University of Veterinary Medicine Budapest
Department of Animal Hygiene, Herd Health and Mobile Clinic

Embryo Transfer in Dairy Cattle, Current Trends and Future Possibilities.

Holly Mary O'Connell

Supervisor: Dr. Pribenszky Csaba

2024

Abstract

This thesis presents a critical evaluation of the benefits and limitations of Embryo Transfer (ET) in dairy cows, through an extensive review of the existing literature. It also points out key areas that require further investigation and technological advancement, to guarantee that ET continues to make a sustainable, profitable, and ethical contribution to the future of dairy production around the globe.

By facilitating quicker genetic advancements and raising dairy herd production, embryo transfer (ET) technology has emerged as a crucial instrument in the dairy sector. With an emphasis on the developments in reproductive technologies, such as genomic selection, cryopreservation, and superovulation protocols, this thesis examines current trends surrounding ET in dairy cows.

The thesis simultaneously explores ET's potential for the future, namely how this might include the integration of cutting-edge technologies like artificial intelligence and CRISPR-Cas9 gene editing. These developments present encouraging opportunities to improve reproductive control, boost climatic resilience, and strengthen genetic selection in dairy cows. There remain issues, however, such as the need to increase the success rates of in vitro-produced embryos, the financial obstacles facing smaller dairy farmers, and concerns regarding the welfare of the donor and recipient cows involved in ET procedures.

Absztrakt

Ez a dolgozat kritikai értékelést ad az embriótranszfer (ET) előnyeiről és korlátairól tejelő teheneknél, a meglévő szakirodalom átfogó áttekintésén keresztül. Rámutat továbbá kulcsfontosságú területekre, amelyek további vizsgálatot és technológiai fejlesztést igényelnek annak garantálása érdekében, hogy az ET továbbra is fenntartható és nyereséges legyen világszerte.

A gyorsabb genetikai előrehaladás elősegítésével és a tejelő állomány termelésének növelésével az embriótranszfer technológia kulcsfontosságú eszközzé vált a tejágazatban. A dolgozat továbbá a tejelő teheneknél alkalmazott asszisztált reprodukciós eljárások jelenlegi trendjeit vizsgálja tenyésztési, szelekciós (genomikai), mélyhűtési és a szuperovulációs protokollok fejlesztésére helyezve a hangsúlyt.

A dolgozat egyidejűleg feltárja az ET jövőbeli lehetőségeit, nevezetesen, hogy miként integrálhatja az olyan élvonalbeli technológiákat, mint a mesterséges intelligencia és a CRISPR-Cas9 génszerkesztés. Ezek a fejlesztések biztató lehetőségeket kínálnak a szaporodás szabályozásának javítására, a hőtűrő képesség fokozására és a tejelő tehenek genetikai szelekciójának megerősítésére. Továbbra is vannak azonban problémák, mint például az in vitro előállított embriók beültetésének sikerességi arányának növelése, a kisebb tejtermelők előtt álló pénzügyi akadályok, valamint az ET eljárásokban érintett donor és recipiens tehenek jólétével kapcsolatos aggályok.

Contents

Table of Figures

Abbreviations

ET	Embryo Transfer
IVF	In Vitro Fertilisation
IETS	International Embryo Technology Society
FSH	Follicle Stimulating Hormone
IVP	In Vitro Production
BCS	Body Condition Score
CF	Crude Fibre
FTAI	Fixed-time Artificial Insemination
INF-t	Interferon-tau
AI	Artificial Insemination
hCG	Human Chorionic Gonadotropin
ART	Assisted Reproductive Technologies
FET	Frozen Embry Transfer
ICSI	Intracytoplasmic Sperm Injection
FTET	Fixed Time Embryo Transfer
MOET	Multiple Ovulation Embryo Transfer
IVM	In-Vitro Maturation
ROS	Reactive Oxygen Species
mSOF	Synthetic Oviductal Fluid Modified
RRI	Responsible Research and Innovation
THI	Temperature Humidity Index
GHG	Greenhouse Gas
FGF2	Fibroblast Growth Factor 2
LIF	Leukaemia Inhibitory Factor
IGF1	Insulin-Like Growth Factor 1
OHSS	Ovarian Hyperstimulation Syndrome

1. Introduction

ET has become an essential resource for improving and streamlining the reproductive management of livestock within the dairy industry. The need for high producing, sustainable and genetically superior dairy cows has increased simultaneously with the rising population of our planet and the subsequent heightened demand for dairy products by consumers. ET holds quite an advantage in that it helps in the rapid spread of the desirable genetic traits, therefore enhancing the productivity and general welfare of the cows involved in the procedures. Reproductive technologies such as ET have transformed the practice of selective breeding by enabling the acquisition and delivery of embryos from genetically superior donor cows to recipient cows, thus speeding up the process of genetic enhancement and improved herd performance [1].

Since its inception, the application of ET in dairy cows has undergone several significant changes. Breakthroughs in the 1970s and 1980s paved the way for ET to be utilised in the dairy farming sector. Increasing milk production, improving fertility and strengthening resistance to disease are noted as being some of the main driving forces behind these developments. In recent years, advancements in cryopreservation technologies, handling protocols for embryos and hormonal synchronisation methods have raised the success rates of embryo transfers even further. Genetic improvements have been made even more precise through the integration of genomic selection in conjunction with ET. This has enabled farmers to select for the variety of traits which lend themselves to increased productivity and environmental adaptability, as well as improved cow welfare [2].

With the potential to further improve genetic benefits, more advanced reproductive technologies such as gene editing and in vitro fertilisation (IVF) are contributing to the current trends in ET. Research is being aimed at ensuring that breeding protocols are in line with evolving global sustainability goals and also that the welfare standards of the cows involved in ET procedures remain high. There is considerable interest in using ET to breed calves that are more tolerant to stresses such as heat stress, water shortages and other environmental challenges, considering the increasing impact of climate change on dairy farming and the impact of dairy farming on climate change. The potential positive outcomes from these practices highlight just how crucial it is to continue researching and improving reproductive biotechnologies, especially as a means of ensuring the long-term sustainability of the dairy industry across the globe [3].

The purpose of this thesis is to investigate the current trends and future possibilities of ET in dairy cows. This thesis will investigate the problems, practical uses and the technological developments related to its use. This study aims to evaluate the efficacy of ET in enhancing the genetics and productivity of dairy herds through an extensive review of the existing literature. Additionally, it will identify the critical aspects that contribute to the performance of ET and investigate potential areas for future improvement. This thesis sheds light on how ET offers an opportunity to support the dairy industry's competitive edge in a modern agricultural environment that is continuously changing.

2. Objectives

The main goals of this thesis are to investigate and assess ET technologies in dairy cows as they are today, with a particular emphasis on the potential of ET, its' applications and the limitations involved. Through a thorough assessment of the literature, this study seeks to identify gaps in the existing literature, summarise important discoveries in relation to the development and efficacy of ET and also to evaluate the trends that influence its application in modern dairy farming. This literature review also aims to elucidate the methods through which the developments in ET might support sustainable dairy farming practices, boost production and make genetic improvements more accessible within dairy herds.

This thesis aims to provide a comprehensive summary of the most important concepts, theories and findings that exist in the research relating to ET in dairy cattle. Following this objective, it is hoped that a greater understanding of the current trends and future possibilities of ET in dairy cattle will be achieved, by consolidating the facts that already exist while emphasising the areas that require further research or investigation.

An imperative objective of this thesis is to compare and analyse the numerous sources of material on ET in dairy cows. This will entail an in-depth review of the various research articles, studies and the professional opinions that have shaped our knowledge of ET technology. Contradictory findings have been found in the literature as a result of, for instance, the large variations in ET success rates that occur based on the breed in question, environmental factors, or the hormonal synchronisation that is used. Throughout this approach, this thesis aims to present a broad take on the current understanding of ET in dairy cows, with the aim of guiding future practice and research.

This thesis reviews the literature and attempts to pinpoint important gaps in the existing knowledge about ET in dairy cows. Although ET has advanced significantly in recent years, there are still certain areas of the subject where the information is scarce or contradictory. Further research is needed, for example, on the economic viability of the widespread implementation of ET in small scale dairy farms, as well as the heterogeneity in success rates in various breeds and the long-term impacts of intense genetic selection. This study's identification of such gaps will enable future research efforts a clear pathway forward and the presentation of new opportunities and innovations which have the potential to enhance the effectiveness, viability and availability of ET technology in the dairy sector.

One of the main objectives of this thesis is to evaluate the techniques used in previous research on ET in dairy cows. This will require an examination of the numerous experimental methodologies and protocols which have been utilised to boost the success of ET in these animals. These include synchronisation plans, superovulation protocols and embryo handling procedures. This study will identify areas with flaws in their methodologies or certain limiting factors and seek to identify the best practices. This can be undertaken by contrasting the advantages and disadvantages of various techniques. This assessment will also take into account how new technologies, such as cryopreservation and genetic selection, affect the success rates of ET. Comprehending the efficacy and relevance of these approaches is imperative in order to enhance present ET practices and promote these technologies throughout the future of the dairy industry.

The main advancements in ET technology for dairy cows will be investigated in this thesis. The breakthroughs in reproductive technologies that have taken place in recent years have improved the effectiveness and availability of ET for dairy farmers [1]. Through an analysis of these changes, this study will investigate how ET in dairy cows has progressed from being a marginal practice used by a minority of farmers, to a more commonly adopted strategy for the enhancement of genetics within dairy herds. In addition to this, new advances will be investigated to identify how they might impact the future of ET in dairy cows. In conjunction with highlighting the state of ET currently, this review will provide insights into the future possibilities of the ET field.

This thesis also aims to contextualise the research within the larger academic and practical environments. This will allow the study to advance existing knowledge while also addressing the current issues in relation to this topic. The research will be contextualised to demonstrate how improvements in ET can be utilised to boost wider efforts globally to drive sustainable farming practices, especially within the dairy industry.

3. Literature Review

3.1 ET Throughout History

Due to international trade and health regulations, continental cattle breeds transported into countries such as Canada were very rare and extremely valuable. A way to quickly multiply their numbers was provided by ET. The individuals who created the technology for the usage of the procedures were small commercial enterprises and individual veterinary practitioners who applied laboratory skills to real-world situations. These scientists faced numerous challenges when applying ET in reality and they established the International Embryo Technology Society (IETS) to encourage open dialogue and information flow, which they believed was essential to the advancement of the biotechnologies [4].

It took almost 60 years after the first successful transfer of mammalian embryos in 1890 for the fundamental technology of embryo transfer in cattle to show signs of substantial advancement. In cattle, ET has been widely used to increase the reproductive rates of genetically superior female animals [5]. There is a general understanding that Walter Heape's 1890 live birth was the first recorded live birth from mammalian ET. By the beginning of the 1970's, technological advancements in the area had let to the point where numerous nations were capable of establishing ET protocols. Currently, many trusted and well rooted techniques, such as superovulation or cryopreservation are used as part of ET procedures on a worldwide scale. Over the years, there has been minimal variation in the average numbers of embryos produced via superovulation, however the changes can be seen in synchronisation and hormonal therapies, which have improved. Worldwide, more than a million embryos are generated each year [6].

Over the past century, advances in reproductive technologies have made significant improvements to the dairy industry. Cattle were successfully inseminated artificially for the first time in the 1900's. The implementation of semen extenders and progeny testing throughout the 1930's and 1940's, along with the discovery of glycerol as a sperm cryopreservation medium in

1949 were subsequent significant advancements. In the 1980's, the cytometric separation of X- and Y- sperm began and by the end of the 20th century, the birth of calves produced from sexed semen was taking place [7]. The switch from surgical to nonsurgical embryo recovery techniques during the 1970's and the introduction of the use of Follicle Stimulating Hormone (FSH) to increase embryo production are notable turning points in the development of ET in dairy cattle. Furthermore, the improvement of embryo evaluation based on the morphology and quality standards set out by the IETS also proved to be an imperative advancement in the field [8].

3.2 The General Procedure of ET in Dairy Cows

It has been established that ET in bovines has had an extensive history and that it has developed throughout time due to advancements in methodologies and technologies. Cows that are considered phenotypically and genetically superior are chosen as donors. These donors then undergo superovulation protocols to generate numerous embryos. These embryos are then recovered non-surgically and their quality is assessed. They are delivered to recipient cows who have been synchronised for their reception. As a result of their ability to provide more frequent ovum collection, cryopreservation and IVF, procedures such as the in vitro production (IVP) of embryos and embryo cloning have transformed the science of embryology. These advancements have opened the door for the utilisation of sex-sorted semen, as well as presenting new avenues for marketable ET services [8].

One of the most crucial elements of an effective ET program is cattle management. In order to ensure balanced nutrition, management entails the selection and upkeep of donor and recipient cows who are healthy and in good reproductive health, with a suitable body condition score (BCS). Certain essential nutrients must be consumed by the cows in question in order to ensure the best performance. This includes, in particular, a good quality roughage, which will provide an adequate source of crude fibre (CF) for rumen function. It is vital that the requirements of the recipient cow are considered carefully prior to any superovulation or the recovery of embryos. When an embryo in transferred into a uterine environment that best resembles its original one, the highest conception rate is attained [9].

3.3 The Donor

Nowadays, the selection of a donor cow may be made based on any number criteria. Oftentimes these may be unrelated to the reproductive health, age, or endocrinology of the cow, such as industry standing or market variation [10]. The veterinarian or the practitioner plays a large role in the management concerns surrounding the donor cow also. This relates to their knowledge, abilities, experience, equipment and the facilities available to them. It must be emphasised that consistent training to develop tried and trusted protocols, such as for hormonal or synchronisation processes, is very important to prevent any management issues. The practitioner should aim to keep accurate and consistent records for each donor, as this will support the implementation of management adjustments that are based on reality and not merely speculation [8]. A 2016 study explored the metabolic and endocrine distinctions between Bos taurus and Bos indicus cows, emphasising the management consequences for reproduction. These distinctions are essential for improving the procedures for both in vitro and in vivo fixed-time artificial insemination (FTAI) and embryo formation. Bos Indicus cattle have distinct reproductive differences in comparison to Bos Taurus breeds. Bos Indicus cattle have smaller follicular diameters while they are more sensitive to gonadotropins and experience a shorter oestrus. ET programs intended for use in Bos Indicus breeds should take these distinctions into account [11].

The age of the donor cow is also one of the key elements which influence the efficacy of ET. Studies have revealed that cows of younger ages typically respond less intensely to superovulation, due to hormonal abnormalities, such as higher levels of oestradiol. In comparison, while older cows may produce a higher quantity of embryos, the quality of these can differ greatly depending on the animals reproductive health [12].

Hormonal treatments are administered to donor cows in order to promote the growth of numerous follicles. Usually, FSH is used in combination with other hormones to promote superovulation. Uterine flushing is a non-invasive technique which is used to extract embryos from the donor cows and the practice includes the use of a particular medium. The quality of the embryos gathered is then examined under a microscope. Naturally, only healthy, good quality embryos are chosen for transfer [13].

3.4 The Recipient

The oestrus cycles of the recipient animals are synchronised, so that they match the stage of development of the embryo [13]. Dairy recipients should fall within the BCS range of 5.5-6.5. Conception rates are often lower in animals that are severely underweight or overweight, than in a cow or heifer of the ideal BCS. Other nutritional considerations, in addition to increasing fibre or starch intake, include the availability of trace minerals and ensuring that feed has not been contaminated by mycotoxins [14]. Another primary element of an ET program is recipient selection. Considerations must be made, for example, if a practitioner is working within an open or a closed herd. It is also important to note that while using heifers as recipients is a widespread practice, there are also risks involved. While heifers usually give birth at rates approximately 10% to 20% higher than cows, the drawback is that the genetics of these females may not be selected for ease of calving. Thus, an emphasis must be placed on calving management in cases where heifers are used as recipients [15].

3.5 Maternal Recognition of Pregnancy

The process of maternal recognition of a pregnancy will guarantee that an implanted and developing embryo is supported by the maternal environment, which is imperative for the continuation of the pregnancy. The embryo and the maternal system exchange a number of biochemical signals in order for the mother to recognise her pregnancy. Interferon-tau (IFN-τ), a protein released by the embryo's trophoblast cells, is the main mediator of this process in cows. IFN-τ prevents the corpus luteum from regressing, which subsequently keeps progesterone production continuing and this is crucial for sustaining pregnancy. Maternal pregnancy recognition following ET may be influenced by a number of circumstances. These include the recipient cow's health and nutritional condition, the synchronisation procedures employed, and the embryo's stage during transfer [16].

3.6 The Current Applications of ET in Dairy Cows

ET has numerous applications in modern dairy herds. One of most predominant of these is its use in repeat breeder dairy cows. A number of variables can lead dairy cows to become repeat breeders. These include subpar oocyte quality, early embryo mortality and an unsuitable uterine environment. When compared to artificial insemination (AI) on its own, ET has improved the rates of conception promisingly in repeat breeder dairy cows [13]. The possibility that performing ET after AI may enhance the secretion of INF-t from the embryos, which, in turn, aids in the maternal recognition of pregnancy, is yet another reason as to why it is thought to boost conception rates in these cows. Some particular reasons behind the infertility of some cows, such as unsuccessful fertilisations and embryonic losses, may be avoided by performing ET [17]. It has also been demonstrated that the use of fresh embryos for ET increases the likelihood of conception in repeat breeder dairy cows. Various studies have examined the pregnancy rates in repeat breeder cows following AI and ET, with ET appearing to generate greater pregnancy rates. In a study conducted on Holstein Friesians, the conception rates obtained were 17.9% after AI and 41.7% following ET [13].

Due to the fact that ET places the embryos into the uterus at a less vulnerable developmental stage, the procedure can also lessen the detrimental effects of heat stress on fertility. Research has shown that recipient dairy cows' oestrous expression frequency and intensity can influence the success of subsequent ET pregnancies. In a study that was undertaken on dairy farms in Brazil, the relationship between the expression of oestrus and fertility in dairy cows who were recipients of an ET procedure, was assessed. The study discovered that the pregnancy rate for each ET was impacted by the frequency and strength of oestrous expression. It also highlighted that oestrous cows outnumbered non-oestrous cows. Furthermore, in comparison to cows who received embryos at the morula stage, cows that were given blastocyst or early blastocyst stage embryos had better rates of fertility [18].

In dairy cows, ET can also be used as a preventative measure against twin pregnancies. Twin pregnancies can have an adverse effect on herd health and economics. They lead to higher pregnancy loss rates, as well as an increased number of disorders of reproduction in these dairy cows. Two basic strategies are commonly suggested in order to lower the occurrence of twin pregnancies. The first includes transferring one embryo to a recipient cow that has not been inseminated. The second includes the draining of the antral follicles at the precise moment of insemination, thus reducing the risk of a twin pregnancy without lowering the fertility of the animal. Twin prevention techniques in dairy cows may also benefit from the use of sexed semen. While historically, sexed semen was advised for use in heifers, it can also prove useful in cows, by lowering the frequency of dystocia, caused by larger male calves [19].

For the purpose of increasing fertility in dairy cows that are undergoing ET, hormonal ovulation inducers such as human chorionic gonadotropin (hCG) are used. Research has indicated that the development and maintenance of pregnancy depends heavily on the time of the progesterone surge following ovulation. Early in pregnancy, progesterone levels have been known to rise through the induction of an auxiliary corpus luteum with hCG or GnRH. The uterine environment for embryo elongation can be improved by progesterone supplementation during the early stages of embryo development. A study was conducted that examined how administering GnRH or hCG just before ET affected recipient dairy heifers and cows receiving in IVP embryos in terms of pregnancy per ET, pregnancy loss, and calving per ET. According to the study, there were no appreciable differences between the GnRH or hCG treatment groups and the control group of animals. It was discovered instead that parity affected the results of reproduction. It was found that primiparous and multiparous cows had lower pregnancies per ET and calvings per ET in comparison to nulliparous cows [20].

AI and ET are also becoming increasingly popular in tropical regions, where the use of breeding bulls of unknown genetic origin is commonplace in order to gain significant genetic advancement. The use of ET, AI, and natural mating in Costa Rican cow herds was compared in terms of lactational performance, age at puberty, and postpartum reproductive efficiency. Information from 28 dairy farms was included in the data analysis, with an emphasis placed on herds situated throughout various locations. The data disclosed that reproductive parameters such as the calving to conception interval, age at first calving and milk yield, were strongly influenced by the method of conception. Such reproductive parameters were also correlated with other variables such as genetic background, days in milk and year of calving. These results demonstrate the possible advantages of employing ET to increase the productivity and enhance the reproductive parameters in breeding protocols and management on dairy farms [21].

3.7 Advances in ET Technology

3.7.1 The Cryopreservation of Embryos

Technological developments in embryo transfer, especially with regard to cryopreservation, have had a major impact on assisted reproductive technologies' (ART) success rates. Research has focused on comparing fresh and frozen embryo transfers (FET), providing detailed insights into the differences in their relative results. Methods of cryopreservation, particularly vitrification, have transformed the storage and transfer of embryos. When compared to conventional, slow freezing procedures, vitrification, which is a rapid technique for the freezing of embryos, has been demonstrated to increase the embryo survival rates after thawing [22]. These notable improvements in post thaw survival rates have strengthened the reliability of frozen embryos [23]. It has also been recorded that ET's using frozen-thawed embryos may produce comparable or even better conception outcomes following intracytoplasmic sperm injection (ICSI) [24]. In bovines, slow freezing methods often delivered lower pregnancy rates in comparison to vitrification methods, due to the damage that can be caused to the cellular components during slow freezing [25].

The long-term viability of these embryos renders it possible to create 'embryo banks.' These storage facilities enable increased breeding performances spanning a numerous geographic regions, promoting genetic variety [26]. In addition, these increased success rates which are associated with the vitrification of embryos, have led the way towards the introduction of policies aimed towards encouraging 'single embryo' transfers. Single pregnancies are already shown to cause fewer risks to cows and heifers, especially surrounding parturition [27]. Studies in humans have also highlighted that improved synchronisation between the embryo itself and the endometrial environment during cycles of FET can lead to increased implantation rates. Generally, the embryos that are selected for freezing are of superior quality, thus contributing to more the more favourable outcomes associated with FET [28].

Nonetheless, there remains issues surrounding the standardisation of the cryopreservation procedures throughout the various laboratories. This may cause variations in the success rates [29]. This calls for continued research in order to improve and standardise vitrification methods and to increase the competency of embryo development [30].

3.7.2 Genetic Testing and Genomic Selection

Utilising genomic data to forecast the embryos' breeding values is what is referred to as 'genomic selection' and it has the potential to significantly accelerate genetic advancement in dairy herd breeding operations. Research has shown that implementing genomic selection prior to implantation has the potential to speed up genetic advancement in cattle, especially when applied in conjunction with methods such as blastomere separation [31]. By using this technique, a small sample of cells from the embryo can be removed for genetic analysis, thus making it possible to identify embryos with desired characteristics prior to transfer [32]. This enables dairy farmers to make well-informed decisions which support the long-term genetic objectives of the herd, such as enhancing resistance to diseases and boosting productivity [33]. Screening embryos for the presence of any genetic abnormalities or diseases prior to conducting the ET improves both the economics of the operation, as well as animal welfare [34].

Another noteworthy development within the discipline is the application of genetic testing for determining the sex of bovine embryos. This can be properly ascertained using methods such as PCR. This proves to be of significant importance for dairy herd breeding plans in particular, where farmers may prefer to produce heifer calves over bull calves [35].

In contrast to conventional methods of evaluating embryo morphology, time-lapse technologies have shown promise as useful instruments for evaluating the viability and potential of embryos for implantation. These developments further highlight the significance of including these innovative biotechnologies in ET protocols [36].

The utilisation of genomic selection and genetic testing has become essential for optimising the efficacy and efficiency of ET operations in cows. By combining these technologies, it makes it possible to choose embryos that possess exceptional genetic qualities, increasing the overall productivity and quality of the dairy herd.

3.7.3 Hormonal Synchronisation and Superovulation Protocols

Hormonal synchronisation increases the chances of obtaining successful conceptions, as it ensures that the recipients are ready for the implantation of the embryos. Researchers have also demonstrated that that fixed time embryo transfer (FTET) can lead to similar, if not even a higher yield of conceptions, when compared with conventional AI techniques. Hormones such as hCG and GnRH have been used alongside ET protocols to increase pregnancy rates [37].

The issue which commercial dairy farmers oftentimes face is choosing the most economical and profitable course of action. Profitability has been found to be higher in farms that employ more intense reproductive programmes. These instances are more economical, despite the fact that they require more hormone treatments. The same remains true even when the prices of such treatments rise to abnormally high levels [38].

3.8 Current Trends in ET

3.8.1 Utilisation of IVF and IVP Embryos

The use of IVP-ET technologies enables practitioners to produce and implant embryos from genetically superior donor cows, into recipient cows from the same, or different herds. Utilising these systems is becoming more and more common in dairy herds. It serves to hasten the genetic advancement of the herd, by intensifying selection and boosting the accuracy of selection [39]. The higher expense of IVP-ET when compared to AI is one of its major drawbacks [40]. IVF embryos typically reach the blastocyst stage with greater success. According to some studies, a much higher percentage of IVF embryos reached the blastocyst stage by Day 8, compared to non-IVF embryos. This implies that IVF methods could be more successful in promoting the development of early embryos [41]. The sector is expanding even more rapidly as a result of the rising acceptance of IVP technology. Some livestock producers are switching from traditional multiple ovulation embryo transfer (MOET) to IVP as a result of an excess of viable embryos generated compared to those transferred, according to the IETS [42]. There are many procedures involved in the IVP of embryos. These include the retrieval and selection of oocytes, in-vitro maturation (IVM), the preparation of sperm, IVF and culturing the zygotes to the blastocyst stage [43].

In dairy herds, IVP-ET technologies are gaining popularity because they make it possible to produce and implant embryos from genetically superior donor cows, which speeds up genetic improvement. IVP-ET's higher cost is still a major disadvantage, even though it gives better success in early embryo development than conventional techniques. Due to the abundance of viable embryos, many producers are switching from MOET to IVP.

3.8.2 Oocyte Collection and Selection

Oocyte collection is crucial for IVF because it preserves and makes use of anovulatory follicles that, in vivo under physiological settings, would eventually become atretic follicles as a result of endocrine regulatory feedback [44]. There are four main procedures used for the collection of oocytes. The first option involves aspirating the follicles [45]. From females over six months of age, ultrasound guided transvaginal aspiration can be used to collect the oocytes [46]. From females aged less than six months, the laparoscopic techniques can be implemented to collect the oocytes [47]. Finally, slicing can also be used to obtain the oocytes from the ovaries of cows in slaughterhouses [48]. Following collection, suitable oocysts are selected based on three main characteristics. These include the appearance of the cytoplasm, the diameter of the oocyte itself and the appearance of the clusters which surround it [49]. Oocytes that display characteristics for higher rates of fertilisation are usually composed of multiple cell layers and are surrounded by a cluster [50].

3.8.3 IVM

Increasing the effectiveness of in-vitro embryo development requires first ensuring that the oocytes are sufficient. One of the key reasons behind a drop in the quality of oocytes that are matured in-vitro is oxidative stress. IVP is not without its complications, such as the noted decrease in oocyte quality following IVM [51]. Oocytes that are produced during IVM often lack the protection of certain antioxidant enzymes, meaning that the reactive oxygen species (ROS) produced during oxidative stress cannot be broken down. Instead, these ROS accumulate and eventually lead to the destruction or apoptosis of the cell [52]. Synthetic oviductal fluid modified (mSOF) is cultured from the cells of the oviduct and is used for the maturation of bovine oocytes [53].

3.8.4 Preparation of Sperm

The sperm of mammals expresses strong heterogeny in its nuclear stability, motility and morphology. Cervical mucous acts as a barrier during natural breeding and it allows only sperm that possess these characteristics to migrate [54]. After thawing, the proportion of progressive motility in frozen bull spermatozoa is reduced to between 30% to 70%, although the proportion of morphologically normal sperm cells in thawed ejaculate remains equal to that of fresh semen [55]. To produce a high percentage of suitable, adequate quality spermatozoa for IVF, frozen bovine semen must be free of extender ingredients, such as cryoprotectant. Even from the same bull, methodological elements like sperm preparation could significantly affect the IVF outcomes [56]. The sperm used for IVF must be evaluated based on its motility and morphology in order to ensure it is of the adequate quality. For the in-vitro preparation of sperm, a number of different systems may be used, such as self-migration, the swim-up or swim-down method, or density gradients. These methods do not include the capacitation of the sperm, this step is undertaken at a later point in the process [57].

3.8.5 IVF

The effectiveness of IVF procedures, compared with alternative reproductive technologies, and the variables affecting IVF success rates in cows are only a sample of the topics covered in the literature on this subject. It is often compared with procedures such as AI or ET [41]. Mature oocytes are incubated with chosen sperm in a process called in vitro fertilisation. Similar to what occurs in vivo or under physiological settings, sperm can reach their fertilising potential in-vitro. In order for this to be successful, however, the physiological events such as capacitation, acrosome response activation and the penetration of the zona pellucida must take place [58].

3.8.6 Culturing Embryos

From the zygote until the blastocyst stage, the mammalian embryo experiences significant changes in its physiology and energy metabolism. The in vitro induced cessation at the 8- to 16-cell stage was lessened and blastocyst growth was aided, by the introduction of co-culture procedures in the mid-1980. These procedures involved incubating sheep and bovine embryos with somatic cells. However, intricate tissue culture media supplemented with serum were necessary for the co-culture systems. It has since been demonstrated that such serum is linked to the birth of calves with noticeably higher birth weights than usual, which makes it harder to manage these pregnancies safely and increases the rate of neonatal mortality. In a culture, the ideal embryo development occurs in two or more media, each of which is tailored to meet the evolving needs of the developing embryo in question [59]. To ensure that the embryo retains access to the optimal environment for development, the culture conditions should mimic the physiological environment of the oviduct. Many additional elements such as amino acids, energy molecules, vitamins etc. are often required components of the culture media [43].

3.9 Technological Advancements

Precision breeding, automation and even more recently, artificial intelligence, are revolutionising the modern dairy industry. These developments are essential for tackling issues such as milk yield optimisation and the management of herd health and welfare.

3.9.1 Precision Breeding

The breeding of dairy animals has shifted from using traditional breeding practices to molecular breeding techniques, due to the evolving development of molecular biology and associated fields and these trends are becoming commonplace in the field [60]. Approximately half of the reported variations in animal performance traits in carefully planned breeding programs can be attributed to genetics. Some of the main performance indicators taken into account include milk yield, reproductive performance, conformation and the overall general health of the animal. Animal health is under-represented in most breeding objectives, and characteristics including the quality of the milk produced, feed efficiency, and environmental impact were not specifically considered in many plans in recent years. The repercussions of neglecting or failing to keep track of such specific traits are highlighted by the historical decline in reproductive performance in the global Holstein population [61]. Rapid genetic gains can be made possible by genomic selection. This technique, when used as an element of precision breeding has the potential to revolutionise the breeding of dairy cattle. The ability to select for superior genetic traits enables farmers to select for greater milk outputs for example, thus, improving the health and welfare of the dairy herd. Research indicates that when compared to conventional progeny testing, genomic selection can increase genetic gain by as much as 108% [62]. Genome predictions have also been applied to crossbred dairy cows, which can gain from the genetic knowledge already obtained from purebred assessments [63].

3.9.2 Artificial Intelligence

In a bid to improve performance and operational productivity, dairy farming operations are progressively incorporating artificial intelligence technologies. Recent years have seen a growth in livestock numbers and an overall operational expansion within the dairy industry. This has brought with it significant challenges for farmers in relation to managing the reproductive health of their cows, since accurately detecting oestrus and timing ET procedures correctly are imperative to produce calves [64].

Predictive modelling for reproductive management, in which algorithms evaluate data to forecast ideal breeding periods and raise conception rates, is one example of how artificial intelligence is applied in dairy production systems [65]. The solution to enhancing fertility estimation may lie in carefully examining the sperm and investigating the data acquired using artificial intelligence applications. Since artificial neural networks have been used already to forecast fertility in human patients, this technique for evaluating the outcomes of biotechnologies such as IVEP and ET may prove useful [66].

The breeding and management of dairy cattle is changing significantly as a result of developments in artificial intelligence and molecular biology. By addressing past issues like the loss in reproductive performance in certain dairy populations, these changes enable more focused breeding techniques. When combined, these technologies have the opportunity to revolutionise modern dairy farming's sustainability and productivity.

3.10 Important Considerations of ET

3.10.1 Ethical Considerations

ET in dairy cows raises a number of complex ethical issues, including those related to animal welfare, the economy, and public opinion. It is essential to assess these factors as the dairy sector proceeds to use more sophisticated reproductive technologies, in order to guarantee ethical and responsible operations. Weighing the possible risks to cow welfare against the ethical ramifications is imperative. For example, although ET can improve productivity and fertility, if it is not properly managed it can result in greater health issues and stress for the animals involved [67].

The influence on animal welfare is one of the main ethical issues associated with ET in dairy cows. The views and actions of farmers have an impact on how the general public perceive animal welfare, highlighting the importance of providing dairy animals with humane treatment and sufficient care, especially while undergoing procedures such as ET [68]. Reproductive technologies and other management approaches have a direct impact on the welfare of dairy cattle. For example, using embryos created in vitro can result in the incidence of twin pregnancies. These are, in turn, linked to increased chances of dystocia and a diminished general health status in cows [37]. Twin pregnancies have been shown to reduce a cow's productivity and raise the risk of metabolic disorders. This raises serious welfare concerns. While a twin pregnancy may result in shorter gestational periods and more revenue for farmers from extra calves, twinning reduces milk production, lowers the birth weight of calves, lowers fertility, raises the risk of freemartin heifers, and raises overall culling risks [69].

For the bovines involved, the ET procedure can prove intrusive and stressful. Research has already shown that dairy herds in particular frequently experience health problems such as lameness. The physical symptoms of conditions such as chronic lameness can be made worse as a result of the additional physical requirements of ART [70]. Addressing these ethical issues requires incorporating responsible research and innovation (RRI) concepts. RRI highlights the importance of stakeholder participation and transparency in the advancement of agricultural technology and reproductive biotechnologies, such as ET [71].

In the ethical discussion of ET, the effect of environmental variables, such as heat stress, on the reproductive abilities of these dairy cows or heifers is crucial. It has already been demonstrated that heat stress negatively impacts oocyte development and overall reproductive effectiveness, therefore the environmental circumstances in which dairy cows are residing, as well as the possible effects on their health and welfare of exposing them to reproductive technologies like ET must be taken into consideration [72]. The ability to avoid the early stages of embryonic development and the temperature-sensitive period of oocyte maturation may be an additional benefit of ET. One effective tactic to minimise stress caused by environmental temperatures may involve the selective breeding of livestock species that are thermotolerant [73].

The dairy industry can more effectively negotiate the socio-ethical conundrums raised by sophisticated reproductive technologies by encouraging communication between farmers, researchers, customers and the general public. This strategy can work to guarantee that technological developments are in line with moral principles and allay public worries about animal welfare. It is crucial to implement procedures that place animal welfare to the fore while also meeting societal and financial demands as the dairy sector continues to develop in tandem with ever evolving technological improvements.

3.10.2 Economic Considerations

In addition to the ethical discussion of ET, economic factors are also quite important. The public frequently support methods that put animal welfare first, especially since the activities involved in dairy farming are facing more societal scrutiny than ever before. This calls for dairy farmers to adopt more ethical practices, ones which meet the growing consumer expectations, as this is likely to influence their long term financial viability [74]. It is necessary to balance the possible risks to the welfare of the cattle involved with the actual economic feasibility of employing ET. Many studies have highlighted that ET might not always be financially viable for all farmers when compared to conventional breeding techniques, regardless of the genetic improvement and reproductive efficiency that it lends itself to [75].

ET shortens the generation interval and raises the herd's total genetic merit by facilitating the quick transfer of superior genetic traits. This is especially important in high-production dairy systems where the cow genetics have a big impact on total milk quality and yield. Increased milk quantities can generate more income for a dairy farmer, which would serve to balance the expenses of the ET operations [76].

The ability to select and breed for genetic features which correlate with reduced methane emissions, while simultaneously boosting productivity allows for improved long term profitability for the dairy farm in question [77]. In the perspective of environmental sustainability, ET's financial advantages are undoubtably apparent. ET can help to lower the production of greenhouse gases per unit of milk produced by facilitating more effective breeding techniques. This is especially pertinent given the increase in international initiatives aimed at lessening the effect that the agricultural and dairy industry has on climate change [78].

Important factors that can change the cost-benefit balance of deploying such technologies must be included in a thorough economic analysis of ET. External conditions including input costs, market volatility and specific regulations have in influence on ET’s economic impact. Examples of such may include alterations in milk prices, milk quotas, or veterinary bills, all of which have a big impact on overall profitability [76]. Depending on a number of variables, most notably the scale of the dairy farming operation, the cost and the economies of scale of ET in dairy cows can vary greatly. Since there are often fewer donors or recipients in small-scale farms, the costs may be higher than the procedures’ economic viability for these farmers [79].

Incorporating ET into dairy farming methods not only increases output, but also supports larger environmental and climate change objectives. The foreseeable future of the dairy industry will be significantly shaped by the tactical use of ET to guarantee that it will continue to evolve and become more sustainable, economically feasible and environmentally friendly.

3.11 Future Possibilities for ET

The demand for dairy products will keep increasing sharply over the coming years, in line with further growth in the world's population. It is also to be expected that the global climate will continue in a trend of rising temperature as well as becoming more diversified geographically.

According to climate change projections, the average world temperature may rise by 2.6–4.8°C by 2100 [80]. The heat stress brought on by this kind of warming trend is likely to have a negative impact on livestock production by changing the quality of forage and roughage, limiting water availability, therefore reducing the reproductive health and productivity of the animals [81]. The highest limit for the thermoneutral zone of dairy cattle is said to be between 25 degrees Celsius and 26 degrees Celsius. Dairy cows are the most vulnerable to heat stress of any domesticated production animal. They are usually bred extensively and over a lengthy period of time in order to increase their milk output, which, in turn, raises their metabolic temperature [82]. As a measure of heat stress, the Temperature-Humidity Index (THI) is a crucial instrument for evaluating how animals react to changing weather. Higher THI may be associated with decreased milk production and weakened health, according to research [83]. The energy consumption that is required by these animals in order to regulate their body temperature while under heat stress can subsequently cause a reduction in their milk yield and even an alteration in the total fat and protein content of their milk [84] [85].

Long-term and effective breeding methods are needed since adaptability to the local climate typically has a low heritability and may have antagonistic genetic connections with milk output. Among the various characteristics that make up adaptation, those that impact fitness, like longevity and resistance to disease, are common. Usually low in heredity, these characteristics tend to decrease as milk output increases [86]. A variety of techniques can be used to increase resilience. Increasing resilience through the selection of specific genes in breeding efforts is one example. Unlike management improvements, genetic selection has the advantage of being 'cumulative'. The benefits will pass down to all generations thereafter [87]. The colour of the cows' hair coat could be another possible breeding trait. It has been shown by some researchers that keeping Holstein cows who possess a prominently black coat colour, in a warmer environment, will only mildly dimmish their milk yield and its composition and it will not affect their ability to reproduce [88]. Although they only offer short-term respite, technological innovations such as sprinklers, fans, and shaded structures can help reduce many of the negative impacts of heat stress. However, increasing thermotolerance through genetic selection is a feasible option that will produce more long-term, progressive benefits [89].

Enhancing breeding techniques to increase livestock's tolerance to local climates, especially in dairy cattle, depends heavily on ET. Given the difficulties caused by the low heritability of qualities related to resilience, like longevity and disease resistance, ET enables the quick transmission of advantageous genetic features, across multiple generations. Farmers can hasten the increase of these resilience traits, by selecting for embryos with the necessary genetic characteristics, such as coat colour or heat tolerance. In addition to promoting the production of cattle that will remain productive even under stress, this approach also lends itself to avoiding the compromise between versatility and substantial milk production. ET is an effective instrument in the process of the incremental selection of genes, which will eventually support a more resilient and sustainable dairy industry.

3.11.1 Breeding to Combat Climate Change

Aside from being particularly vulnerable to high ambient temperatures due to the thermogenic effect of lactation, dairy cows also generate methane, which is a known greenhouse gas. Therefore, boosting dairy cows' capacity to withstand heat and breeding for lower greenhouse gas emissions, particularly methane, have emerged as crucial characteristics to take into account in breeding initiatives [90]. In recent years, the public have become increasingly concerned about the greenhouse gas (GHG) emissions from dairy farms and dairy farming’s volatile relationship with climate change and global warming. The breakdown of dung and the microbial fermentation in the rumen produce this methane [91].

Understanding the genetic links between methane qualities and other commercially important features is essential if dairy farmers wish to include methane reduction into their breeding goals. Over the past number of years, a variety of studies have shown that the heritability of methane traits in dairy cattle is low to moderate [92]. Lowering the GHG emissions from dairy cattle can be accomplished effectively and sustainably by genetically selecting cows that release less methane [93]. Some research has been undertaken to estimate the genetic parameters for methane characteristics in cows and the genetic correlations between methane traits and productivity and performance variables using a database consisting of multiple locations worldwide [94].

For dairy farmers, the most significant impact of heat stress is most likely fertility deficits. This decrease in fertility and conception rates due to anoestrus and the increase in open days for these dairy animals are just some of the ways in which the effects of heat stress on reproductive health can manifest. While later stage embryos such as blastocysts are somewhat 'thermotolerant', heat stress can lead to issues surrounding follicular and oocyte development [95].

ET will certainly become increasingly relevant in the area of breeding dairy cows for reduced GHG emissions, such as methane. As public concerns surrounding the impact of dairy farming and its associated activities on the environment continue to grow, the ability to select embryos from genetically superior, lower methane producing cows, will become imperative. This strategy can help to reduce these harmful effects on the global climate, while hastening the integration of such traits into dairy herds. Farmers and practitioners can also select embryos with a greater knowledge of the genetic relationships between methane qualities and other financially significant characteristics, thus, preventing productivity gains from being offset by pollution improvements. It is evident that ET can be employed as tactical instrument to improve the profitability and sustainability of dairy farms, which is consistent with the increased focus on sustainable agriculture and environmentally conscious farming.

3.11.2 The Implementation of ET in Developing Countries

By improving genetic quality and production, ET technology has great potential for the future of dairy farming in underdeveloped nations. It is anticipated that technological developments like cryopreservation and IVF will increase the effectiveness and accessibility of ET, enabling the production and storage of superior embryos that can be transported to more remote regions as required [96]. For small scale farmers, this may result in higher milk output, better food security, and financial gains [97]. Due to the financial gains involved cattle production and the global flow of information, advances in reproductive biotechnologies, especially ART's like ET, are becoming more and more commercialised in developing nations. Due to its ease of use and effectiveness, AI continues to be the most popular ART used in these areas, despite notable developments in MOET and ET technologies. ET will need to be as simple to implement and reliable to use as AI in order for it to be widely implemented in developing nations [98].

Most livestock systems in Africa have not yet gained as much from ARTs as industrialised nations have, especially when it comes to selective breeding strategies and genetic improvement. There aren't many instances of sustainable breeding programs in Africa currently and only certain livestock industries use reproductive technology like AI. The small scale of the dairy farms in these countries, along with infrastructure, government policy and management that is oftentimes inadequate, likely contribute to this lack [99]. However, decision-makers are becoming more aware of the potential of genetic improvement to boost livestock productivity and many African nations have now specifically included genetic improvement in their national livestock development strategies [100].

In locations such as Asia and Africa, most farmers operate smallholder systems, with smaller herd sizes. These farms rarely record their pedigrees or genetic lineages and as a result, they are at the disadvantage of not having access to systems of genetic evaluation [101]. In underdeveloped nations like Brazil, breed groups or organisations handle the majority of genotyping work [102]. Alternatively, the genotyping is undertaken as part of a project, such as the 'African Dairy Genetic Gains Cattle' or the 'East Africa Dairy Development' project [103]. Numerous and varied, African livestock varieties are generally well-suited to the challenging environmental circumstances in which they function. For millennia, they have been utilised to ensure food and nutritional security as well as livelihoods. In these developing nations, the demand for foods derived from animal origin is rising quickly. For instance, it is predicted that the demand for milk in these countries is set to rise by approximately 136% by the year 2030. Significant increases in animal production in these developing nations will be necessary to guarantee that this demand is satisfied, which represents an opportunity for the implementation of ET [104].

Future developments in ET technology offer promising prospects for improving dairy production in developing countries. ET and cryopreservation are two innovations that can serve to enhance genetic quality and provide access to superior embryos, which could boost milk output and help small-scale farmers to ensure their food security. Policy makers in these regions are beginning to recognise just how crucial genetic enhancement is to bovine productivity, opening the door to the wider rollout of ET in the future.

3.11.3 The use of CRISPR Technologies in Combination with Bovine ET

CRISPR is a commonly used abbreviation for CRISPR-Cas9. The name refers to specific segments of DNA. Cas9 is a protein that can function as a type of molecular scissors, to cut strands of DNA. CRISPR stands for 'clustered regularly interspaced palindromic repeats.' It enables the quick, easy, and affordable correction of genomic mistakes as well as the on or off switching of genes in the organisms and cells of individuals. Among its many scientific uses are the quick creation of animal and cellular models, functional genomic assays, and real-time cellular genome imaging [105]. It emerged from a naturally occurring defence mechanism of bacteria against viruses and its essential elements include Cas9 and RNA [106].

New directions in animal genetic engineering have been made possible by the combination of CRISPR technology and bovine ET. This combination makes it possible to precisely alter genes, boosting desired characteristics and raising the general calibre of the herd. Due to its capacity to create precise, targeted modifications to the genome, CRISPR/Cas9 has completely transformed the field of genetic engineering. In bovine breeding, ET is a tried-and-true method for spreading desired genetic features. However, researchers can also create genetically altered embryos by fusing ET and CRISPR. These embryos are subsequently inserted into recipient cows. In addition to speeding up the breeding process, this technique guarantees that particular genetic alterations will be passed down through the generations in a dairy herd. This technology has been applied to livestock to provide advantageous qualities. One example of this application includes improved livestock welfare, where CRISPR has been used to create genetic changes that increase cows' resistance to stress and heat. These modifications improve the welfare and production of cattle by enabling them to better respond to environmental challenges. The introduction of traits that provide protection against and resistance to certain illnesses like Bovine TB or mastitis also improves animal health and serves to lessen the need for antibiotic usage on dairy farms. Improved productivity characteristics can also be achieved, for instance, through the modification of certain genes associated with milk production characteristics [107].

By concentrating on the use of CRISPR in non-humans, the field of ethical discussion has undergone and will continue to undergo substantial changes. Recognising the reality that many CRISPR alterations are not transgenic, for example, has significant ramifications for the ethical discussions, especially given the widely held belief by many individuals and authorities that genetic engineering is unethical. The many effects of CRISPR on the wellbeing of animals indicates that the increased accuracy which it boasts, leads to less unexpected welfare issues, when compared to earlier methods. This increased adaptability suggests that in the future, a greater number of animals will be genetically modified than ever before [108].

An 'Advanced Therapies Journal' article has listed a number of CRISPR/Cas9 biotechnology concerns. Complications in which unwanted portions of the genome are altered and have unanticipated repercussions, are a significant worry. This impreciseness may lead to mutations that affect vital genes or have negative consequences. Furthermore, a major obstacle is the immune reaction brought on by the body's exposure to CRISPR elements, which may result in rejection of the modified cells or inflammation. Additionally, ethical issues are quite important, especially when it comes to the long-term effects of genetic alterations. These difficulties show how much more study is required to enhance the safety and ethical standards pertaining to CRISPR technology [109].

CRISPR has the potential to revolutionise dairy farming by accelerating selective breeding and facilitating the integration of coveted traits such as disease resistance and enhanced milk production. Yet, there are also significant safety and ethical issues with this technique, especially in relation to immunological reactions and unexpected genetic changes. A significant advancement in the realm of reproductive technologies, the combination of CRISPR technology and bovine ET holds great promise for the future of genetic engineering in dairy farming by precisely modifying genetic features that enhance cattle welfare, production, and health. As we move forward, ethical considerations must be re-evaluated, factoring in non-transgenic modifications to find a suitable balance between utilising CRISPR for reproductive improvements, animal welfare concerns, and societal obligations. Further investigation will be crucial to address these challenges and guarantee the safe and responsible use of CRISPR in animal breeding.

3.12 Research Gaps

Although bovine ET research has made great strides in recent years, there are still a number of limitations that prevent the procedure from reaching its full potential and several gaps still remain in the research surrounding ET in dairy cows.

3.12.1 Improving the Success Rates of IVP

It is evident an important component of ET is the in vitro production of embryos. Nevertheless, these embryos frequently have lower pregnancy rates than in vivo-produced embryos. To better replicate the in vivo environment, more study is needed to optimise culture parameters, such as medium composition, incubation durations and oxygen content. Enhancing IVP embryos' developmental competency and lowering the rate of early embryonic loss following transfer should be the main objectives of future research [110].

3.12.2 The Reliability and Success of Superovulation Protocols

Each individual dairy cow can respond differently to superovulation therapies, which results in varying quantities of suitable embryos. To create more reliable, regular, and economical procedures specifically for dairy cows, further research is required to comprehend the hormonal and genetic elements affecting superovulation. Investigating novel medications or combination therapies that enhance oocyte yield and quality but avoid excessively stimulating the ovaries is part of this [111]. Further study is also required to investigate the effects of previous superovulation on dairy heifer fertility, since some research has highlighted that those heifers which underwent two superovulations produced more complete and viable embryos than those that only underwent one. Though they were inseminated later than the other animals in their herd, heifers that were superovulated twice had comparable reproductive outcomes in terms of conception rates and services per pregnancy [112].

3.12.3 Cryopreservation Techniques for Dairy Embryos

Despite advancements, there are still issues with post-thawing viability and developmental capacity when it comes to cryopreserving dairy embryos, especially those created by IVP. Cryopreservation methods (such as slow freezing vs. vitrification) need to be improved, and procedures that preserve the survival of dairy embryos following storage must be created. It is essential to have a better grasp of the causes of cryo-damage and how to lessen its impacts. To improve the cryopreservation results of IVP bovine embryos, for example, one study investigates the use of fibroblast growth factor 2 (FGF2), leukaemia inhibitory factor (LIF), and insulin-like growth factor 1 (IGF1). The integrity of the cytoskeleton, the development of the embryos to the blastocyst stage, and the survival rates after thawing were all considerably enhanced by the researchers' use of such cytokines throughout oocyte development and embryo culture. Following gradual freezing, the incorporation of these substances reduced cell apoptosis and the amount of lipids in the blastocysts, thus, indicating a feasible strategy to lessen cryopreservation damage and enhance embryo survival [113].

Another publication deals with the molecular changes occurring during cryopreservation of embryos or gametes. It is pointed out that freezing can cause serious cell damage because the formation of ice crystals, especially in larger cells like oocytes, can be highly destructive. The article also discusses how the process of freezing and epigenetic modifications brought on by cryoprotective chemicals can impact the survival of cells along with potential for development. Establishing more efficient cryopreservation procedures which aim to reduce harm and preserve the integrity of embryos requires a deeper knowledge of these molecular alterations and, in turn, additional research in this particular field [114].

3.12.4 Welfare and Health Implications for Donors and Recipients

Little concrete information is known regarding how donor cows are affected by recurrent superovulation and embryo retrieval, as well as how recipient cows are impacted by embryo transfer procedures. Research is required to determine how ET affects the long-term health and wellbeing of recipient and donor cows. This entails evaluating the operations' physical stress and the recuperation period, as well as any possible effects on metabolism or reproduction. Although superovulation can result in the production of more high-quality embryos, some researchers have discovered that it also carries dangers, including ovarian hyperstimulation syndrome (OHSS) and related reproductive problems. The study emphasises the necessity of well-balanced procedures that optimise embryo output while lowering health hazards. It also highlights how crucial it is to keep an eye on the metabolic health of these animals because recurrent hormone therapies can result in metabolic abnormalities [115].

The welfare effects of ET procedures on recipient cows are also examined in another paper. It discusses how the handling and specialised procedures involved in the ET process cause physical stress to the animals involved. The post-transfer healing phase and the possibility of heightened vulnerability to infection are problems that are also investigated in this study. To increase the welfare standards for recipient cows, the authors of this particular paper advocate for better handling practices and follow-up care [116]. All of this highlights how important it is to consider the immediate as well as long-term health and wellbeing of donor and recipient cows involved in ET.

3.12.5 Long Term Genetic Effects

The long-term genetic and epigenetic effects of altering dairy cow embryos, particularly through in vitro procedures and superovulation, are unknown. These could have an impact on the offspring's health, milk production, and fertility. More research is needed to determine whether manipulating embryos can result in epigenetic changes and how such changes might affect future generations. This covers research on the health, productivity, and reproductive abilities of the progeny throughout their lives.

Epigenetic remodelling during embryonic differentiation has been shown by research to impact the transmission of genetic characteristics to offspring, influencing factors including reproductive capacity and milk supply. In order to enhance dairy cow breeding methods, the studies highlight the necessity of a deeper comprehension and recognition of epigenetic biomarkers [117]. The manipulation of dairy cow embryos can have unexpected genetic and epigenetic effects. Superovulation and in vitro manipulation can result in epigenetic modifications that could alter the phenotype of the progeny. More thorough research is needed to comprehend these impacts and create procedures that reduce adverse effects [118].

The health and production of the bovines involved may be impacted throughout their lives by the inheritable responses to stress brought on by embryonic interventions. Existing studies emphasise how crucial it is to carefully evaluate the stressors associated with embryo manipulations in order to protect future generations' welfare and productivity [119].

3.12.6 Costs and Accessibility

Many dairy producers, particularly those with small or medium-sized farms, are unable to acquire ET due to its high cost, especially IVP-ET. To make ET a more attractive alternative for a wider variety of dairy farmers, research into more cost-effective methods is required. This includes technological advancements that lower the procedure's labour, time, and medication expenses [120].

3.12.7 Environmental Sustainability and Milk Quality

Few researchers have examined how ET affects milk quality, such as protein and fat content, or its other possible consequences in relation to environmental sustainability, despite the fact that ET strives to increase genetic features related to milk productivity. Further studies are required to assess the environmental impact of ET-assisted reproduction in terms of resource consumption, GHG emissions, and the long-term viability of dairy farming, as well as to determine how ET can be utilised to improve milk quality features in addition to yield in a cost-effective manner [121].

4. Materials and Methods

4.1 Search Strategies

Searches of various databases were performed to review the literature related to the topic. Some of the databases used include PubMed and Google Scholar. Keywords included "in vitro fertilisation," "dairy cows," "bovine embryo transfer," and "genetic improvement," among many more combined in various ways. Journal articles and scientific papers were the main focus of the search, which was limited to studies published between 2000 and 2024.

4.1.1 Screening and Selection of Research

The initial search for scientific papers relating to this thesis yielded in excess of 150 relevant results. Further reviews of these papers were then conducted, which then resulted in the inclusion of 121 papers for the final analysis.

4.1.2 Limitations of Research

Only English-language publications were included in the review and a small number of potentially relevant research papers were unavailable pursuant to subscription restrictions.

4.2 Field Observation of Human ART Practices

An in-person visit to a Hungarian, human ART lab was also conducted by the author on the 13th of June 2024. The aim of this visit was to gain firsthand knowledge and a practical grasp of methods and procedures that might be analogous or applicable to bovine ET procedures.

5. Results

5.1 Clearer Understanding of ART Technologies

While visiting a human ART lab, the author had the chance to witness the application of cutting-edge reproductive technologies, such as IVF, cryopreservation, and oocyte retrieval. The technologies utilised there and those used in bovine ET share many similarities. The practical experience improved the comprehension of the technical facets and difficulties associated with these processes. It is challenging to fully understand the procedures, equipment, and techniques associated with ET from reviewing the literature alone, therefore, this exposure served to enhance the authors' insight into such.

5.2 Species Comparisons

Although the objectives of human ART and bovine ET are similar (a healthy offspring and successful embryo implantation), the precise methods, timelines, and biological considerations of each undoubtedly vary. Observing the operation of a human ART lab resulted in a recognition of the need to contrast these methods and evaluate whether specific techniques or innovations utilised in human ART may be refined or modified for dairy cows.

5.3 Ethics and Regulatory Standards

Human ART laboratories are subject to stringent legal and ethical requirements. Examining these procedures may help shed light on how related ethical issues, like welfare and the long-term effects of reproductive technologies, might be handled in relation to bovine ET. This reproduction clinic is governed the Hungarian healthcare laws and the regulations of ART procedures and legal aspects of gamete and embryo cryopreservation.

5.4 Studying Laboratory Standards and Procedures

To guarantee the effectiveness of reproductive treatments, the ART lab works in a carefully regulated setting with strict quality control processes. The author was able to watch these procedures, such as sample handling, sterilisation, and quality control measures in action during the in-person visit, which may have an influence on preserving the uniformity and the success rates of bovine ET. Understanding these procedures may be helpful in identifying best practices that could be implemented or altered for laboratories working with bovines, hence increasing productivity and decreasing errors.

5.5 Establishing a Practical Perspective

Seeing lab processes carried out in person may offer a greater degree of practical insight in comparison to reading about them in the literature alone. The author was able to observe the workflow, problem-solving techniques, and real-time decision-making that takes place throughout the intricate reproductive procedures involved in ET, by visiting the human ART lab. This practical expertise can help to grasp the technical difficulties and procedural intricacies in the studies examined in the literature on ET in dairy cows, offering depth to the examination of the methodology utilised in bovine ET research.

5.6 Insights from Experts

The opportunity to engage with professionals and specialists in the subject was provided by the visit. The embryologists answered specific questions, shared their knowledge, and made recommendations that were not readily accessible in the published literature on bovine ET.

Figure 1.

Inverted Microscope – For micromanipulation techniques such as intracytoplasmic sperm injection (ICSI)

Figure 2.

Insulated Container- For the transportation of biological materials, including cryopreserved samples, embryos, and semen straws, insulated containers such as this are used to maintain a controlled temperature in both human and bovine ART labs.

5.7 Success Rates of ET in Dairy Cows

The reviewed literature indicates that ET's success rates in dairy cows have varied, particularly when combined with innovative biotechnologies like IVP. Studies comparing in-vitro versus in vivo-produced embryos typically reveal lower pregnancy rates with IVP embryos because of the challenges with embryo growth and implantation. However, the post-thawing survivability has been greatly improved by advancements in cryopreservation methods, particularly vitrification, which has raised pregnancy rates.

5.8 Current Trends in ET Technologies

Innovative technologies like IVP, genetic selection, and enhanced cryopreservation techniques have greatly revolutionised the utilisation of ET in dairy cows. IVP-ET is becoming more and more popular, especially since IVP embryos are more likely than standard procedure embryos to reach the blastocyst stage. The increasing use of precision breeding methods, which allow for more precise genetic trait selection, is another factor propelling this trend. Notwithstanding these developments, IVP-ET still has problems, such as lower pregnancy rates than in vivo-produced embryos, which suggests that culture conditions and embryo handling procedures still need to be researched further and improved.

Studies show that improved post-thaw survival rates of frozen embryos are made possible by vitrification, a fast-freezing cryopreservation method, which produces more reliable pregnancy results. The increasing trend towards single-embryo transfers, which lowers the hazards of multiple pregnancies such dystocia and decreased productivity, is also supported by this change. The capacity to store and export embryos globally through the use of embryo banks has significantly extended the reach and effect of these technologies, making it easier for dairy farmers to acquire superior genetics across geographies.

5.9 Future Possibilities

Future possibilities for improving the accuracy of genetic alterations in dairy cows include the CRISPR-Cas9 gene-editing technique. CRISPR has the ability to more quickly introduce traits like disease resistance, heat tolerance, and increased productivity into herds when paired with ET. However, safety issues like immunological responses and unexpected genetic alterations continue to be problems that require further study.

Simultaneously, by enhancing reproductive control, artificial intelligence is becoming more popular in dairy production. In order to increase conception rates and efficiency, farmers are using AI-driven systems for oestrus detection and fertility prediction to optimise the time of embryo transfers. By providing more accurate insights into reproductive health, the integration of AI into ET programs has the potential to completely transform dairy herd management.

6. Discussion

This thesis highlights ET's potential within the dairy industry by examining its present trends and prospective future applications in dairy cows. ET has become a vital tool for expediting genetic improvement, enhancing productivity, and guaranteeing herd sustainability as the demand for dairy products rises globally due to population expansion and the mounting effects of climate change.

The practice of selective breeding has been transformed by recent developments in ET technology, especially the incorporation of genomic selection and improvements in cryopreservation methods. Desired features including improved disease resistance, higher milk yield, and resilience to environmental stressors like heat can be precisely targeted through genomic selection. Advances in embryo handling, such as the use of vitrification, which has greatly increased embryo survival rates after thawing, have contributed to the success of these technologies. These developments demonstrate how ET is being continuously modified to satisfy the productivity and sustainability requirements of contemporary dairy production as it gains broader use throughout the globe.

ET is becoming a more dependable technique for dairy farmers as a result of fixed-time embryo transfer, which synchronises the hormonal cycles of donor and recipient cows and further increases conception rates. Since single embryo transfers lessen the dangers involved with multiple pregnancies, they also support important, welfare-driven objectives. ET is proving to be extremely helpful in resolving particular reproductive issues in dairy cattle. ET can be utilised to improve fertility results and herd health, as evidenced by its usage in repeat breeder cows and its function in preventing twin conceptions. This is in line with current developments in the sector that optimise reproduction technology for welfare as well as efficiency and productivity.

Notwithstanding these achievements, economic factors continue to play a significant role in determining the further adoption of ET, especially for small-scale farmers. Even as the technology advances, the expenses of hormone treatments, embryo transfer procedures, and superovulation regimens can be unaffordable for some dairy enterprises, particularly those operating on a smaller scale. However, farms that use ET in conjunction with precision breeding methods have demonstrated that these initial expenses can result in higher milk production and better genetic improvements over the long run. The continuous trend of using ET in high-production systems where efficiency is essential is shown in the cost-benefit balance of these enterprises.

Evidently, ET's potential in the future shows significant promise when combined with technologies like artificial intelligence and CRISPR-Cas9 gene editing. Breeding dairy cows with characteristics like improved heat tolerance and lower methane emissions is made possible by CRISPR, which enables precise genetic modifications. This could potentially address issues related to productivity and environmental protection, especially in light of the effects of climate change and the role that dairy farming plays in that narrative. Artificial intelligence, on the other hand, has the potential to completely transform the management of reproductive health by increasing the precision of oestrus detection and maximising the timing of ET, which would serve to improve overall herd efficiency and conception rates.

ET has an encouraging future, but there are still obstacles to overcome. More research is still needed to determine the trustworthiness of IVP embryos, which often have lower pregnancy rates than in-vivo produced embryos. As technology develops, issues surrounding the welfare of donor and recipient cows, especially those subjected to repeated superovulation and ET's, must also be considered. To guarantee responsible and informed implementation of ET going forward, the ethical ramifications of widespread genetic manipulation, particularly with new technologies like CRISPR, will need to be investigated thoroughly.

The dairy sector is undergoing changes as a result of the current ET trends, which enable quicker genetic improvements and increase herd resilience against economic and environmental stresses. ET may continue to play a significant role in the development of dairy farming, as evidenced by the revolutionary potential of these prospects, particularly those pertaining to gene editing and the incorporation of artificial intelligence. However, in order to fully realise these potentials, further research is needed to increase success rates, remove financial obstacles, and make sure that ethical and animal welfare issues are taken into account when implementing these advancements.

The visit to a human ART lab by the author provided invaluable insights into the similarities between human and bovine reproductive technologies. However, it equally highlighted numerous advanced techniques that could be implemented to enhance bovine ET practices. By incorporating sophisticated tools such as time-lapse embryo monitoring and the strict quality control methods from human ART labs like temperature and pH monitoring for embryo culture environments, the field of bovine ET could make significant progress in efficiency, accuracy, and overall success.

7. Conclusion

With major benefits for genetic improvement, herd productivity, and sustainability, ET in dairy cows has become a key tool in the dairy farming sector of today. As demonstrated in this thesis, recent developments in ET technology, including improvements in cryopreservation, genomic selection, and superovulation techniques, have increased the extent of genetic progress in dairy herds while simultaneously enhancing the effectiveness of reproductive management. These advancements make it possible to select for features that improve animal welfare, production, and adaptability with greater precision.

New technologies like artificial intelligence and CRISPR-Cas9 gene editing are strongly correlated with ET's potential going forward. In order to improve the endurance of dairy farming in the face of climate change, for example, these advancements have the potential to drastically alter the dairy industry by making it possible to precisely select and modify desirable genetic traits like reduced methane emissions and increased heat tolerance. The promise of technological and biotechnological advancements in dairy production is further indicated by the role of artificial intelligence in improving reproductive control and timing ET protocols.

Even given these developments, there are still persistent obstacles to the broader use of ET, especially in smaller-scale dairy farms, where the viability of such technologies from an economic standpoint is sometimes questioned. Even though hormonal therapies and sophisticated embryo handling methods have long-term biological and economic benefits, their costs can be unsustainable for smaller farms. However, ET's significance in herd management, especially in high-production systems, is reinforced by its ability to address certain reproductive difficulties, such as enhancing conception rates among repeat breeder dairy cows.

The existing gaps in both the practice and research of ET in dairy cows must be recognised and overcome. Careful consideration must be given to the ethical ramifications of genetic changes, the welfare issues raised by repeated superovulation and ET, and the dependability of in vitro-produced embryos. It will be essential to address these issues if ET is to continue to be sustainable, and profitable in the future.

In conclusion, ET in dairy cows is at the nexus of crucial scientific development and practical implementation. Its transforming effect and increasing significance in attaining genetic advancement in dairy herds are shown in the current trends of its application. There are tremendous opportunities and future possibilities to transform the dairy sector through the integration of ET with gene editing and artificial intelligence technology. ET will continue to be a vital component of modern dairy production, promoting productivity, sustainability, and resilience in the years to come, with careful study, ethical analysis, and strategic implementation.

8. Summary

This thesis critically examines the current trends and future possibilities of ET technology in dairy cows, with a focus on the role it plays in genetic improvement, productivity enhancement, and sustainable dairy farming practices. It explores advancements in areas such as vitrification, genomic selection, and superovulation protocols, which have broadened the possibilities, increased the efficiency and reliability of ET. Possibilities for the implementation of ET include addressing fertility issues in repeat breeders, mitigating the effects of environmental stress, and preventing twin pregnancies with the aim of improving cow welfare. The future possibilities investigated in this thesis highlighted the integration with Artificial Intelligence for precision reproductive management and CRISPR-Cas9 gene editing for traits like heat tolerance and reduced methane emissions, which addresses climate change challenges. Challenges remain, however, including lower success rates for in-vitro-produced embryos, ethical concerns around animal welfare and genetic manipulation, and the financial accessibility of ET services for small-scale dairy farmers. The research emphasises the transformative potential of ET in modern dairy farming while underscoring the need for ethical, technical, and economic considerations to ensure that it can be adopted sustainably.

Bibliography

1. Mebratu B, Fesseha H, Goa E (2020) Embryo Transfer in Cattle Production and Its Principle and Applications. 7:40–54

2. Sirard M-A (2018) 40 years of bovine IVF in the new genomic selection context. https://doi.org/10.1530/REP-18-0008

3. Hansen PJ, Block J (2003) Towards an embryocentric world: the current and potential uses of embryo technologies in dairy production. Reprod Fertil Dev 16:1–14. https://doi.org/10.1071/RD03073

4. Mapletoft RJ (2018) History and perspectives on bovine embryo transfer. Hist Perspect Bov Embryo Transf 10:168–173

5. Jelani G, Kalwar Q, Kaka A, Channo A, Ahmed A, Soomro M, Soomro H, Bhuptani D, Jalbani Y (2022) Historical Background and Significance of Embryo Transfer Technology in Cattle with its Relevant Applications. Pak J Zool 55:. https://doi.org/10.17582/journal.pjz/20220403060427

6. Hasler JF (2014) Forty years of embryo transfer in cattle: A review focusing on the journal *Theriogenology*, the growth of the industry in North America, and personal reminisces. Theriogenology 81:152–169. https://doi.org/10.1016/j.theriogenology.2013.09.010

7. Moore SG, Hasler JF (2017) *A 100-Year Review:* Reproductive technologies in dairy science. J Dairy Sci 100:10314–10331. https://doi.org/10.3168/jds.2017-13138

8. Phillips PE, Jahnke MM (2016) Embryo Transfer (Techniques, Donors, and Recipients). Vet Clin North Am Food Anim Pract 32:365–385. https://doi.org/10.1016/j.cvfa.2016.01.008

9. Curtis JL (1992) Cattle embryo transfer procedure: an instructional manual for the rancher, dairyman, artificial insemination technician, animal scientist, and veterinarian. Academic Press, San Diego, Calif. u.a

10. Santos JEP, Cerri RLA, Sartori R (2008) Nutritional management of the donor cow. Theriogenology 69:88–97. https://doi.org/10.1016/j.theriogenology.2007.09.010

11. Baruselli PS, de Sá Filho MF, Martins CM, Nasser LF, Nogueira MFG, Barros CM, Bó GA (2006) Superovulation and embryo transfer in *Bos indicus* cattle. Theriogenology 65:77–88. https://doi.org/10.1016/j.theriogenology.2005.10.006

12. Isnaini N, Febrianty SA, Andri F, Edwar E, Darlian F (2024) Effects of Age and Season on in vivo Embryo Production of Friesian Holstein Cows. Am J Anim Vet Sci 19:58–62. https://doi.org/10.3844/ajavsp.2024.58.62

13. Nowicki A (2021) Sciendo. J Vet Res 65:231–237. https://doi.org/10.2478/jvetres-2021-0018

14. McDermott K (2012) American Embryo Transfer Association

15. Baruselli PS, de Sá Filho MF, Martins CM, Nasser LF, Nogueira MFG, Barros CM, Bó GA (2006) Superovulation and embryo transfer in *Bos indicus* cattle. Theriogenology 65:77–88. https://doi.org/10.1016/j.theriogenology.2005.10.006

16. Wolstenholme GEW, O'Connor M (2009) Foetal Autonomy. John Wiley & Sons

17. Dochi O, Takahashi K, Hirai T, Hayakawa H, Tanisawa M, Yamamoto Y, Koyama H (2008) The use of embryo transfer to produce pregnancies in repeat-breeding dairy cattle. Theriogenology 69:124–128. https://doi.org/10.1016/j.theriogenology.2007.09.001

18. Madureira AML, Burnett TA, Marques JCS, Moore AL, Borchardt S, Heuwieser W, Guida TG, Vasconcelos JLM, Baes CF, Cerri RLA (2022) Occurrence and greater intensity of estrus in recipient lactating dairy cows improve pregnancy per embryo transfer. J Dairy Sci 105:877–888. https://doi.org/10.3168/jds.2021-20437

19. Transfer of a single embryo versus drainage of subordinate follicles to prevent twin pregnancies in dairy cows. Why not both? https://www.jstage.jst.go.jp/article/jrd/66/4/66_2020-014/_article. Accessed 5 Oct 2024

20. Azzi MSE, Cardoso JL, Landeo RA, Pontes JHF, Souza JC de, Martins JPN (2023) Effect of inducing accessory corpus luteum formation with gonadotropin-releasing hormone or human chorionic gonadotropin on the day of embryo transfer on fertility of recipient dairy heifers and lactating cows. JDS Commun 4:155–160. https://doi.org/10.3168/jdsc.2022-0286

21. Martínez JF, Galina CS, Rojas J, Vargas B, Romero-Zúñiga JJ (2023) Comparative productive performance of cows born through embryo transfer, artificial insemination and natural mating in dairy and dual-purpose herds raised in tropical conditions. Reprod Domest Anim 58:1104–1113. https://doi.org/10.1111/rda.14409

22. Wang Y, Chen D, Cai B, Huang D, Xu Y, Ding C (2022) Effects of Repeated Cryopreservation of Cleavage-Stage Embryos on Pregnancy and Perinatal Outcomes of Single Embryo Transfer. Clin Exp Obstet Gynecol 49:244. https://doi.org/10.31083/j.ceog4911244

23. Zheng X, Chen Y, Yan J, Wu Y, Zhuang X, Lin S, Zhu J, Lian Y, Qiao J, Liu P (2017) Effect of repeated cryopreservation on human embryo developmental potential. Reprod Biomed Online 35:627–632. https://doi.org/10.1016/j.rbmo.2017.08.016

24. Wang X, Wu H, He X, Jiang H, Wu L, Xu Y, Zhou P, Wei Z, Cao Y (2018) Retrospective Study to Compare Frozen-Thawed Embryo Transfer with Fresh Embryo Transfer on Pregnancy Outcome Following Intracytoplasmic Sperm Injection for Male Infertility. Med Sci Monit 24:2668–2674. https://doi.org/10.12659/MSM.907229

25. Do VH, Taylor-Robinson AW (2020) Cryopreservation of in vitro-produced bovine embryos by vitrification: In pursuit of a simplified, standardized procedure that improves pregnancy rates to promote cattle industry use. Biotechnol Anim Husb 36:251–270

26. Marinho L, Sanches B, Rosa C, Tannura J, Rigo A, Basso A, Pontes J, Seneda M (2015) Pregnancy Rates to Fixed Embryo Transfer of Vitrified IVP Bos indicus, Bos taurus or Bos indicus × Bos taurus Embryos. Reprod Domest Anim 50:807–811. https://doi.org/10.1111/rda.12591

27. Rienzi L, Gracia C, Maggiulli R, LaBarbera AR, Kaser DJ, Ubaldi FM, Vanderpoel S, Racowsky C (2017) Oocyte, embryo and blastocyst cryopreservation in ART: systematic review and meta-analysis comparing slow-freezing versus vitrification to produce evidence for the development of global guidance. Hum Reprod Update 23:139–155. https://doi.org/10.1093/humupd/dmw038

28. Raya YSA, Bilgory A, Aslih N, Atzmon Y, Shavit M, Estrada D, Sharqawi M, Shalom-Paz E (2023) High initial β-hCG predicts IVF outcomes accurately and precludes the need for repeated measurements. https://doi.org/10.1530/EC-23-0189

29. Do VH, Taylor-Robinson AW (2020) Cryopreservation of in vitro-produced bovine embryos by vitrification: In pursuit of a simplified, standardized procedure that improves pregnancy rates to promote cattle industry use. Biotechnol Anim Husb 36:251–270

30. Mogas T (2019) Update on the vitrification of bovine oocytes and in vitro-produced embryos. Reprod Fertil Dev 31:105–117. https://doi.org/10.1071/RD18345

31. Fujii T, Naito A, Moriyasu S, Kageyama S (2021) Potential of preimplantation genomic selection using the blastomere separation technique in bovine in vitro fertilized embryos. J Reprod Dev 67:155–159. https://doi.org/10.1262/jrd.2020-153

32. Silvestri G, Canedo-Ribeiro C, Serrano-Albal M, Labrecque R, Blondin P, Larmer SG, Marras G, Tutt DAR, Handyside AH, Farré M, Sinclair KD, Griffin DK (2021) Preimplantation Genetic Testing for Aneuploidy Improves Live Birth Rates with In Vitro Produced Bovine Embryos: A Blind Retrospective Study. Cells 10:2284. https://doi.org/10.3390/cells10092284

33. VanRaden PM (2020) Symposium review: How to implement genomic selection. J Dairy Sci 103:5291–5301. https://doi.org/10.3168/jds.2019-17684

34. Oliveira CS, Camargo LSA, Silva MVGB da, Saraiva NZ, Quintão CC, Machado MA (2023) Embryo biopsies for genomic selection in tropical dairy cattle. Anim Reprod 20:e20230064. https://doi.org/10.1590/1984-3143-AR2023-0064

35. Quang TT, Pham TD, Nguyen NT, Vinh T, Pham QD, Pham MC, Nguyen NTT, Tran HY, Nguyen VT, Le TM (2021) Rapid sex determination of preimplantation bovine embryo using direct-PCR from a single blastomere. Sci Technol Dev J 24:2077–2083. https://doi.org/10.32508/stdj.v24i3.2576

36. Magata F (2023) Time-lapse monitoring technologies for the selection of bovine in vitro fertilized embryos with high implantation potential. J Reprod Dev 69:57–64. https://doi.org/10.1262/jrd.2022-131

37. López-Gatius F, Saleri R, De Rensis F, Llobera-Balcells M, Garcia-Ispierto I (2022) Transfer of a single fresh in vitro-produced embryo may prevent twin pregnancy without compromising the fertility of the cow. Reprod Domest Anim 57:450–455. https://doi.org/10.1111/rda.14079

38. Ricci A, Li M, Fricke PM, Cabrera VE (2020) Short communication: Economic impact among 7 reproductive programs for lactating dairy cows, including a sensitivity analysis of the cost of hormonal treatments. J Dairy Sci 103:5654–5661. https://doi.org/10.3168/jds.2019-17658

39. Kaniyamattam K, Block J, Hansen PJ, De Vries A (2017) Comparison between an exclusive in vitro–produced embryo transfer system and artificial insemination for genetic, technical, and financial herd performance. J Dairy Sci 100:5729–5745. https://doi.org/10.3168/jds.2016-11979

40. E. S. Ribeiro KNG (2018) Economic aspects of applying reproductive technologies to dairy herds. Econ Asp Appl Reprod Technol Dairy Herds 9:370–387

41. Funeshima N, Noguchi T, Onizawa Y, Yaginuma H, Miyamura M, Tsuchiya H, Iwata H, Kuwayama T, Hamano S, Shirasuna K (2019) The transfer of parthenogenetic embryos following artificial insemination in cows can enhance pregnancy recognition via the secretion of interferon tau. J Reprod Dev 65:443–450. https://doi.org/10.1262/jrd.2019-026

42. Deb GK, Miraz MFH, Kabir MA, Nahar TN, Hossain SMJ, Ali MS (2024) Birth of First In vitro Produced Calves in Bangladesh and Their Reproductive Fitness. Eur J Vet Med 4:4–7. https://doi.org/10.24018/ejvetmed.2024.4.2.122

43. Rodríguez V, Sinchipa OA, Cárdenas FAR, Escobar KV (2024) In Vitro Embryo Production: Overview. Evol Stud IMAGINATIVE Cult 1160–1179. https://doi.org/10.70082/esiculture.vi.1348

44. Neuregulin 1 (NRG1) modulates oocyte nuclear maturation during IVM and improves post-IVF embryo development - ScienceDirect. https://www.sciencedirect.com/science/article/pii/S0093691X22004551 . Accessed 7 Oct 2024

45. Dode MAN, Caixeta FMC, Vargas LN, Leme LO, Kawamoto TS, Fidelis AAG, Franco MM (2023) Genome transfer technique for bovine embryo production using the metaphase plate and polar body. J Assist Reprod Genet 40:943–951. https://doi.org/10.1007/s10815-023-02758-3

46. Carrillo-González DF, Hernández-Herrera DY, Maldonado-Estrada JG (2023) The role of L-carnitine in bovine embryo metabolism. A review of the effect of supplementation with a metabolic modulator on in vitro embryo production. Anim Biotechnol 34:413–423. https://doi.org/10.1080/10495398.2021.1938593

47. Grycmacher K, Boruszewska D, Sinderewicz E, Kowalczyk-Zięba I, Staszkiewicz-Chodor J, Woclawek-Potocka I (2019) Prostaglandin F2α (PGF2α) production possibility and its receptors expression in the early- and late-cleaved preimplantation bovine embryos. BMC Vet Res 15:203. https://doi.org/10.1186/s12917-019-1939-0

48. Boruszewska D, Kowalczyk-Zieba I, Suwik K, Staszkiewicz-Chodor J, Jaworska J, Lukaszuk K, Woclawek-Potocka I (2020) Prostaglandin E2 affects in vitro maturation of bovine oocytes. Reprod Biol Endocrinol 18:40. https://doi.org/10.1186/s12958-020-00598-9

49. https://www.indianjournals.com/ijor.aspx?target=ijor:ijar1&volume=56&issue=5&article=008

50. Travnickova I, Hulinska P, Kubickova S, Hanzalova K, Kempisty B, Nemcova L, Machatkova M (2021) Production of sexed bovine embryos in vitro can be improved by selection of sperm treatment and co-culture system. Reprod Domest Anim 56:864–871. https://doi.org/10.1111/rda.13926

51. Wang Y, Qi J-J, Yin Y-J, Jiang H, Zhang J-B, Liang S, Yuan B (2023) Ferulic Acid Enhances Oocyte Maturation and the Subsequent Development of Bovine Oocytes. Int J Mol Sci 24:14804. https://doi.org/10.3390/ijms241914804

52. Wang G-F, Dong Q, Bai Y, Yuan J, Xu Q, Cao C, Liu X (2017) Oxidative stress induces mitotic arrest by inhibiting Aurora A-involved mitotic spindle formation. Free Radic Biol Med 103:177–187. https://doi.org/10.1016/j.freeradbiomed.2016.12.031

53. Maternal metabolic status and in-vitro culture conditions during embryonic genome activation deregulate the expression of energy-related genes in the bovine 16-cells embryo | PLOS ONE. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0290689. Accessed 7 Oct 2024

54. Rodriguez-Martinez H, Larsson B, Pertoft H (1997) Evaluation of sperm damage and techniques for sperm clean-up. Reprod Fertil Dev 9:297–308. https://doi.org/10.1071/r96081

55. Parrish JJ, Krogenaes A, Susko-Parrish JL (1995) Effect of bovine sperm separation by either swim-up or Percoll method on success of in vitro fertilization and early embryonic development. Theriogenology 44:859–869. https://doi.org/10.1016/0093-691X(95)00271-9

56. Walters AH, Eyestone WE, Saacke RG, Pearson RE, Gwazdauskas FC (2004) Sperm Morphology and Preparation Method Affect Bovine Embryonic Development. J Androl 25:554–563. https://doi.org/10.1002/j.1939-4640.2004.tb02826.x

57. Metabolism of fatty acids in follicular cells, oocytes, and blastocysts in: Reproduction and Fertility Volume 3 Issue 2 (2022). https://raf.bioscientifica.com/configurable/content/journals$002fraf$002f3$002f2$002fRAF-21-0123.xml?t:ac=journals%24002fraf%24002f3%24002f2%24002fRAF-21-0123.xml. Accessed 7 Oct 2024

58. Reshi QUA, Godakumara K, Ord J, Dissanayake K, Hasan MM, Andronowska A, Heath P, Fazeli A (2023) Spermatozoa, acts as an external cue and alters the cargo and production of the extracellular vesicles derived from oviductal epithelial cells in vitro. J Cell Commun Signal 17:737–755. https://doi.org/10.1007/s12079-022-00715-w

59. Gardner DK (1998) Embryo Development and Culture Techniques. In: Animal Breeding. Routledge

60. Yang Y, Zhou R, Li K (2017) Future livestock breeding: Precision breeding based on multi-omics information and population personalization. J Integr Agric 16:2784–2791. https://doi.org/10.1016/S2095-3119(17)61780-5

61. Berry DP (2015) Breeding the dairy cow of the future: what do we need? Anim Prod Sci 55:823–837. https://doi.org/10.1071/AN14835

62. Calus MPL, Bijma P, Veerkamp RF (2015) Evaluation of genomic selection for replacement strategies using selection index theory. J Dairy Sci 98:6499–6509. https://doi.org/10.3168/jds.2014-9192

63. Eiríksson JH, Byskov K, Su G, Thomasen JR, Christensen OF (2022) Genomic predictions for crossbred dairy cows by combining solutions

from purebred evaluation based on breed origin of alleles. J Dairy Sci 105:5178–5191. https://doi.org/10.3168/jds.2021-21644

64. Nagahara M, Tatemoto S, Ito T, Fujimoto O, Ono T, Taniguchi M, Takagi M, Otoi T (2024) Designing a diagnostic method to predict the optimal artificial insemination timing in cows using artificial intelligence. Front Anim Sci 5:. https://doi.org/10.3389/fanim.2024.1399434

65. Crowe MA, Hostens M, Opsomer G (2018) Reproductive management in dairy cows - the future. Ir Vet J 71:1. https://doi.org/10.1186/s13620-017-0112-y

66. Campanholi SP, Garcia Neto S, Pinheiro GM, Nogueira MFG, Rocha JC, Losano JD de A, Siqueira AFP, Nichi M, Assumpção MEOD, Basso AC, Monteiro FM, Gimenes LU (2023) Can in vitro embryo production be estimated from semen variables in Senepol breed by using artificial intelligence? Front Vet Sci 10:. https://doi.org/10.3389/fvets.2023.1254940

67. Chen F, Hou Y, Zhu X, Mei C, Guo R, Shi Z (2023) Impact of Accessory Corpus Luteum Induced by Gonadotropin-Releasing Hormone or Human Chorionic Gonadotropin on Pregnancy Rates of Dairy Cattle following Embryo Transfer: A META-Analysis. Vet Sci 10:309. https://doi.org/10.3390/vetsci10050309

68. Ventura BA, Keyserlingk MAG von, Wittman H, Weary DM (2016) What Difference Does a Visit Make? Changes in Animal Welfare Perceptions after Interested Citizens Tour a Dairy Farm. PLOS ONE 11:e0154733. https://doi.org/10.1371/journal.pone.0154733

69. Cabrera VE, Fricke PM (2021) Economics of Twin Pregnancies in Dairy Cattle. Animals 11:552. https://doi.org/10.3390/ani11020552

70. Ahsan S, Islam MA, Islam MT (2016) On-farm welfare assessment of dairy cattle by animal-linked parameters in Bangladesh. Res Agric Livest Fish 3:417–424. https://doi.org/10.3329/ralf.v3i3.30733

71. Eastwood C, Klerkx L, Ayre M, Dela Rue B (2019) Managing Socio-Ethical Challenges in the Development of Smart Farming: From a Fragmented to a Comprehensive Approach for Responsible Research and Innovation. J Agric Environ Ethics 32:741–768. https://doi.org/10.1007/s10806-017-9704-5

72. Ahmed A, Tiwari RP, Mishra GK, Jena B, Dar MA, Bhat AA (2015) Effect of Environmental Heat Stress on Reproduction Performance of Dairy Cows- A Review. Int J Livest Res 5:. https://doi.org/10.5455/ijlr.20150421122704

73. Chandra V, Sejian V, Sharma GT (2015) Strategies to Improve Livestock Reproduction Under the Changing Climate Scenario. In:

Sejian V, Gaughan J, Baumgard L, Prasad C (eds) Climate Change Impact on Livestock: Adaptation and Mitigation. Springer India, New Delhi, pp 425–439

74. Nalon E, Stevenson P (2019) Protection of Dairy Cattle in the EU: State of Play and Directions for Policymaking from a Legal and Animal Advocacy Perspective. Animals 9:1066. https://doi.org/10.3390/ani9121066

75. Kaniyamattam K, Block J, Hansen PJ, Vries AD (2018) Economic and genetic performance of various combinations of in vitro-produced embryo transfers and artificial insemination in a dairy herd. J Dairy Sci 101:1540–1553. https://doi.org/10.3168/jds.2017-13475

76. Nehring RF, Gillespie J, Greene C, Law J (2021) The Economics and Productivity of Organic versus Conventional U.S. Dairy Farms. J Agric Appl Econ 53:134–152. https://doi.org/10.1017/aae.2020.34

77. Kalemkeridou M, Nanas I, Moutou K, Amiridis GS, Tsipourlianos A, Dovolou E, Mamuris Z, Giannoulis T (2023) Genetic diversity and thermotolerance in Holstein cows: Pathway analysis and marker development using whole-genome sequencing. Reprod Domest Anim 58:146–157. https://doi.org/10.1111/rda.14274

78. SciELO - Brazil - Applying assisted reproductive technology and reproductive management to reduce CO_2-equivalent emission in dairy and beef cattle: a review Applying assisted reproductive technology and reproductive management to reduce CO_2-equivalent emission in dairy and beef cattle: a review. https://www.scielo.br/j/ar/a/snT5nttZ6KtdCKSZ7VS5KcF/?lang=en. Accessed 8 Oct 2024

79. Sánchez Z, Lammoglia M, Alarcón M, Romero J, Galina C (2015) Is the Production of Embryos in Small-Scale Farming an Economically Feasible Enterprise? Reprod Domest Anim 50:574–579. https://doi.org/10.1111/rda.12526

80. Pachauri RK, Allen MR, Barros VR, Broome J, Cramer W, Christ R, Church JA, Clarke L, Dahe Q, Dasgupta P, Dubash NK, Edenhofer O, Elgizouli I, Field CB, Forster P, Friedlingstein P, Fuglestvedt J, Gomez-Echeverri L, Hallegatte S, Hegerl G, Howden M, Jiang K, Jimenez Cisneroz B, Kattsov V, Lee H, Mach KJ, Marotzke J, Mastrandrea MD, Meyer L, Minx J, Mulugetta Y, O'Brien K, Oppenheimer M, Pereira JJ, Pichs-Madruga R, Plattner G-K, Pörtner H-O, Power SB, Preston B, Ravindranath NH, Reisinger A, Riahi K, Rusticucci M, Scholes R, Seyboth K, Sokona Y, Stavins R, Stocker TF, Tschakert P, van Vuuren D, van Ypserle J-P (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland

81. Rashamol VP, Sejian V, Pragna P, Lees AM, Bagath M, Krishnan G, Gaughan JB (2019) Prediction models, assessment methodologies and biotechnological tools to quantify heat stress response in ruminant livestock. Int J Biometeorol 63:1265–1281. https://doi.org/10.1007/s00484-019-01735-9

82. Liu Z, Ezernieks V, Wang J, Arachchillage NW, Garner JB, Wales WJ, Cocks BG, Rochfort S (2017) Heat Stress in Dairy Cattle Alters Lipid Composition of Milk. Sci Rep 7:961. https://doi.org/10.1038/s41598-017-01120-9

83. Manzanilla C, Vandenplas J, Breukelen A van, Veerkamp R, Gredler-Grandl B (2024) Effect of heat stress on methane emissions of Dutch Holstein cows. Interbull Bull 97–102

84. Bernabucci U, Bani P, Ronchi B, Lacetera N, Nardone A (1999) Influence of Short- and Long-Term Exposure to a Hot Environment on Rumen Passage Rate and Diet Digestibility by Friesian Heifers1. J Dairy Sci 82:967–973. https://doi.org/10.3168/jds.S0022-0302(99)75316-6

85. Dairy cattle in a temperate climate: the effects of weather on milk yield and composition depend on management | animal | Cambridge Core. https://www.cambridge.org/core/journals/animal/article/abs/dairy-cattle-in-a-temperate-climate-the-effects-of-weather-on-milk-yield-and-composition-depend-on-management/12DE4D4A768FC8E383D75C89A1F3D868. Accessed 9 Oct 2024

86. Strandén I, Kantanen J, Russo I-RM, Orozco-terWengel P, Bruford MW (2019) Genomic selection strategies for breeding adaptation and production in dairy cattle under climate change. Heredity 123:307–317. https://doi.org/10.1038/s41437-019-0207-1

87. Berghof TVL, Poppe M, Mulder HA (2019) Opportunities to Improve Resilience in Animal Breeding Programs. Front Genet 9:. https://doi.org/10.3389/fgene.2018.00692

88. Anzures-Olvera F, Véliz FG, de Santiago A, García JE, Mellado J, Macías-Cruz U, Avendaño-Reyes L, Mellado M (2019) The impact of hair coat color on physiological variables, reproductive performance and milk yield of Holstein cows in a hot environment. J Therm Biol 81:82–88. https://doi.org/10.1016/j.jtherbio.2019.02.020

89. Cole JB, Bormann JM, Gill CA, Khatib H, Koltes JE, Maltecca C, Miglior F (2017) BREEDING AND GENETICS SYMPOSIUM: Resilience of livestock to changing environments1. J Anim Sci 95:1777–1779. https://doi.org/10.2527/jas.2017.1402

90. Boettcher P, Viljoen G, Garcia Podesta M (2023) Proceedings of the International Symposium on Sustainable Animal Production and Health: Current status and way forward

91. Bačėninaitė D, Džermeikaitė K, Antanaitis R (2022) Global Warming and Dairy Cattle: How to Control and Reduce Methane Emission. Animals 12:2687. https://doi.org/10.3390/ani12192687

92. Lassen J, Løvendahl P (2016) Heritability estimates for enteric methane emissions from Holstein cattle measured using noninvasive methods. J Dairy Sci 99:1959–1967. https://doi.org/10.3168/jds.2015-10012

93. Garnsworthy PC, Craigon J, Hernandez-Medrano JH, Saunders N (2012) Variation among individual dairy cows in methane measurements made on farm during milking. J Dairy Sci 95:3181–3189. https://doi.org/10.3168/jds.2011-4606

94. Manzanilla-Pech CIV, Løvendahl P, Mansan Gordo D, Difford GF, Pryce JE, Schenkel F, Wegmann S, Miglior F, Chud TC, Moate PJ, Williams SRO, Richardson CM, Stothard P, Lassen J (2021) Breeding for reduced methane emission and feed-efficient Holstein cows: An international response. J Dairy Sci 104:8983–9001. https://doi.org/10.3168/jds.2020-19889

95. Gauly M, Ammer S (2020) Review: Challenges for dairy cow production systems arising from climate changes. animal 14:s196–s203. https://doi.org/10.1017/S1751731119003239

96. Frontiers | Genomic Selection and Use of Molecular Tools in Breeding Programs for Indigenous and Crossbred Cattle in Developing Countries: Current Status and Future Prospects. https://www.frontiersin.org/journals/genetics/articles/10.3389/fgene.2018.00694/full. Accessed 10 Oct 2024

97. Development of genetically improved tropical-adapted dairy cattle | Animal Frontiers | Oxford Academic. https://academic.oup.com/af/article/13/5/7/7311131. Accessed 10 Oct 2024

98. Rodriguez-Martinez H (2012) Assisted Reproductive Techniques for Cattle Breeding in Developing Countries: A Critical Appraisal of Their Value and Limitations. Reprod Domest Anim 47:21–26. https://doi.org/10.1111/j.1439-0531.2011.01961.x

99. Karen M, Stanly T, J J, M T, A M (2016) Better dairy cattle breeds and better management can improve the livelihoods of the rural poor in Senegal. ILRI (aka ILCA and ILRAD)

100. Marshall K, Gibson JP, Mwai O, Mwacharo JM, Haile A, Getachew T, Mrode R, Kemp SJ (2019) Livestock Genomics for Developing

Countries – African Examples in Practice. Front Genet 10:. https://doi.org/10.3389/fgene.2019.00297

101. Kosgey IS, Okeyo AM (2007) Genetic improvement of small ruminants in low-input, smallholder production systems: Technical and infrastructural issues. Small Rumin Res 70:76–88. https://doi.org/10.1016/j.smallrumres.2007.01.007

102. Silva RMO, Fragomeni BO, Lourenco DAL, Magalhães AFB, Irano N, Carvalheiro R, Canesin RC, Mercadante MEZ, Boligon AA, Baldi FS, Misztal I, Albuquerque LG (2016) Accuracies of genomic prediction of feed efficiency traits using different prediction and validation methods in an experimental Nelore cattle population1. J Anim Sci 94:3613–3623. https://doi.org/10.2527/jas.2016-0401

103. Brown A, Ojango J, Gibson J, Coffey M, Okeyo M, Mrode R (2016) *Short communication:* Genomic selection in a crossbred cattle population using data from the Dairy Genetics East Africa Project. J Dairy Sci 99:7308–7312. https://doi.org/10.3168/jds.2016-11083

104. Marshall K, Gibson JP, Mwai O, Mwacharo JM, Haile A, Getachew T, Mrode R, Kemp SJ (2019) Livestock Genomics for Developing Countries – African Examples in Practice. Front Genet 10:. https://doi.org/10.3389/fgene.2019.00297

105. Redman M, King A, Watson C, King D (2016) What is CRISPR/Cas9? Arch Dis Child - Educ Pract 101:213–215. https://doi.org/10.1136/archdischild-2016-310459

106. Contiliani DF, Moraes VN, Passos GA, Pereira TC (2023) What Is the CRISPR System and How It Is Used? In: Passos GA (ed) Genome Editing in Biomedical Sciences. Springer International Publishing, Cham, pp 1–11

107. Bevacqua RJ, Fernandez-Martín R, Savy V, Canel NG, Gismondi MI, Kues WA, Carlson DF, Fahrenkrug SC, Niemann H, Taboga OA, Ferraris S, Salamone DF (2016) Efficient edition of the bovine *PRNP* prion gene in somatic cells and IVF embryos using the CRISPR/Cas9 system. Theriogenology 86:1886-1896.e1. https://doi.org/10.1016/j.theriogenology.2016.06.010

108. Schultz-Bergin M (2018) Is CRISPR an Ethical Game Changer? J Agric Environ Ethics 31:219–238. https://doi.org/10.1007/s10806-018-9721-z

109. Radbakhsh S, Moghaddam HN (2024) Advantages, disadvantages and risks of CRISPR/cas9 technique for gene therapy. 6:

110. Riegler MMKLJDEKMA (2024) Recent advancements to increase success in assisted reproductive technologies in cattle. Recent Adv

Increase Success Assist Reprod Technol Cattle 21:0–0. https://doi.org/10.1590/1984-3143-AR2024-0031

111. Rogan D (2010) New approaches to superovulation in the cow. Reprod Fertil Dev

112. Fernandes CAC, Pereira GHS, Siqueira LGB, Neri HLDH, Viana JHM, Palhao MP, Teodoro RAS (2020) Short communication: Does previous superovulation affect fertility in dairy heifers? J Dairy Sci 103:10862–10866. https://doi.org/10.3168/jds.2020-18386

113. Stoecklein KS, Ortega MS, Spate LD, Murphy CN, Prather RS (2021) Improved cryopreservation of in vitro produced bovine embryos using FGF2, LIF, and IGF1. PLOS ONE 16:e0243727. https://doi.org/10.1371/journal.pone.0243727

114. Cryopreservation of Gametes and Embryos and Their Molecular Changes. https://www.mdpi.com/1422-0067/22/19/10864. Accessed 15 Oct 2024

115. Linstädt J, Thöne-Reineke C, Merle R (2024) Animal-based welfare indicators for dairy cows and their validity and practicality: a systematic review of the existing literature. Front Vet Sci 11:. https://doi.org/10.3389/fvets.2024.1429097

116. Molina FM, Marín CCP, Moreno LM, Buendía EIA, Marín DCP (2020) Welfare Quality® for dairy cows: towards a sensor-based assessment. J Dairy Res 87:28–33. https://doi.org/10.1017/S002202992000045X

117. Wang X, Li W, Feng X, Li J, Liu GE, Fang L, Yu Y (2023) Harnessing male germline epigenomics for the genetic improvement in cattle. J Anim Sci Biotechnol 14:76. https://doi.org/10.1186/s40104-023-00874-9

118. Understanding the Unintended Effects of Genetic Manipulation. In: Nat. Inst. https://www.natureinstitute.org/article/craig-holdrege/understanding-the-unintended-effects-of-genetic-manipulation. Accessed 15 Oct 2024

119. Kisliouk T, Ravi PM, Rosenberg T, Meiri N (2024) Embryonic manipulations shape life-long, heritable stress responses through complex epigenetic mechanisms: a review. Front Neurosci 18:. https://doi.org/10.3389/fnins.2024.1435065

120. Hill J (2024) Science, technology, and innovation in the dairy sector. Int J Food Sci Technol 59:6717–6723. https://doi.org/10.1111/ijfs.17385

121. Fiorillo V, Amico BM (2024) Milk Quality and Economic Sustainability in Dairy Farming: A Systematic Review of Performance Indicators. Dairy 5:384–402. https://doi.org/10.3390/dairy5030031

Acknowledgements

I wish to extend my gratitude to everyone who provided guidance, support and assistance throughout the process of writing this thesis. I wish to recognise and thank Csaba Pribenszky, my thesis supervisor for his expert guidance and encouragement, without which completing this thesis would not have been possible.

A special mention of thanks must be expressed to the embryologists, doctors and nurses at Dunamenti REK Reproduction Centre, namely Dr. Mátyás Szabolcs, for welcoming me so warmly and for taking the time to teach me about their fascinating work.

I am profoundly grateful to my dear friend, and fellow student Savannah-Rose McAuley for her unwavering steadfast friendship over the past five years. You have been a constant reminder that no obstacle is insurmountable, and your positivity and determination have been truly inspiring. Thank you for being by my side through it all, I am grateful to have shared this journey with you.

The unwavering support and encouragement of my family, chiefly my mother, Christina, throughout my five years of university and the completion of this thesis deserves my deepest, heartfelt gratitude. Your belief in me has been my greatest strength, and your guidance and sacrifices have made all of this possible. This accomplishment would not have been achievable without your constant motivation and the strong foundation you have continuously provided. Thank you for always being my pillars of strength and inspiration.

Thesis progress report for veterinary students

Name of student: Holly O'Connell

Neptun code of the student: THDH2B

Name and title of the supervisor: Dr. Pribenszky Csaba

Department: Animal Hygiene, Herd Health and Mobile Clinic

Thesis title: Embryo Transfer in Dairy Cattle, Current Trends and Future Possibilities.

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2022	10	25	First Meeting	Dr. Pribenszky Csaba
2.	2022	11	09	First Review of Articles	Dr. Pribenszky Csaba
3.	2023	10	05	Signing of Thesis Announcement Form	Dr. Pribenszky Csaba
4.	2023	10	09	Discussion of progress	Dr. Pribenszky Csaba
5.	2023	12	02	Discussion of progress	Dr. Pribenszky Csaba

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	02	21	Recommendations on drafts sent via email	Dr. Pribenszky Csaba
2.	2024	02	28	Review of articles and discussion of progress	Dr. Pribenszky Csaba
3.	2024	06	13	Human ART lab visit	Dr. Pribenszky Csaba
4.	2024	10	31	Discussion of Final draft	Dr. Pribenszky Csaba
5.	2024	11	20	Discussion of final draft	Dr. Pribenszky Csaba

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,

…………Dr. Pribenszky Csaba………………..
signature of the supervisor

Signature of the student: Holly O'Connell……

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

Date of handing the thesis in………… ………………..…...