing them in a microcentrifuge tube with a Chelex 100 solution. Cells were lysed by boiling and then stored at minus 20 degrees Celsius until analysis. Before PCR analysis, samples were thawed then centrifuged and then mixed with PCR reagents. Two types of primers- Y-chromosome-specific (BRY1a) and bovine satellite sequence- were used for sex determination. PCR was conducted in 40 cycles and the products were then ran on a gel. Presence of both the BRY 1a band and the satellite sequence indicated a male, while only the satellite sequence band indicated a female. [21]

#### 2.4.2.3. Detection of X linked enzymes

Detection of X linked enzymes is a non-invasive method of embryo sexing. A non-invasive method is preferred as it maintains the embryo's independence, reducing the chances of hindering its movement and successful implantation.

Non-invasive technologies involves the synthesis of X-related enzymes before X chromosome inactivation and the immune response of sex-specific antigen antibodies. This approach is advantageous because it protects the embryo's integrity, thereby decreasing the likelihood of unsuccessful embryo transfers. [23]

Some enzymes, such as glucose-6-phospate dehydrogenase (G6PD), hypoxanthine phosphoribosyl transferase (HPRT) and phosphoglycerate kinase, are X-linked which leads to a higher production level in females since they have two X chromosomes, unless males who have one. The enzyme concentration differences between male and female embryos enable sex determination. X-chromosome inactivation in females occurs near the blastocyst stage, forming a Barr body. This allows differentiation between sexes by comparing X-linked enzyme activity to autosomal enzyme activity, although it does have limitation such as reduced embryo viability and potential diagnostic errors. [20]

#### 2.4.2.4. Detection H-Y antigens

Detection of H-Y antigens is also a non-invasive method. The H-Y antigen was identified by Eichwald and Silmser in 1955 as a weak male-specific transplantation antigen system. It is present in the cell membranes of nearly all nucleated male mammalian cells and shows a high degree of evolutionary conservation, with mouse H-Y antibodies capable of cross-reacting with the H-Y antigen from about seventy different species. [24]

### 2.4.3. Cryopreservation

“Cryopreservation is the use of very low temperatures to preserve structurally intact living cells and tissues.” [25]

The primary biological impact of cooling is water freezing, which leads to an increased solute concentration in the remaining liquid. Theories of freezing damage suggest that it can be due to ice crystals directly harming cells through mechanical action or due to altered solute concentrations affecting the cells. Cryoprotectants help by raising solute levels, thus reducing ice formation at lower temperatures. For them to be effective, they must enter cells and remain minimally toxic. [25]

Cryopreservation is a key technique for preserving and expanding populations of animals with highly valuable genetic traits. Cryobiologists focus on reducing damage from intracellular ice formation during freezing and thawing processes by applying methods that combat this issue. Intracellular ice formation in cells is influences by the cooling rate and the surface area-to-volume ratio (Mazur, 1963,1970). Cattle embryos are typically cryopreserved by using a slow, controlled freezing technique while a faster method called vitrification has been introduced (Vajta & Nagy, 2006). The primary differences between these methods lie in cryoprotectant concentration and cooling rate (Leibo & Loskutoff, 1993; Massip, 2001). [26] Common cryoprotectants include glycerol, dimethyl sulfoxide (DMSO), ethanediol and propanediol. [25]

Slow freezing allows extracellular ice to form, causing dehydration of cells, while vitrification uses high concentrations of cryoprotectants and ultra-rapid cooling to prevent ice formation within cells. [26]

Damage during cryopreservation arises from temperature changes, known as thermal stress, leading to ice crystal formation both inside and outside the sperm

cells (Morris et al.,2012). Changes in osmolality also occur, such as hyperosmotic environment during freezing which can cause the cells to lose water and therefore shrink to balance the solute levels (Yeste, 2016). Conversely, thawing creates a hypotonic environment which leads to a water influx and an increase cell volume (Pommer et al., 2002). [27]

## 2.5. Factors affecting the success of embryo transfer

There are numerous factors which can influence either the success or failure of the embryo transfer process. Embryo transfer is just one step in a series of stages. There are many other steps such as collecting the embryos, isolating the embryos, storage of embryos and also the freezing of the embryos. [28]

### 2.5.1. Superovulation

Over the past 25 years, advancements in cattle superovulation has been very limited. Date coming from EM Tran, Inc. indicated that the average number of embryos recovered from super ovulated cattle was 4.6 in 1979 and 4.8 in 1999, despite about 20% of donors yielding no viable embryos. Although individual embryo production per donor has not significantly improved, overall efficiency has increased due to the use of progesterone-releasing devices. Additionally, it has been found that allowing two oestrous cycles between superovulation is unnecessary, enabling repeated superovulation every 40 days with effective outcomes. [28]

### 2.5.2. Embryo Recovery

Since the mid 1980s, when non-surgical embryo recovery, known was flushing was widely used, the procedures for embryo retrieval have seen little innovation.

Most practitioners use Foley-type catheters with inflatable cuffs, typically flushing the uterus with one to two litres of fluid with a low gravity flow. Opinions vary on whether to flush the uterine body or individual horns, though recovery rates seem comparable. Recent developments include silicone catheters designed for embryo retrieval, which offer advantages such as autoclave sterilisation and an improved cuff design. [28]

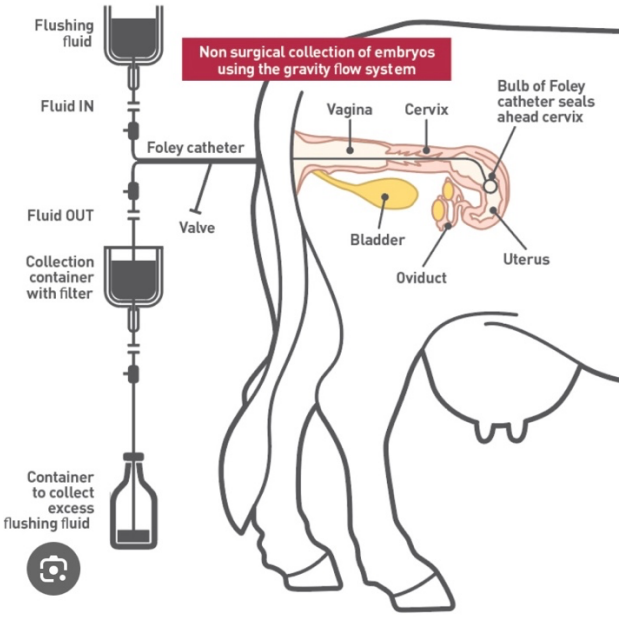


Figure 3: Collection of embryos [33]

### 2.5.3. Embryo transfer

Embryo transfer success rates in commercial settings are typically high, often exceeding 70%, with nearly 80% when the quality of the fresh embryos are used in the appropriate recipients. While embryo quality is crucial, recipient factors offer opportunities to improve the pregnancy rates. Studies show comparable rates among beef cows and heifers but lower rates in dairy cows. Oestrus synchrony is important but not critical, as slight asynchrony does not affect the outcomes.

Despite various hormonal treatments explored to enhance pregnancy rates, recent trials indicate that low doses of eCG may be beneficial. [28]

## 2.6. Factors Affecting Offspring

Pregnancy loss in cattle is more common during the embryonic period, which is days 1-42 compared to the foetal, which is days 42-280 and neonatal periods which is up to day 28. Approximately 40% of all losses occur due to early embryonic loss which is often related to maternal failure to recognise the pregnancy before day 18. The rate of embryo mortality following the transfer of in vivo-derived embryos is comparable to that seen after artificial insemination, with abortion rates dropping below 10% after two months. In contrast, in vitro-produced embryos exhibit significantly higher rates of loss during early embryonic, foetal and perinatal stages. [30]

### 2.6.1. Pregnancy Rates

According to a study I read, which analysed factors which affect the pregnancy rate. The pregnancy rate was unaffected by the donor's breed, fertility, lactational status or the timing of super ovulatory treatment within the oestrous cycle, however, embryos belonging to cows that were older than 15 years of age had a lower success rate. While the total number of ova recovered and fertilisation rates had very little impact, the number of fertilised ova did significantly influence the outcomes. Morphological quality and development stages of the embryos, as well as the timing of the recovery post-oestrus, were also significant factors. Recipients synchronised with the donor via prostaglandin exhibited higher pregnancy rates than those in natural synchrony. Various interactions among factors influence pregnancy outcomes, such as embryo quality and also oestrus synchrony. [31]

Another study concluded, that the data collected at the time of the embryo transfer included the recipient's parity, be it a cow or a heifer, whether oestrus was natural or synchronised using PGF2α, cloprostenol or CIDR and methods for confirming oestrus, be it standing heat, rectal palpation or a mucous discharge. The study assessed the effects of these factors on pregnancy rates using CATMOD procedures in SAS. Overall, pregnancy rates with the heifers showed higher than with the cows. Higher pregnancy rates were linked to confirming standing heat, conducting two CL examinations, using high-quality frozen embryos and transferring embryos on day 8 of the oestrous cycle.

3. Conclusion

There are various factors involved in the successful transfer of an embryo. Nowadays, we are very lucky in the technology available to us in assisting us with a successful process and transfer. There are also several factors which can aid the process, but on the other hand there are several factors which can lead to the process failing.

In conclusion, the literature on embryo transfer shows us the significant advancements in our understanding of this process. As previously highlighted above, the importance of factors such as the quality of the embryo, embryo handling and storage are of significant important. The recipient cow and the donor cow are also of utmost importance in the process too.

We are also at an advantage nowadays with the technology available to us such as karyotyping, polymerase chain reaction and detection of the H-Y antigen. The processes have further lead us to a more successful embryo transfer.

Despite these advancements, gaps still remain in our understanding of some mechanisms that may influence the success of implantation. There is certainly room for more research and criteria to refine protocols.

4. Acknowledgments

I would like to take this opportunity to thank my supervisor Dr. Somoskoi Bence for both his advice and support throughout the writing of my thesis. I would like to thank my friends and family, in particular, my mum and dad, John and Avril, for their never ending love and support over the last 5.5 years.

5. References


1. Seidel, G.E. Jr., 2002. Applications of embryo technologies to dairy cattle. Journal of Dairy Science, 85(4), pp. 1047-1058.
2. Troxel, T.R. (2007): Embryo transfer in cattle. Cooperative Extension Service, University of Arkansas Division of Agriculture, US Department of Agriculture, and county governments cooperating
3. Betteridge, K.J. (1981): An historical look at embryo transfer. Reproduction 62:1–13. doi:10.1530/ jrf.0.0620001
4. Allen, W.T. and Wilsher, S., 2020. Historical aspects of equine embryo transfer.Journal of Equine Veterinary Science 89:102987. doi:10.1016/ j.jevs.2020.102987.
5. Kraemer, D.C. (2013): A history of equine embryo transfer and related technologies. Journal of Equine Veterinary Science 33:305–308. doi:10.1016/ j.jevs.2013.03.007
6. Baruselli, P.S., Ferreira, R.M., Sá Filho, M.F., Nasser, L.F.T. & Rodrigues, C.A., 2013. Bovine embryo transfer in the Americas. Reproduction, Fertility and Development, 26(1), pp.1-14.
7. Jahnke, M. & Dohlman, T., 2017. Bovine embryo transfer helps ISU veterinarians improve herd genetics. Iowa State University College of Veterinary Medicine.
8. Food and Agriculture Organization (FAO), 1995. Embryo transfer in livestock: a tool for animal breeding.
9. Mapletoft, R.J., Bó, G.A., 2011. The evolution of improved and simplified superovulation protocols in cattle. Theriogenology, 76(9), pp.1581-1589.
10. Partners in Reproduction, n.d. MOET and IVF.
11. Santos, J.E.P., Rutigliano, H.M. & Sá Filho, M.F., 2009. Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows. Journal of Dairy Science, 92(11), pp. 5702-5712. Available

at: https://www.sciencedirect.com/science/article/pii/S0022030215009443 (Accessed: 10 October 2024).

12. Borchersen, S. & Peacock, M., 2009. Semen technologies in bovine breeding: A review of the current status. Journal of Dairy Science, 92(10), pp. 4731-4741.

13. Bó, G.A. & Mapletoft, R.J., 2010. Historical perspectives and recent research on superovulation in cattle. Reproduction, Fertility and Development, 22(1), pp. 1-13. Available at: https://www.publish.csiro.au/RD/RD09214 (Accessed: 10 October 2024).

14. Chebel, R.C., Santos, J.E.P., Reynolds, J.P., Cerri, R.L.A., Juchem, S.O. & Overton, M., 2004. Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows. Journal of Dairy Science, 87(11), pp. 3227-3236. Available at: https://www.sciencedirect.com/science/article/pii/S002203020470065X (Accessed: 10 October 2024)

15. Mohammed, A., 2018. Artificial insemination and its economical significance in dairy cattle: A review. Available at: https://www.researchgate.net/profile/Ahmed-Mohammed-4/publication/325472932_Artificial_Insemination_and_its_Economical_Significancy_in_Dairy_Cattle_Review/links/5b1007570f7e9b4981fefe06/Artificial-Insemination-and-its-Economical-Significancy-in-Dairy-Cattle-Review.pdf (Accessed: 10 October 2024).

16. MSD Veterinary Manual, n.d. Embryo Transfer in Cattle

17. Fesseha, H. (2020). Embryo Transfer in Cattle Production and Its Principle and Applications. Available at: https://www.researchgate.net/profile/Haben-Fesseha/publication/340815285_Embryo_Transfer_in_Cattle_Production_and_Its_Principle_and_Applications/links/5eabd4c4a6fdcc70509dfdcb/Embryo-Transfer-in-Cattle-Production-and-Its-Principle-and-Applications.pdf [Accessed 10 Oct. 2024].

18. Tanga, B.M., Alexander, L., Lee, B., Cantrell, L., Price, J., & Hamilton, D. (2022). Article Title. Journal of Dairy Research, 1-11. Available at: https://www.tandfonline.com/doi/full/10.1080/24750263.2022.2077994 (Accessed: 10 October 2024).

19. Betteridge, K.J., Nino-Soto, M.I., Eaglesome, M.D. and Flood, P.F. (1988). Embryo bisection in cattle. Theriogenology, 29(1), pp.339-353. Available at: https://www.sciencedirect.com/science/article/abs/pii/0093691X8890249X (Accessed: 10 October 2024).

20. Bashiri, M. and Gholami, H., 2020. A review of reproductive biotechnology in livestock: methods and applications. International Journal of Livestock Research, [online] Available at: https://journals.iau.ir/article_671543.html [Accessed 10 October 2024].

21. Schmidt, M., & Cellini, R. (2001) 'Genome Analysis and Embryo Production in Cattle', Proceedings of the 17th International Congress of Animal Reproduction, pp. 122-128. Available at: https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=115a26209157cd7227e68be1844e6df84eea1d32 (Accessed: 11 October 2024).

22. Kim, H., Lee, J. Y., Lee, H. S., & Park, C. K. (2022). Title of the Article. Journal of Animal Reproduction and Biotechnology, 37(4), pages. Available at: https://www.e-jarb.org/journal/view.html?uid=2614&vmd=Full [Accessed 11 Oct. 2024].

23. Hosseini, S.M., Rahimi, G., Fathi, S. and Aref, A. (2022) 'Review of embryo sexing in farm animals', Eurasian Journal of Animal and Veterinary Advances, 14(3), pp. 175-185. Available at: https://www.e-jarb.org/journal/view.html?uid=2614&vmd=Full (Accessed: 11 October 2024).

24. Avery, B. and Schmidt, M. (1989) 'Sex determination of bovine embryos using H-Y antibodies', Acta Veterinaria Scandinavica, 30, pp. 155-164.

25. Mitalipov, S. and Wolf, D. (2015) 'Introduction: Principles of Mammalian Embryo Development', in Mammalian Embryo Development: Methods and Protocols. Methods in Molecular Biology, vol. 1330, Springer, New York, NY, pp. 1-12. Available at: https://link.springer.com/protocol/10.1007/978-1-4939-2193-5_1 (Accessed: 17 October 2024)

26. Elahi, R., Sudhakar, K. & Mamat, R., 2016. Prospect of concentrating solar power in Malaysia: A review. Physica A: Statistical Mechanics and its Applications, 451, pp.196-

211. Available at: https://www.sciencedirect.com/science/article/abs/pii/S0378432015300695

27. Martínez-Pastor, F., Fernández-Domínguez, I., Alvarez-Rodríguez, M., Martínez-Alborcia, M.J. and Anel-López, L., 2021. Cryopreservation of farm animal sperm: From freezing protocols to optimal storage conditions. Physica A: Statistical Mechanics and its Applications, 565, p.125518. Available at: https://www.sciencedirect.com/science/article/pii/S0378432021002190 [Accessed 17 Oct. 2024].

28. Hasler, J.F. (2001) 'Factors influencing the success of embryo transfer in cattle', available at: https://www.researchgate.net/publication/228484750_Factors_influencing_the_success_of_embryo_transfer_in_cattle(Accessed: 17 October 2024)

29. Makinde, O. J., Adediran, S. A., & Ojo, O. A. (2015). Effect of cryopreservation on spermatozoa and fertility of farm animals: A review. University of Jos Agricultural Sciences, 11(1), 1-10. Available at: https://www.ajol.info/index.php/ujas/article/view/128071 (Accessed: 17 October 2024).

30. Davis, R.W. and Hurst, L.N., 2007. Factors affecting bovine embryo quality: a review. Reproductive Biology and Endocrinology, 5(1), p.24. Available at: https://www.sciencedirect.com/science/article/abs/pii/S0093691X07004979[Accessed 17 Oct. 2024].

31. Pavlok, A., Babcock, D., Ciegler, J. and Pritchard, R. (1987) 'The effect of superovulation on the fertilization of bovine ova', Theriogenology, 27(1), pp. 21-26. doi: 10.1016/0093-691X(87)90075-6.

32. ResearchGate, 2023. Multiple ovulation embryo transfer technology. Available at: https://www.researchgate.net/figure/Multiple-ovulation-embryo-transfer-technology-3-Sperm-Sexing-Livestock-producers-are_fig1_370871968 [Accessed 11 Dec. 2024]

33. Highland Genetics, 2024. In Vitro Fertilization (IVF) Embryo Transfer. [online] Available at: https://www.highlandgenetics.com/blogs/news/in-vitro-fertilization-ivf-embryo-transfer [Accessed 11 December 2024].

UNIVERSITY OF VETERINARY MEDICINE, BUDAPEST
founded in 1787, EU-accredited since 1995
INTERNATIONAL STUDY PROGRAMS
secretary, student@univet.hu

# Thesis progress report for veterinary students

Name of student: EMEK U. KABAKE

Neptun code of the student: G1816M

Name and title of the supervisor: DR. BENCE SOMOSKEI

Department: DEPARTMENT OF REPRODUCTION AND FARM ANIMAL CLINIC

Thesis title: EMBRYO TECHNOLOGY IN DAIRY CATTLE

## Consultation – 1st semester

| | Timing | | | Topic / Remarks of the supervisor | Signature of the supervisor |
|---|---|---|---|---|---|
| | year | month | day | | |
| 1. | 2024 | 02 | 21 | TOPIC CLEARING | |
| 2. | 2024 | 03 | 21 | LITERATURE REVIEW | |
| 3. | 2024 | 04 | 13 | LITERATURE REVIEW | |
| 4. | 2024 | 05 | 14 | METHODOLOGY/ART TRENDS | |
| 5. | 2024 | 05 | 21 | METHOD DISCUSSION | |

**Grade achieved at the end of the first semester:** 5

## Consultation – 2nd semester

| | Timing | | | Topic / Remarks of the supervisor | Signature of the supervisor |
|---|---|---|---|---|---|
| | year | month | day | | |
| 1. | 2024 | 09 | 25 | LIT REVIEW | |
| 2. | 2024 | 10 | 28 | LIT. REVIEW | |
| 3. | 2024 | 11 | 14 | DISCUSSION ON NEW TRENDS | |
| 4. | 2024 | 11 | 18 | DISCUSSION ON ADVANCED TECH | |

| | | | | | |
|---|---|---|---|---|---|
| 5. | 2024 | 11 | 20 | FINAL Lecture + Discussion | |

**Grade achieved at the end of the second semester:** 5

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

.....
signature of the supervisor

Signature of the student: .....

Signature of the secretary of the department: .....

Date of handing the thesis in: 2024. 12. 13.