ISSN Print: 2664-6188
ISSN Online: 2664-6196
Impact Factor: RJIF 5.39
IJCBB 2026; 8(1): 01-06
www.biochemistryjournal.net
Received: 10-11-2025
Accepted: 15-12-2025

PA Patel
Private Practitioner, Lunawada, Mahisagar District, Gujarat, India

PD Patel
Animal Nutritionist, CFP Khandiya, Panchamrut Dairy, Godhra, Gujarat, India

RB Khant
Veterinary Officer, Panchamrut Dairy, Godhra, Gujarat, India

Corresponding Author:
PA Patel
Private Practitioner, Lunawada, Mahisagar District, Gujarat, India

Principles and applications of bovine embryo transfer: A comprehensive review of procedural advancements

PA Patel, PD Patel and RB Khant

DOI: https://www.doi.org/10.33545/26646188.2026.v8.i1a.113

Abstract
Embryo Transfer (ET) has emerged as a transformative assisted reproductive technology in the dairy and beef industries, enabling the rapid multiplication of high-quality genetics from elite donor females. This review examines the fundamental principles and procedural advancements that have shaped contemporary bovine ET. Key technical stages are discussed, including the rigorous selection of clinically healthy donor cows, superovulation protocols using PMSG or FSH to induce multiple ovulations, and specialized insemination techniques. Particular emphasis is given to the transition from invasive surgical recovery to modern nonsurgical transcervical flushing, typically optimized for the day 6-8 post-estrus window to ensure maximum embryo viability and ease of handling. Additionally, the article highlights the comparative benefits of *in vivo* and *in vitro* fertilization (IVF) and the role of ET in global biosecurity and genetic dissemination. By bypassing natural reproductive limits, ET continues to be a primary driver of economic value and genetic progress in modern livestock production.

Keywords: Embryo transfer, bovine reproduction, genetic improvement

Introduction

Embryo Transfer (ET) serves as a powerful reproductive tool where an embryo is retrieved from a high-quality "donor" female and placed into a "recipient" female. The recipient then carries the pregnancy to term and handles the birthing process. For breeders of registered purebred livestock, this technology is a major profit driver. Its primary advantage is that it allows a genetically elite female to produce significantly more offspring than she ever could through natural breeding alone. By bypassing the standard one-calf-per-year limit, producers can rapidly expand the influence of their best animals and maximize their herd's genetic value.

The scientific and technological advances achieved during the past decades in animal reproduction have resulted in the development of a variety of tools commonly referred to as assisted reproductive technologies (ART). The primary focus of these tools is to maximize the number of offspring from genetically superior animals and disseminate germplasm worldwide (Berglund, 2008) [2]. In recent decades, the field of animal husbandry has been completely transformed by rapid breakthroughs in biology, embryology, and the study of hormones. These scientific leaps have moved us far beyond traditional breeding, leading to a new era of "biotechnologies" that allow farmers to manage their livestock with incredible precision. Rather than relying on nature's slow pace, modern producers use tools like heat synchronization and artificial insemination to gain better control over when and how animals are born. More advanced methods, such as Multiple Ovulation and Embryo Transfer (MOET), allow a single elite female to pass on her superior traits to dozens of offspring at once.

Today, the industry has even moved into laboratory-based solutions, including *in vitro* embryo production where life begins in a petri dish and nuclear transfer, which allows for the actual cloning of top-tier animals. Together, these innovations have made animal breeding faster, more efficient, and much more profitable by ensuring that only the strongest genetics are carried forward into the next generation.

Historical Development of Embryo Transfer Technology

Embryo transfer (ET) represents one of the earliest assisted reproductive technologies, with its origin dating back to experimental studies conducted in rabbits in 1890. Subsequent progress was achieved in small ruminants, with successful embryo transfer reported in sheep and goats during the 1930s. The application of ET in cattle advanced further when the first bovine embryo transfer was reported in 1949, followed by the birth of the first calf through this technique in 1951 (Willet, 1951) [16]. Despite these early successes, commercial application of embryo transfer did not occur until the early 1970s in the United States (John, 2008).

In its initial stages, embryo transfer was performed exclusively through surgical procedures. Embryo recovery commonly involved mid-ventral laparotomy carried out under halothane anaesthesia. These surgical techniques restricted the widespread adoption of ET, particularly in dairy cattle, where the anatomical structure of the udder complicated surgical access. However, by the mid-1970s, several research groups developed non-surgical embryo recovery and transfer methods that demonstrated efficiency comparable to surgical approaches, leading to increased adoption of ET technology (Gordon, 2003) [6].

Reproductive efficiency in livestock significantly influences the economic development of a nation. As a result, scientists continuously sought advanced reproductive technologies to enhance animal productivity and genetic improvement. Embryo transfer technology, developed and refined since 1890, became an effective strategy to accelerate genetic progress. Commercial embryo transfer programs in cattle were initially established in North America and later expanded to other regions during the early 1970s, primarily to rapidly propagate superior and exotic beef cattle genetics.

The demand for embryo transfer services often surpassed the availability of trained veterinarians and reproductive specialists. To address the low genetic potential of indigenous cattle populations, selective breeding and crossbreeding with highly productive exotic breeds were considered practical solutions (Tadesse, 2008) [15]. This strong economic motivation led to rapid advancements in superovulation protocols, surgical embryo recovery techniques, and embryo transfer procedures, along with the establishment of specialized ET service centers. However, as the economic value of ET-derived offspring depended largely on their genetic scarcity, the expansion of the technology ultimately reduced exclusivity, rendering early commercial efforts self-limiting.

Challenges and Economic Advantages of Bovine Embryo Transfer: While embryo transfer is a transformative tool, its success is often dictated by variables that fall outside the technician's immediate control. Factors such as the poor selection of donor cows or incompatible service sires can significantly hinder results. Furthermore, environmental stressors including extreme weather patterns or sudden storms during the critical superovulation and synchronization windows can disrupt the hormonal balance of the animals, leading to failed embryo recovery or unsuccessful transfers. These biological and environmental hurdles remain some of the most persistent challenges in the field Genzebu, D. (2015) [5]. Despite these risks, the commercial incentives for adopting embryo transfer technology are substantial. For the domestic livestock industry, it serves as a primary engine for genetic progress by allowing elite parents to produce a much higher volume of superior offspring than natural breeding allows. Beyond simple multiplication, the technology has revolutionized international trade; because embryos can be frozen and transported globally, the need to ship live animals across long distances is greatly reduced. This not only lowers biosecurity risks but also allows breeders to market high-quality genetics on a global scale, maximizing the reach and profitability of top-tier breeding stock (Lamb *et al.*, 2005) [9].

Fundamental Principles of Embryo Transfer

Selection of Donor Cow: Selection of a suitable donor cow is the first and most critical step in embryo transfer. The donor should possess genetic superiority aligned with the objectives of the program, such as improved milk production, milk composition, growth rate, calving ease, and disease resistance, along with the ability to produce a large number of usable embryos. The donor must be clinically healthy, cyclic, and have a history of high fertility, as such animals respond better to superovulation. Cows selected as donors should be at least two months postpartum, as embryo yield is higher compared to cows closer to calving. Young cows generally produce slightly more transferable embryos than heifers under certain conditions. Proper nutritional management is essential; both over-conditioned and under-conditioned cows exhibit reduced fertility. Therefore, the donor cow should be maintained at an optimum body condition score (BCS) at the time of embryo transfer. Selecting the male is usually more important than selecting the donor female because males will normally be bred to many females and can be selected more accurately than females. Since male required for 50% of the genetic value, it is extremely important to use genetically superior bulls, i.e. it is necessary to select fertile bulls and fertile semen which makes it especially important to use high quality semen (David, A. & S. Hamilton, 2016) [4].

Superovulatory treatment of the donor cow

Superovulation refers to the hormonally induced release of multiple ova during a single estrous cycle and represents the second critical step in an embryo transfer (ET) program. Properly treated cows or heifers may ovulate ten or more viable oocytes in a single estrus. Approximately 85% of normal, fertile donor animals respond favorably to superovulatory treatment, yielding an average of five transferable embryos per collection. Donor cows may undergo repeated superovulation at intervals of approximately 60 days; however, a gradual decline in embryo yield has been observed with successive treatments.

Two principal methods of superovulation are commonly employed. One widely used protocol involves the administration of a single intramuscular injection of pregnant mare serum gonadotropin (PMSG), also known as equine chorionic gonadotropin (eCG), at a dose of 2,000-2,500 IU, typically on day 10 of the estrous cycle (with day 0 defined as the day of observed estrus). This is followed 2-3 days later by two injections of prostaglandin $F_2\alpha$ (dinoprost or cloprostenol) administered 12-24 hours apart to induce luteolysis. The superovulatory response induced by eCG treatment is often greater than that induced by FSH; however, more embryos of transferable good quality are produced on average after FSH treatment (Selk, G. 2013)

[13]. Advantages of using PMSG are that it is cheaper and easier to obtain than FSH and the single dose is enough. Disadvantages are that there is a huge variation in the quality of the collected embryos and has residue problem after administration (Çİzmecİ *et al.*, 2018) [3].

Insemination of Donor Cow

The onset of standing estrus in the donor cow is considered the reference point for the timing of insemination. Owing to the ovulation of multiple oocytes from numerous superstimulated follicles, donor cows are routinely inseminated with at least two doses of semen administered at 12-hour intervals. Embryo recovery is subsequently carried out by nonsurgical uterine flushing on day 7 post-insemination, coinciding with the morula to blastocyst stages of embryonic development.

The use of high-quality semen, characterized by a high proportion of morphologically normal and progressively motile spermatozoa, is a critical determinant of fertilization success in embryo transfer programs. Correct semen deposition in the uterine body, located immediately anterior to the cervix (approximately 1-2 cm), is essential for optimal fertility. Excessive advancement of the insemination catheter leading to unilateral semen deposition in a uterine horn may compromise fertilization rates, particularly when ovulations occur in the contralateral ovary.

Production of embryo

***In vivo* fertilization**

During normal *in vivo* embryonic development, blastomeres undergo a series of mitotic cleavage divisions. Following fertilization, the zygote sequentially develops into the 2-cell, 4-cell, 8-cell, and subsequently the 16-cell stages. As cleavage continues, the blastomeres form a compact, grape-like structure in which individual cells can no longer be distinctly identified; this developmental stage is referred to as the morula. With further development, the embryo prepares for its first major differentiation event, known as blastulation. Immediately prior to blastulation, the morula undergoes compaction, during which blastomeres establish tight intercellular junctions and become spatially segregated into inner and outer cell populations (Jahnke *et al.*, 2014) [7]. These stages of *in vivo* embryonic development enable the production of multiple viable embryos, which can subsequently be recovered from the donor uterus by nonsurgical transcervical flushing using a two-way or three-way Foley catheter and transferred to synchronized recipient females.

***In vitro* fertilization**

This procedure usually comprises four separate steps *in vitro*: oocyte maturation, capacitation of sperm, fertilization, and culture of embryos until they can be frozen or transferred to the uterus. The actual IVF step is the easiest of the four, but success requires that the other steps work well. Oocyte maturation, capacitation, and culture of embryos can all be done *in vivo*, but as the number of *in vivo* steps increases, the practicality decreases greatly [21]. Oocytes are aspirated from ovarian follicles; then matured (*in vitro* maturation), mixed with capacitated sperm (*in vitro* fertilization) and zygote is cultured (*in vitro* culture) for 8-9days to obtain blastocysts for transfer to the uterus of recipient. For the purpose of embryo culturing, we use media like Krebs-ringer's bicarbonate, modified bulbeccos (Walkite *et al.*, 2019).

Nonsurgical Embryo Recovery from Donor Cows

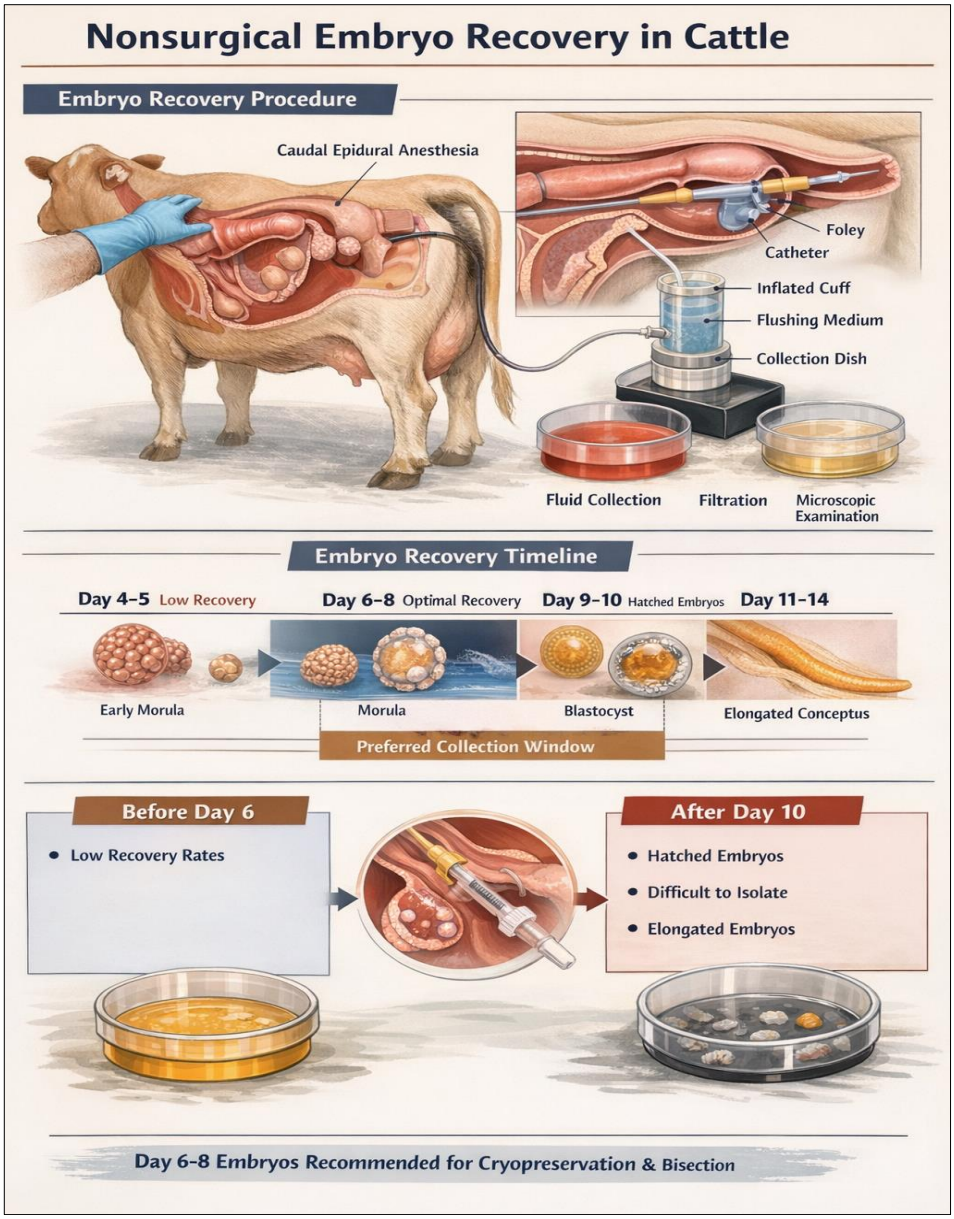
Contemporary embryo transfer procedures for embryo recovery are predominantly performed using nonsurgical techniques, typically around day 7 post-breeding. The recovery process is relatively straightforward and can usually be completed within one hour when conducted by trained personnel using appropriate equipment. The procedure requires specialized instrumentation and technical expertise. Prior to uterine flushing, donor cows are administered a caudal epidural anesthesia at the sacrococcygeal or first intercoccygeal space to minimize straining and facilitate catheter placement.

A flexible rubber catheter is introduced transcervically into the uterine body. The inflatable cuff of the catheter is expanded with sterile saline to secure its position and to prevent retrograde fluid loss. Flushing medium is then infused into each uterine horn through distal perforations located anterior to the cuff. The fluid-filled uterine horn is gently massaged per rectum to facilitate dislodgement of embryos, after which the flushing medium containing embryos is recovered through the catheter. The effluent is passed through an embryo collection filter and collected into a sterile graduated cylinder or Petri dish. Recovered embryos are subsequently identified and retrieved by microscopic examination under a stereomicroscope.

Embryos may be recovered nonsurgically as early as day 4 after estrus in certain donor cows; however, recovery rates prior to day 6 are consistently lower compared with those obtained between days 6 and 8 post-estrus. Although embryo collection can be performed between days 9 and 14 after estrus, embryos typically hatch from the zona pellucida around days 9-10, rendering them more difficult to identify, isolate, and handle, and increasing their susceptibility to microbial contamination. Beyond day 13, embryos undergo rapid elongation, which may result in physical damage during recovery or entanglement with other conceptuses. Furthermore, standard procedures for embryo cryopreservation and micromanipulation, including embryo bisection, have been optimized for embryos recovered between days 6 and 8, providing an additional justification for selecting this recovery window.

Surgical embryo collection method

Early embryo collection techniques relied on invasive approaches, including the slaughter of donor females followed by excision of the oviducts, or surgical removal of the oviducts from live animals approximately 72 hours post-ovulation to facilitate embryo recovery by flushing. Surgical recovery methods were developed initially and involved performing a laparotomy via flank or midline abdominal incision to exteriorize the reproductive tract. To enable directed flushing, the distal one-third of the uterine horn was occluded manually using a clamp or digital pressure applied with the thumb and forefinger. Culture medium was then infused into the uterine horn, forcing the fluid retrogradely through the oviduct by gentle milking, with embryos collected at the level of the infundibulum. The flushing medium was introduced either through a puncture at the utero-tubal junction or directly via the oviduct until uterine distension was achieved.



Following exteriorization of the reproductive tract, the uterine wall was punctured using a blunt needle connected to a flexible catheter. Hydrostatic pressure generated by the infused flushing medium facilitated its rapid flow through the catheter, creating sufficient turbulence to dislodge and transport embryos into a sterile collection vessel. Although these surgical techniques enabled recovery of a high proportion of embryos, their repeated application was limited by procedure-induced tissue trauma and subsequent formation of postoperative adhesions. Such adhesions progressively hindered adequate exposure of the reproductive tract, thereby restricting the number of repeat surgical collections to approximately three per donor. In cattle, embryos intended for commercial embryo transfer programs are typically recovered between days 6 and 9 following estrus.

Selection of Recipient Cows: Selection of embryo transfer recipients was based on established reproductive and performance criteria. Recipient cows were required to be reproductively sound, exhibit a history of calving ease, and possess adequate milking capacity and maternal behavior. Estrus synchronization was performed to ensure appropriate physiological alignment with donor embryo age. Prior to embryo transfer, recipient cows were examined by transrectal palpation to confirm the presence of a functional corpus luteum, indicative of luteal-phase synchronization suitable for embryo acceptance (Ongubo *at el*., 2015) [11].

Synchronization of Recipient Cows

Recipient cows synchronized using prostaglandin $F_2\alpha$ ($PGF_2\alpha$) are typically treated 12-24 hours earlier than donor cows, as $PGF_2\alpha$-induced estrus occurs approximately 60-72 hours post-treatment in recipients, compared with 36-48 hours in super ovulated donor cows. Synchronization protocols are most effective in recipient females that are already cyclic (Mebratu at el., 2020) [10].

Estrus synchronization of recipient cows may be performed using protocols similar to those applied to donor cows and within the same management schedule. The critical requirement for successful embryo transfer is precise temporal alignment between donor insemination and recipient estrus, ensuring comparable uterine environments at the time of embryo transfer, typically seven days later.

Anestrous or non-cycling cows, particularly those in poor body condition or at an insufficient interval postpartum, are suboptimal recipients and are generally excluded from embryo transfer programs (Baruselli *at el*., 2011) [1].

Importance of embryo transfer

Embryo transfer (ET) offers multiple advantages, including accelerated genetic improvement and evaluation, facilitation of planned matings, enhanced disease control through reduced animal movement, cost-effective import and export of genetic material, and increased farm profitability. The technology is widely employed for the production of artificial insemination (AI) sires derived from genetically superior donor cows and elite AI bulls (Baruselli *at el*., 2011) [1].

In several countries, ET has been integrated into structured breeding programs through the establishment of nucleus herds, where female progeny are subjected to juvenile multiple ovulation and embryo transfer (MOET) schemes, while male offspring are selected for future AI bull development. Implementation of such strategies has been shown to substantially accelerate genetic progress, with estimates suggesting up to a two-fold increase in genetic gain. Currently, the predominant application of embryo transfer in livestock production systems is the rapid multiplication of animals expressing superior or economically desirable phenotypes.

Embryo transfer (ET) provides a unique opportunity to disseminate the genetics of elite, proven females, with the timing of reproduction precisely controlled by management practices. This technology allows the development of herds composed of genetically valuable females, including full- or half-siblings, and has been instrumental in rapidly expanding limited gene pools. While artificial insemination (AI) has enabled the widespread use of superior bulls, ET has similarly facilitated the propagation of highly valuable females.

In vivo-produced embryos present a lower risk of infectious disease transmission when proper handling procedures are followed (Stringfellow *at el*., 2004) [14] Extensive studies have demonstrated that bovine embryos with an intact zona pellucida, when appropriately washed, do not transmit infectious agents (Baruselli *at el*., 2011) [1]. The International Embryo Transfer Society (IETS) has classified various disease agents according to their risk of transmission via embryos. Notably, pathogens such as *Enzootic bovine leukosis*, *Foot-and-mouth disease virus*, *Bluetongue virus*, *Brucella abortus*, *Infectious bovine rhinotracheitis virus*, and *Bovine spongiform encephalopathy* are not transmitted by *in vivo*-produced bovine embryos, provided standard handling and washing protocols are adhered to. Consequently, ET has been recommended as a strategy to salvage valuable genetics during disease outbreaks (Wrathall *at el*., 2004) [17].

Costs and Commercial Significance of Embryo Transfer

The costs associated with embryo transfer (ET) programs vary widely across and within countries, influenced by multiple factors including labor, management, and feed requirements. While the direct costs of ET services or technology may be relatively low, the overall expense is increased due to the need to maintain healthy, reproductively sound cows or heifers as recipients, often temporarily removing them from production. Despite these costs, ET programs are frequently economically viable, provided that a careful analysis demonstrates that the benefits exceed the expenditures. Programs where the cost-benefit ratio is unfavourable should generally be avoided.

Commercial ET in cattle has become a well-established industry worldwide. Although the absolute number of offspring produced annually remains limited, the impact on livestock genetics is substantial due to the high genetic merit of the animals produced. ET is increasingly employed for genuine genetic improvement, particularly in the dairy sector, where a significant proportion of AI bulls originate from embryo-derived progeny.

An additional advantage of *in vivo*-produced embryos is that they can be rendered pathogen-free through validated washing procedures, making ET an effective tool for disease control and for the safe international movement of genetic material. Over the past six decades, advancements in techniques have enabled frozen-thawed embryos to be transferred to synchronized recipients with efficiency and ease comparable to artificial insemination. While *in vitro* embryo production and embryo or semen sexing have achieved success, their widespread adoption is currently limited by time and cost constraints.

The most common application of bovine ET in modern livestock production combines the use of proven donor cows inseminated with semen from elite bulls, followed by industry-wide dissemination of superior genetics via artificial insemination. This integrated approach maximizes genetic gain while maintaining efficiency and biosecurity in herd improvement programs.

References

1. Baruselli PS, Ferreira RM, Sales JNS, Gimenes LU, Sá Filho MF, Martins CM, *et al.* Timed embryo transfer programs for management of donor and recipient cattle. Theriogenology. 2011;76(9):1583-1593.
2. Berglund B. Genetic improvement of dairy cow reproductive performance. Reprod Domest Anim. 2008;43(Suppl 2):89-95.
3. Çizmeci SÜ, Güler M. Superovulation in cows: A review. J Vet Sci Res. 2018:65-68.
4. David A, Hamilton S. Hamco Cattle Co.: 18th Annual Angus Bull Sale. Glenboro: Manitoba, Canada; 2016.
5. Genzebu D. A review of embryo transfer technology in cattle. Glob J Anim Sci Res. 2015;3(2):562-575.
6. Gordon I. Laboratory production of cattle embryos. 2nd ed. Ireland: Biotechnology in Agriculture; 2003. p. 13-17.
7. Jahnke MM, West JK, Youngs CR. Evaluation of *in vivo*-derived bovine embryos. In: Bovine Reproduction. Hoboken: Wiley-Blackwell; 2014. p. 733-748.
8. Grimes JF. Utilization of embryo transfer in beef cattle agriculture. Agric Nat Res. 2008;17:8.
9. Lamb C, Larson J, Marquenzini G, Vasconcelos J. Factors affecting pregnancy rates in an IVF embryo transfer program. In: Proc Joint AETA-CETA Meeting; 2005. p. 31-36.
10. Mebratu B, Fesseha H, Eyob GE. Embryo transfer in cattle production: principles and applications. Int J Pharmacol Biomed Res. 2020;7(1):40-54.
11. Ongubo MN, Rachuonyo HA, Lusweti FN, Kios DK, Kitilit JK, Musee K, *et al.* Factors affecting conception rates in cattle following embryo transfer. Uganda J Agric Sci. 2015;16(1):19-27.
12. Selk G. Embryo transfer in cattle. DASNR 102. Stillwater (OK): Oklahoma State University; 2013. ANSI-3158.

13. Stringfellow DA, Givens MD, Waldrop JG. Biosecurity issues associated with current and emerging embryo technologies. Reprod Fertil Dev. 2004;16(2):93-102.
14. Tadesse B. Calf sex ratios in artificially inseminated and naturally mated crossbred dairy cattle. In: Proc 13th Annu Conf Ethiopian Soc Anim Prod; 2008. Addis Ababa, Ethiopia. p. 227.
15. Willett EL. Embryo production in animals. Science. 1951;114:411.
16. Wrathall AE, Simmons HA, Bowles DJ, Jones S. Biosecurity strategies for conserving valuable livestock genetic resources. Reprod Fertil Dev. 2004;16(2):103-112.