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Review Article

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Review on embryo transfer in cattle and its application

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Abstract

Currently in animal husbandry, the new biotechnologies have become the most powerful tool for animal scientists and breeders to improve genetic construction of their animal herds and increase quickly elite animals. Embryo transfer is a biotechnological process by which an embryo is collected from a donor female and then transferred into a recipient female where the embryo completes its development. This technology involves the selection of desired male breed, the selection and management of donor and recipient animals and the collection and transfer of embryo within a narrow window of time following oestrus. Recipients must have a proven reproductive performance, free of congenital or infectious diseases to obtain high conception rates and have a sturdy body size to avoid problems of dystocia. Both embryo collection and Embryo transfer techniques can operate in surgical and non-surgical. Surgical methods for embryo collection and transfer are increasingly replaced by nonsurgical methods, which are much more practical and less expensive in selection program. The application of this method is numerous in cattle production to amplify reproductive rates of valuable females, genetic improvement, twining, disease control, planned mating, increased farm income, and others. Advances in research on *in vitro* fertilization, production of twins or multiples, sexing, manipulation of embryo genetic stock and other techniques are also applied. The success and economics of cattle embryo transfer program is dependent on several factors that include super ovulation system, management and weather impacts, failure at embryo recovery, embryo transfer technique failure and bio security problem The greatest challenges often associated with this in the developing world are lack of adequate technology due to high cost and technological skills associated with the entire process. Despite the fact that this technology is not commercially available in developing countries, ET technology could provide opportunities for the conservation and the development of minor breeds.

Keywords: Application, Cattle, Donor, Embryo, Embryo transfer, Recipient

1. Introduction

In concern of its use modern reproductive technologies have opened way to study, treat and manipulate the reproductive phenomenon of both *in vitro* and *in vivo* to improve reproductive performance in various domestic species of livestock [1]. Based on the advance in scientific knowledge of endocrinology, reproductive physiology, biology and embryology during the last five decades new biotechnologies have been developed and entered in to animal breeding and husbandry. Among them are estrus synchronization (induction), artificial

insemination, multiple ovulation induction and embryo transfer (MOET), *in vitro* embryo production (IVP) and cloning by nuclear transfer (NT) [2]. The aims of these reproductive technologies were initially to speed up the genetic improvements of farm animals by the increase of offspring of selected males and females, reduction of the generation intervals, experimental purposes, enhancement of the uniformity of herds for an easier management and multiplication of transgenic animals after gene-targeting [3].

Embryo transfer (ET) is the most advanced reproduction techniques by which embryos are collected from a donor female and are transferred to recipient females, which serve as surrogate mothers for the remainder of pregnancy [4]. It has become the most power full animal scientist and breeders to improve genetic construction of their animal herds and increase quickly elite animal numbers which have recently gained considerable popularity with seed stock dairy and beef producers [2].

Reviewing the report from the previous literatures, (Habtie, 2019) [5] the first successful transfer of a mammalian embryo occurred in rabbits in 1890, the first transfer of a bovine embryo was reported in 1949 and the first calf from embryo transfer in 1951.The most modern applicable embryo transfer technology was developed in the 1970's. In North America, the commercial ET industry began in the 1970's due to the demand for "exotic" breeds of cattle that had been imported from Europe (Hasler, 2003). Nowadays, embryo transfer technology is considered to be the principal technique which is very much necessary for achieving success in various assisted reproductive technologies, especially in case of *invitro* fertilization and animal cloning [2].

The primary use of embryo transfer in cattle has been to amplify reproductive rates of valuable females. Ideally, embryo transfer can be used to enhance genetic improvement and to increase marketing opportunities with purebred cattle. Because of their relatively low reproductive rate and long generation interval, embryo transfer is especially useful with cattle [4]. Actual transfer of an embryo is only one step in a series of processes that may include some or all of the following: super ovulation and insemination of donors, collection of embryos, isolation, evaluation and storage of embryos, micromanipulation and genetic testing of embryos, freezing of embryos and embryo transfer. Initially, all collections and transfer were performed surgically through mid-ventral exposure of the uterus and ovaries [4].

However, non-surgical embryo recovery and transfer techniques were developed in the mid-1970. Advancement in ET technology that occurred in the mid 1970's was the ability to successfully cryopreserve bovine embryos [6]. The transfer of bovine embryos today commonly involves oestrus synchronization and super ovulation of a donor animal, insemination of the donor animal, and collection of embryos from the donor approximately 7 days after oestrus, then transferred to recipients (fresh) or frozen and transferred at a later date. Almost all transfers today are performed non-surgically and most are performed on-farm, rather than in a central clinic (surgically) [6]. The goals of animal genetic improvement are to accelerate genetics progress (improvement speed) as well as to add new genetic trait to animal body [7].

Many factors may influence the embryo transfer technology. Inappropriate selection of specific donor cows and service sires, long term weather problems or storms during the super ovulation/recipient synchronization process are beyond the control of anyone [4], superovulation system, failure at embryo collection and transfer techniques failure [8]. The commercial advantages of embryo transfer in domestic animal include: facilitating genetic improvement in the domestic animal industry by obtaining a large number of desirable progeny from parents of high genetic quality; enabling embryos to be moved from country to country in the frozen state, thereby reducing the need for long distance domestic animal movement; permitting high quality breeding stock to be available for sale in much larger number than was previously possible; and exploiting developments in reproductive

technology, such as embryo sexing and embryo splitting. Split or bisected embryos have the ability to develop into identical twins [9].

Therefore, the objectives of this review paper are: to review embryo transfer and its application and to understand the current status of the embryo transfer technology in cattle.

2. History of embryo transfer

From historical perspective on assisted reproduction, the first successful transfer of mammalian embryos was performed by Walter Heape in 1890. Heape transferred two four-cell Angora rabbit embryos into an inseminated Belgian doe, which subsequently gave birth to four Belgian and two Angora young. Embryo transfer in food animals began in the 1930s with sheep and goats, but it was not until the 1950's that successful embryo transfers were reported in cattle and pigs by Jim Rowson at Cambridge, England. The first successful embryo transfers in cattle were reported by Umbaugh in 1949. He produced four pregnancies from the transfer of cattle embryos, but all the recipients aborted before the pregnancies reached full term. In 1951, the first embryo transfer calf was born following the surgical transfer of an abattoir-derived day-5 embryo [10]. Application of embryo transfer to the cattle industry began in the early 1970's when European dual-purpose breeds of cattle became popular in North America, Australia and New Zealand. The bovine embryo transfer industry as we know it today arose in North America in the early 1970's [11].

Table 1: First young born after transfer of frozen thawed embryo

Year	Species	Researchers
1971	Mouse	Whittingham *et al*.
1973	Cow	Wilmut and Rowson
1974	Rabbit	Bank and Maure
1974	Sheep	Willadsen
1975	Rat	Whittingham
1976	Goat	Bilton and Moore
1982	Horse	Yamamoto *et al*
1984	Human	Zeilmaker *et al*
1985	Hamster	Ridha and Dukelow
1988	Cat	Dresser *et al*
1989	Pig	Hayashi *et al*
1989	Rhesus monkey	Wolf *et al*.

Source: Gordon (2004) [12].

3. Principles and application of embryo transfer in cattle

3.1. Selection of Donor Cow

It is the first step in embryo transfer. The following requirements of donor cow should be met to be selected. These include genetic superiority, for having genetic objectives of the programmed milk production, milk composition, growth rates, calving ease and disease resistance and likelihood of producing large numbers of usable embryos; healthy, cycling cattle with a history of high fertility make the most successful donors; donors at least two months post-partum because they produce more embryos than those closer to calving; young cows as they seem to yield slightly more usable embryos than heifers under some conditions; appropriate nutrition for her size and level of milk production since both the very obese cow and the thin cow will have reduced fertility, so it is important that the donor cow be in an appropriate body condition score at the time of embryo transfer [5].

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 [13].

3.2. Superovulation of Donor Cow

Superovulation is the release of multiple eggs at a single oestrus. It is the second step in the embryo transfer process. Cows or heifers properly treated can release as many as 10 or more viable egg cells at one estrus. Approximately 85% of all normal fertile donors will respond to superovulation treatment with an average of five transferable embryos. Some cows are repeatedly treated at 60-day intervals with a slight decrease in embryo numbers over time [4].

Popular methods of superovulation are two. One method consists of administering a single IM injection (2,000–2,500 IU) of PMSG (pregnant mare serum gonadotropin) or commonly known as equine chorionic gonadotropin (eCG), typically on day 10 of the estrous cycle (where day 0 is defined as the day cows are observed in estrus), followed 2-3 days later by two injections of prostaglandin (PG) 2α (dinoprost or cloprostenol) 12–24 hour apart. 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 [14]. 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 [15].

The other commonly used method consists of administering follicle stimulating hormone (FSH) [14]. FSH is a pituitary gonadotropin which is usually obtained from horse, pig and sheep. Application dose is 25-50 mg depending on live weight of cow [16]. The half-life of FSH is approximately 2 hours thus it must be administered subcutaneously in repeated doses. The biological half-life of FSH is short; hence it is administered two doses in a day. Researchers reported that the best time to start FSH administration is the 9th – 14th days of the cycle. The reasons for the hormone to be effective around those days are both the presence of a mature CL; also, it is the best time of follicular wave for beginning superovulation applications [15]. Commercially available preparations of FSH are injected twice daily for four days at the middle or near the end of a normal estrous cycle, while a functional corpus luteum (CL) is on the ovary. A prostaglandin injection is given on the third day of the treatment schedule which will cause CL regression and a heat or estrus to occur approximately 48 to 60 hours later. At this time, the cow will usually produce 7-20 or more viable eggs with an average of 5-15 or more that are transferable [17].

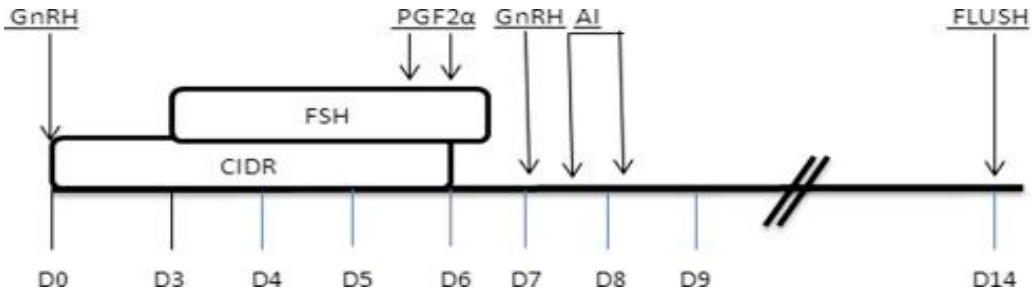

Figure 1: Superovulation schedule using CIDRs and GnRH. PGF2α, prostaglandin

Source: *https://doi.org/10.1016/j.cvfa.2016.01.008* [18]

3.3. Insemination of Donor Cow

The time when the donor is first observed in standing oestrus is the reference point for insemination. As the release of many ova from the multiple follicles on the ovary, superovulated donor in heat is inseminated, usually with at least two straws of semen 12h apart, and 7 days later the uterus is flushed to recover the embryos [5]. Using high quality semen with a high percentage of normal, motile cells is a very critical step in any embryo transfer program. The correct site for semen placement is in the body of the uterus. This is a small target (1/2 to 1 inch) that is just in front of the cervix. There seems to be a tendency for inseminators to pass the rod too deep and deposit the semen into one of the uterine horns, thereby reducing fertility if ovulations are taking place at the opposite ovary [14].

3.4. Production of Embryo

3.4.1. *In vivo* fertilization

During normal *in vivo* embryonic development, blastomeres go through a series of cleavage divisions. The ovum, after fertilization, divides and develops into a 2-cell embryo, the 2-cell develops into a 4-cell, the 4-cell into an 8-cell, the 8 in to 16 etc. when the blastomeres appear like a cluster of a grapes and individual blastomeres can`t be differentiated, this stage of embryonic development is known as the morula stage. As the embryo further develops, it prepares to undergo its first differentiation event known as blastulation. Just prior to this differentiation event, cells of the morula "compact" and are allocated to the "inside" and "outside" parts of the embryo [19]. Through these we can produce many embryos and then collect from the donor through trans-cervical 2-way or 3-way folly catheter, then transfer to the recipient [3].

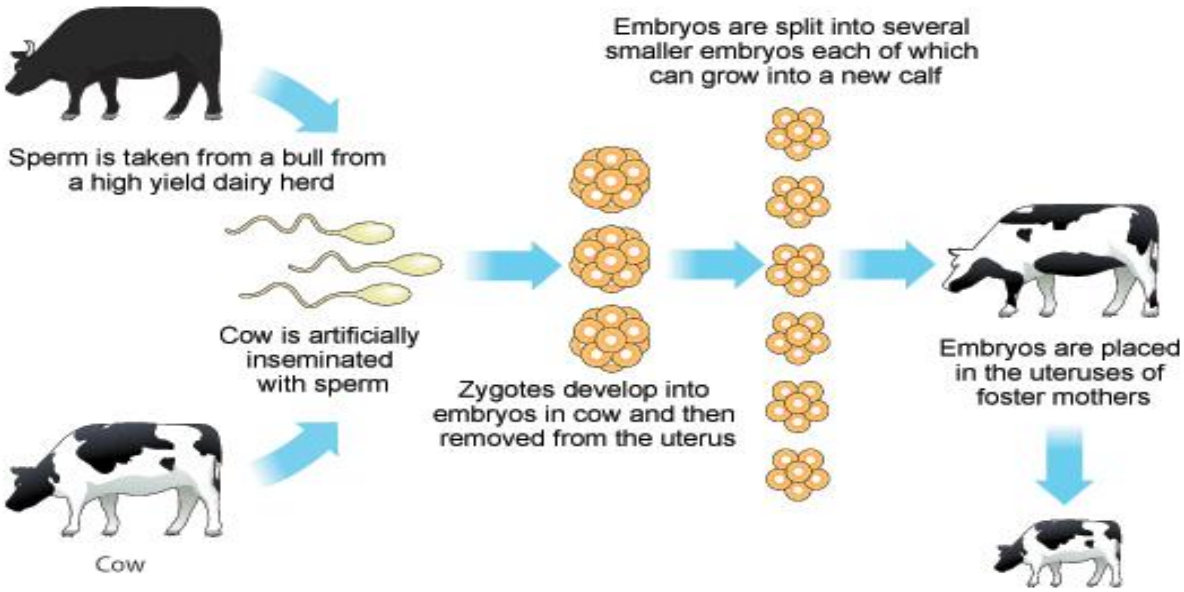

Figure 2: *In vivo* fertilization and embryonic development.
Source: (Jahnke *et al*., 2017) [20]

3.4.2. *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 (*invitro* fertilization) and zygote is cultured (*invitro*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 [3] (Walkite *et al*., 2019).

Jordana *et al*., 2020 [22] also reported that ovaries from crossbred beef cycling heifers and cows were collected at the slaughterhouse. Then follicles between 2–8 mm sized were aspirated and intact cumulus-oocyte complexes (COC) were selected for *in vitro* maturation (IVM). Groups of 50 COCs were cultured in maturation medium that consisted of TCM-199 supplemented with 10% fetal calf serum and 10 ng/mL epidermal growth factor for a period of 24 h at 38.5°C, under 5%CO2 and high humidity (Jordana*et al*., 2020). Frozen semen straws were thawed in a water bath at 38°c. Matured oocytes were washed in Fert-TALP medium, transferred to a new dish and inseminated with spermatozoa. Fertilization was left to occur during 18–20 h, at 38.5°C, under 5% CO2 and high humidity [22].

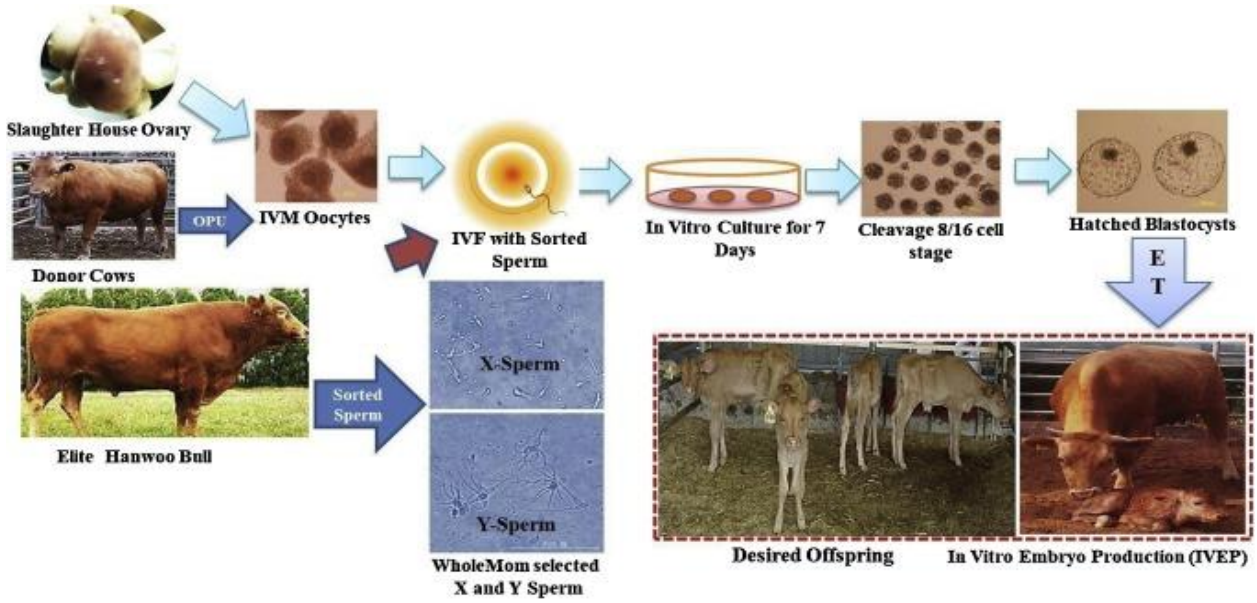

Figure 3: *In vitro* fertilization and embryonic development.
Source: (Chowdhury, *et al*., 2019) [23].

3.5. Embryo Recovery

3.5.1. Non surgical embryo collection method

Current embryo transfer procedures for embryo recovery or flushing are generally accomplished through non-surgical techniques at approximately seven days after breeding. The recovery process is relatively simple and can be completed in well under an hour. This process requires specific instrumentation and training. Initially, the donor is given an epidural block at the tail head to prevent straining.

A flexible rubber tube catheter is passed through the cervix and into the body of the uterus. The cuff is inflated with saline solution to hold the catheter in place and to prevent backflow is flushed into the uterine horns through holes at the tip of the catheter that precede the cuff. The solution-filled uterine horn is gently massaged and the fluid containing the embryos is drawn back out through the catheter. This solution is collected through a filter and into a cylinder or dish. Embryos are then located retrieved by examination under a microscope [24].

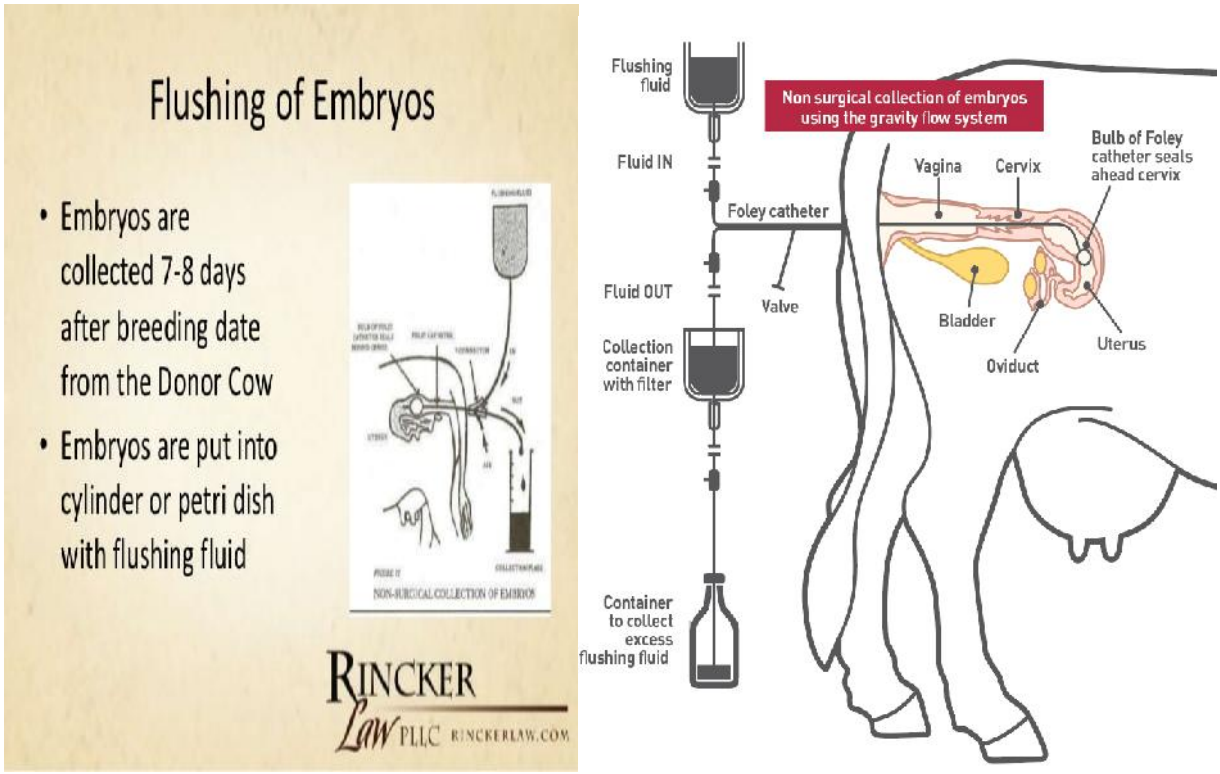

Figure 4: Non-surgical collection of embryo
Source: (Rincker, 2013) [17].

Embryos can be recovered non-surgically as early as four days after oestrus from some cows, but prior to day 6 recovery rates are lower than on days 6 to 8. Embryos can also be recovered on days 9 to 14 after oestrus however, they hatch from the zonapellucida on day 9 or 10, making them more difficult to identify and isolate and more susceptible to infection. After day 13, embryos elongate dramatically and are sometimes damaged during recovery or become entangled with each other. Procedures for cryopreservation and bisection have been optimized for day 6–8 embryos, which is another reason for choosing this time [21].

3.5.2. Surgical embryo collection method

Early collection techniques involved either slaughtering the females and excising the oviducts, or surgically removing the oviducts from live females at 72 hours post ovulation so that the embryos could be recovered by flushing. A surgical method was developed first. This is done by performing a laparotomy (flank or midline abdominal incision) to expose the reproductive tract. A clamp or the thumb and forefinger can be used to block the distal one third of the uterine horn, so that fluid injected into that segment can be forced through the oviduct with a gentle milking action and collected at the infundibulum. Culture medium is introduced through a puncture at the utero-tubal junction or through the oviduct until the uterus is turgid.

The uterus is then punctured with a blunt needle attached to a flexible catheter. The pressure will cause the medium to gush through the catheter, with enough turbulence to carry the embryos into a collection tube. These procedures allow for the recovery of a high percentage of embryos. However, because of the surgical trauma and resulting adhesions they can be repeated only a few times. Adhesions make it difficult, if not impossible, to expose the reproductive tract repeatedly, and limit surgical interventions to a maximum of around Three. In cattle, embryos for commercial purposes are usually recovered 6 to 9 days after estrus [5].

3.6. Selection of Recipient Cow

Selection of embryo transfer recipients was based on breeding parameters and using the following criteria; cows that are reproductively sound, those that exhibit calving ease, have good milking and mothering ability. Their estrus was synchronised and before the transfer of embryos, the recipient cows were palpated for presence of the corpus luteum [25].

3.7. Synchronization of Recipient

Recipients synchronized with prostaglandin F2α (PGF2α) must be treated 12 to 24 hours before donor cows because PGF2α-induced estrus will occur in recipients in 60 to 72 hours [4] and in super ovulated donors in 36 to 48 hours [26]. Synchronizing products are more effective on recipient females that are already cycling [27]. Synchronization of the recipients can be done in a similar manner and at the same working time as the donor cows. The critical point regarding recipient cow estrous synchronization is the timing must match the time of insemination of the donor cow so that the donor and the recipients have a similar uterine environment 7 days later when the transfer takes place. Synchronizing products are more effective on recipient females that are already cycling. Anestrus or non-cycling cows that are too thin or too short in days postpartum will not make useful recipients [28].

3.8. Embryo Handling, Evaluation and storage

3.8.1. Embryo handling

Once an embryo is identified in the searching dish, it is immediately transferred to a small Petri dish (35 × 10 mm) containing fresh, filtered (0.22–0.45 μ pore size), sterile medium. As a holding medium, generally phosphate buffered saline (PBS) containing penicillin plus 10–20 percent heat inactivated serum is used. Embryos are then serially rinsed through at least three different dishes containing fresh sterile medium using a new sterile pipette for each step. Finally, they are placed in a dish awaiting transfer or cryopreservation. Under certain circumstances, e.g. for export, embryos must be rinsed through ten different dishes containing sterile media. All dishes must be kept covered between searches to avoid contamination, and particularly evaporation, when placed in the incubator [4]. Recommended culture condition for bovine embryo are PH of 7.2-7.6, osmolality of 270-310 mOs M/kg, humidity of 100 %, room temperature(15-25oC) or 37°C in incubator and Buffer of Phosphate or bicarbonate ion (later must be maintained under 5% CO2 atmosphere) [29].

3.8.2. Evaluating embryos

After collection, the embryos are evaluated for quality using a stereoscopic microscope. Shape, color, texture, and size are some factors considered during the evaluation [4].

Mapletof *et al*., 2013 [30] indicated IETS (International Embryo Transfer Society) recommended grades for embryo quality range from "1" to "4" as follows:

Grade1 (Excellent or good): Symmetrical and spherical embryo mass with individual blasto meres (cells) that are uniform in size, colour and density. This embryo is consistent with its expected stage of development. The zonapellucida should be smooth and have no concave or flat surfaces that might cause the embryo to adhere to a Petri dish or a straw.

Grade 2 (Fair): Moderate irregularities in overall shape of the embryo mass or size, color and density of individual cells. At least 50% of the cellular material should be an intact, viable embryo mass.

Grade 3 (Poor): Major irregularities in shape of the embryo mass or size, color and density of individual cells. At least 25% of the cellular material should be an intact, viable embryo mass. Grade 4 (Dead or degenerating): Degenerated embryos, oocytes or 1-cell embryos; non-viable. Generally embryos of excellent and good quality, at the developmental stages of compact morula to blastocyst yield the highest pregnancy rates, even after freezing. Fair and poor quality embryos yield poor pregnancy rates after freezing and should be transferred fresh. It is advisable to select the stage of the embryo for the synchrony of the recipient. It would also seem that fair and poor quality embryos are most likely to survive transfer if they are placed in the most synchronous recipients [30].

3.8.3. Storage

Cryopreservation of embryo is essential part of embryo transfer programs. Blastocysts with a multi-layered trophoblast are best suited for rapid freezing and thawing method. Reliable freezing methods have been developed for bovine and sheep embryo. This method includes a one-step addition of 1.4 M glycerol as cryoprotectant, a 20 minute equilibration period, packaging into 0.25 ml straw, slow cooling (0.3 to 0.1 C/min) down to 35 C and subsequent plunging into liquid nitrogen (-196 C) [31]. The frozen embryo can be used as and when required. Embryos are thawed by placing the straws directly into warm water. Glycerol is removed using sucrose [32].

Short-term storage: Embryos can be stored at room temperature for one day for direct transfer from the donor to the recipients. For periods of 24 to 72 hours, the embryos must be stored at 4°C in PBS, medium 199, or medium L15, each supplemented with 50% FBS. Most media and culture systems are adequate for maintaining the viability of the embryo between donor and recipient [33].

Long-term storage: If embryos are to be transported great distances or suitable recipients are not immediately available, a long-term storage system is essential. Deep-freezing embryos is storage in liquid nitrogen (-196°c) for an indefinite period of time. Long-term storage through freezing usually results in damage of 30% to 50% of the stored embryos. Damage is usually caused by ice crystal formation within the embryonic cell. Although the average survival rate of frozen-thawed embryos is approximately 65%, it is profitable to maintain embryos in long-term storage [5].

3.9. Embryo Transfer Method

The actual embryo transfer process is similar to the method used for artificial insemination, except that the transfer gun is passed well up the uterine horn ipsilateral to the CL. The donor may be inseminated naturally or artificially and embryos will be collected non-surgically six to eight days after breeding. Following collection, embryos must be identified, evaluated and maintained in a suitable medium prior to transfer [21].

3.9.1. Non-surgical embryo transfer method

An epidural injection of 5-7 ml of Lidocaine hydrochloride 2% was administered for smooth handling of the genitalia [25]. And the vulva is washed with water and dried with a paper towel. The sheath is coated with sterile, nontoxic obstetrical lubricant and the sheathed pipette is passed through the vulvar labia while avoiding contamination. Before application the recipient cow should be restrained and the rectum is evacuated of feces. At the same time, the presence and side of a functional corpus luteum (CL) is confirmed [3]. The ovaries of the recipient animal were palpated rectally to determine the ovary that ovulated. An embryo was then loaded into 25 ml insemination straw and put in transfer gun. The transfer gun/ insemination rod was carefully passed through the cervix. The embryo was then deposited in the uterine horn epsilateral to the side

of the CL [25]. More recently, the use of non-surgical embryo transfers has increased the utilization of embryo transfer because of even less elaborate requirements. Non-surgical embryo transfer techniques utilized today involve the use of an artificial insemination pipette and more recently, specialized embryo transfer pipettes [3].

3.9.2. Surgical embryo transfer method

Embryos can be transferred via mid-line abdominal incision to cows under general anaesthesia, but through flank incision is far more practical. Recipients are placed in squeeze chutes that give access to either flank. The CL (corpus luteum) is located by rectal palpation and the flank ipsi lateral to the CL is clipped, washed with soap and water, and sterilized with iodine and alcohol [5]. The recipient is prepared for surgery by shaving an area about 6 inches square located some 6 inches in front of the hip joint. The area prepared should be on the side where the corpus luteum is present. A local anaesthetic is injected at the shaved area. The area is scrubbed with alcohol and a 2 inch incision is made with a scalpel. The uterus and the ovaries are brought near the opening of incision by grasping the uterus with the fingers of a hand. A small incision is made in the exposed uterine horn with a blunt needle. The embryo is drawn into a 0.25 ml straw attached to a small syringe and deposited into the uterus. The incision is closed with a few stitches and antibiotic solution is applied into the stitch area to remove infection [2]. About 60 ml of 2% procaine is given along the line of the planned incision [5].

3.10. Pregnancy Detection and Follow-up until Parturition

Pregnancy should be detected at day 30±3 of gestation by rectal ultrasonography (Easi-Scan™, BCF Technology). Measurement of the crown-rump length (mm) was performed to the conceptuses. Confirmation of pregnancy could be repeated at days 60, 90, 150, and 210 of pregnancy [22]. The first good indicator of pregnancy is failure of the recipients to show oestrus 18–24 days after the pre-transfer oestrus. Progesterone assay of milk or blood samples 22–24 days after the pre-transfer oestrus is 95% accurate in diagnosing non pregnancy and about 80% accurate for pregnancy. Development of sensitive automated inline milk progesterone assays should make this technology amenable to commercial application [22].

The first reliable pregnancy- specific hormone assays were developed to measure placenta-derived proteins. This protein found starting around days 17 to 19 of pregnancy in cattle. Also begin to be reliably detectable in plasma starting at day 24, and by day 28. Palpation recommended at 45–60 days per rectum and at about day 26 of pregnancy in heifers and day 28 in cows, pregnancy can be diagnosed accurately under field conditions by ultrasonography. Finally the cow must manage properly on the bases of nutrition, health [5]. If parturition had not occurred by day 283 ± 2 of gestation, labour was induced with 0,150 mg d-cloprostenol q 24h. Human intervention can be needed when calving is difficult. Calving is considered “easy” if little or no help is necessary and “difficult” if heavy assistance is required [22].

4. Importance of embryo transfer

There are different advantages of embryo transfer that include genetic improvement and genetic testing, planned mating, disease control [34], embryo import and export cost [10] and increased farm income [35].

Embryo transfer is now commonly used to produce AI sires from proven donor cows and bulls in AI service [34]. In several countries around the world nucleus herds are now being developed and heifer offspring are being subjected to "Juvenile MOET", while male offspring are being selected for the next generation of AI bulls. In this way, it has been estimated that genetic gains can be doubled [36].

By far the most common use of embryo transfer in animal production programs is the proliferation of so-called desirable phenotypes. Embryo

transfer provides the opportunity to disseminate the genetics of proven, elite females and task in terms of time has been controlled by man. Embryo transfer also permits the development of herds of genetically valuable females, most of which may be sibs if not full-sibs. Embryo transfer has also been used to rapidly expand a limited gene pool. As AI has led to the very valuable bull, embryo transfer has resulted in the very valuable female [34].

Risk of infectious disease transmission is less by *in vivo*-produced embryos, providing embryo handling procedures were done correctly [37]. Several large studies have now shown that the zona intact, washed, bovine embryo will not transmit infectious diseases [34]. In fact, the IETS has categorized disease agents based on the risk of transmission by a bovine embryo [38]. Diseases include Enzootic bovine leukosis, Foot and mouth disease (cattle), Bluetongue (cattle), *Brucella abortus* (cattle), Infectious bovine rhinotracheitis, and Bovine spongioform encephalopathy are not transmitted by *in vivo*-produced bovine embryos, provided that embryo handling procedures were done correctly. Consequently, it has been suggested that embryo transfer be used to salvage genetics in the face of a disease outbreak [39].

The intercontinental transport of live animal costs several thousands of dollars, whereas an entire herd can be transported, in the form of frozen embryos, for less than the price of a single plane fare. Additional benefits of embryos include reduced risk of disease transmission, reduced quarantine costs, a wider genetic base from which to select, the retention of genetics within the exporting country, and adaptation. Over the last 10 years, embryo import regulations for many countries have been simplified. In the year 2002, approximately 30,000 embryos were frozen in North America for export purposes, and in 2010, more than 13,000 embryos were exported from Canada alone [10].

In most cases, altering milk production has to be considered per day of calving interval, as improvements in reproduction increase the time a cow spends in the dry period, which is considered a non-productive stage of the lactation cycle. Improving reproduction often times results in greater availability of replacement animals, which increases herd turnover [35].

5. Factors affecting the success of embryo transfer in cattle

The superovulation system (Walkite *et al*., 2019) [3], failure at embryo recovery (Ball and Peters, 2004) [40], embryo transfer technique failure (Lamb, 2012) [8], Management and weather (Dhavalkumar *et al*., 2018) [34] and Bio security (Mitchell and Doak, 2004) [24] are considered as the major influencing the success of embryo transfer in cattle.

It was recently shown that the average number of embryos recovered from superovulated cattle have been increased from time to time. A serious problem is that approximately 20% of donors produce non usable embryos. Although embryo production per donor has not improved, there have been increases in embryo production per donor on a per unit time basis. In addition, it has been clearly shown that donors do not benefit from having two estrous cycles between superovulations as was widely formerly believed [3].

Following the widespread adoption of non-surgical recovery, often referred to as flushing of embryos in the mid-1980s, the procedures for recovering embryos have received little attention. The embryo collection methods and the equipments applied during the embryo recovery have the potential of impacting the success of pregnancy rate. Virtually all practitioners utilize foley-type 2 and3 catheters with an inflatable cuff. Efficacy of embryo recovery appears similar for both approaches. In contrast, some practitioners achieve fine results by introducing a very small volume of medium with a syringe. Traditionally foley catheters were composed of rubber or latex used this is the main for failure in embryo recovery [40].

Embryo transfer technique failure has impact on success of embryo transfer. Success rates with embryo transfer in many commercial situations are consistently high, often exceeding 70% pregnancy rates (Ball and Peters, 2004). Site of embryo placement in recipient's uterus, embryo size, quality and stage of development have influence on implantation and conception success [8]. The major factors influencing pregnancy rate are probably embryo quality and recipient suitability. Embryo quality is the most important factor in pregnancy rate. However, practitioners have little choice regarding this variable when it comes time to transfer, whereas numerous variables related to recipients provide the opportunity for influencing pregnancy rate [3]. Comparisons between different studies regarding recipient factors are not always legitimate. There has been a great deal of effort directed at identifying a hormone progesterone, human chorionic gonadotropin (hCG), gonadotropic hormone(GnRH) or drug (banamine,clenbuterol) that improves pregnancy rate in embryo transfer recipients. Recently, the use of a low dose (400 IU) of equine chorionic gonadotropin eCG has resulted in improved pregnancy rates in embryo transfer recipients in several field trials [40].

Probably the single most important variable affecting success in ET is the level of donor and recipient management [34]. Lactation status of recipient animals and time of embryo recovery after insemination influences implantation and overall conception rate [8]. Long term weather problems or storms during the superovulation/recipient synchronization process are beyond the control of anyone and can wreak havoc with ET success [34].

There has been little attention focused on the relationship between disease and embryo transfer on a domestic basis within countries, i.e. bio security play important role. As the international trade in frozen embryos grew rapidly during the 1980s, however, this subject received a good deal of attention and very specific protocols have been developed for the production and handling of embryos destined for movement between countries. These protocols have proven quite effective and there are no indications that any identified microbes have been transported internationally in association with embryos. The protocols in place do not necessarily apply to embryos produced by *invitro* procedures and more research is necessary to develop effective sanitary regulations for the production of *in vitro*, cloned and transgenic embryos [24].

The risk of transmitting genetic disease via embryo transfer is the same as that involved in natural mating or artificial insemination; wise selection of dams and sires is mandatory([34]. In light of recent international outbreaks of foot and mouth and bovine spongiform encephalomalities, it is highly likely that the use of media containing no products of animal origin will be mandated for the handling and freezing of all cattle embryos [24].

6. Current status of cattle embryo transfer

Stroud (2012) [41] reported that 572,432 *in vivo* derived bovine embryos were transferred world-wide in 2011, of which 54% were transferred after freezing and thawing. In addition, 373,836 *in vitro* produced bovine embryos were transferred. The International Embryo Transfer Society reports that total numbers of collections of transferred cattle embryos have increased which brought the total number of embryos transferred around 590,561. The study shows that more than 104,651 donor cows were flushed 732,227, transferable embryos were attained. In 2011, North America continued to lead in commercial embryo transfer activity with collection of 51,735 donor cows and the transfer of more than 253,671 embryos (42.95% of all embryo transfers) [41].

The global production of bovine IVF (*in-vitro* fertilization) embryos is exceeded the half-million mark with a total of 592,450 IVF embryos produced 590,359 by ovum pick-up (OPU) method and 2091 by collection of ovaries at abattoirs in 2014. In 2015, among 671,161 IVF embryos produced 612,709 by ovum pick-up (OPU) method and 58,452 by collection of

ovaries at abattoirs. This is a significant increase by 13.3%. Production of OPU transferrable embryos grew significantly from 590,359 embryos to 612,709 embryos, a significant increase of 3.8% and abattoirs 2091to 58452 this increased by 270% in two successive years 2014 and 2015 respectively [42].

The number of *in vivo* derived (IVD) bovine embryos collected in 2015 was 660,221, this result highest of 614,464 recorded in 2014. The value is increased by 7.45%. Regionally, the number of IVD bovine embryo collected leading by North America (64.66%), Europe (22.4%) and South America (11.09%) [42].

The divergent trends for IVD and IVP embryos in cattle resulted in an increase in the proportion of IVP embryos, which accounted for 72.7% of all transferable embryos in cattle in 2019, compared with 68.7% in 2018. Although the proportion of IVD embryos collected and transferred decreased at similar rates (-17.5% and -17.1%, respectively), the number of transferred IVP embryos increased 7.3%. In fact, more IVP embryos were actually transferred in 2019, compared with 2018 (77.3% vs. 72.2%, respectively), and this trend was observed for all regions. There is no significant change in the use of cryopreservation in IVD embryos (60.8% vs. 60.1% for 2019 and 2018, respectively). Conversely, the proportion of frozen IVP embryos transferred increased from 26.8% in 2018 to 43.9 in 2019, resuming the trend observed from 2013 until 2017.In summary in 2019 the world cattle embryo industry was decrease in the production and transfers of IVD embryos, contrasting with an increase in the number of transfers of IVP embryos [43].

Table 2: The global cattle embryo production and transfer in 2019

Region	Number and proportion of countries submitting data by the region		Cattle embryo production in 2019		Transfer of embryo in 2019 in cattle	
	IVD	IVP	IVD	IVP	IVD	IVP
Africa	1	1	4,413	3,645	3,077	3233
Asia	1	0	94	0	44	0
Europe	21	10	124,892	62,009	99,697	47,010
North America	3	3	218,926	525,078	181,301	335,758
Oceania	0	1	0	10,480	0	10,031
South America	3	8	39,444	430,355	35,842	401,158
Total	29	23	387,769	1,031,567	319,961	797,190
Africa	1	1	4,413	3,645	3,077	3233

Source: (Viana, 2020) [43]

The first successful embryo transfer in Ethiopia, resulted in the birth of a Holstein Jersey calf at the Adami Tulu Animal Research Center in the beginning of May, 2010 and five more calves had born [27]. Still now it's not well known in the societies of Ethiopia and no more research is done on this technology. But embryo transfer is more profitable than artificial insemination by giving well improved product. Even though, some authors have evaluated the efficiency of assisted reproductive technologies in different production systems of Ethiopia, well thought-out information is not available [3].

Boran cattle breed is also the only indigenous breed exposed to assisted reproductive technology other than AI in the process of breed improvement, even though reproductive technologies are mostly developed for *Bostaurus* breeds. Boran breeds showed a relatively better potential for application of embryo technology in terms of ovarian follicular growth, superovulatory response, and embryo production [44].

7. Conclusion and Recommendations

Embryo transfer technology applied to animal breeding have the important role of increasing the impact of superior genotype in the population. In cattle, embryo transfer has grown into a mature international business with high success rates and it becomes a well-established industry. Its impact is large because of the quality on animals being produced. Superovulation and embryo transfer are used to increase the number of offspring from genetically outstanding females as well as superior sires. Enough process for production, selection of donor and recipients, embryo recovery and transfer procedures of embryos as well as in recipient management made before embryo transfer provides an effective and economical method to improve fertility in herds. The technology has its own importance regarding to economic development as its application results genetic improvement, genetic implementing, and genetic testing; circumvent infertility, disease control strategies as well as for import and export. Most semen used today comes from bulls that have been produced by embryo transfer. The techniques involved with embryo transfer are not extremely difficult or confusing. A generally successful approach is to build embryo transfer on a programme that has been successful for artificial insemination. Facilities and logistics of handling animals are similar for both techniques. With sufficient training and experience embryo transfer can be a very viable method for genetic improvement or manipulation within any situation. Although embryo transfer is generally costly, it is profitable in both developed and developing countries.

Therefore, based on the above conclusion the following points are recommended:

- There should be collaboration among cattle production industries, universities, institutions and other concerning bodies to create well trained technician for embryo transfer technology and they should have to play role to expand the technology.

- It will have amenable result if used in our local breeds, since the technology enhances the genetic potential and thus the productivity of animals.

- Researchers should concentrate on the embryo transfer to apply the technology properly.

- Awareness should be created in the community and companies about the profitability of embryo transfer.

8. References

1. Choudhary, K., K, Kavya, A, Jerome and R, Sharma, 2016. Advances in reproductive biotechnologies; Published Online 2016 Apr 18. Document, 9(4), 388-395.
2. Kennady, V., S, Vijayalakshmy, J, Manimegalai, V, Ranjeet and R, Vikaschaudhiry 2018. Embryo transfer technology in animals.*Journal of Entomology and Zoology Studies*; **6**(5): 2215-2218.
3. Walkite,F., G, Mustafa and D, Misgana, 2019. Review on Embryo Transfer and Its Application in Animal Production. *Asian Journal of Medical Science Research Review*; **01**: 04-12.
4. Genzebu, D., 2015. A Review of Embryo Transfer Technology in Cattle. *Global Journal of Animal Scientific Research;***3**(2): 562-561.

5. Habtie, A.K., 2019. Review on Growth and Development of Multiple Ovulation and Embryo Transfer Technology in Cattle. Ethiopian Institute of Agricultural Research, Pawe Agricultural Research Center,*World Scientific News;* **127**(3): 191-211.
6. Hasler, J. F., 2003. The current status of oocyte recovery, in vitro embryo production, and embryo transfer in domestic animals, with an emphasis on the bovine. *Journal of AnimalScience* ;**76**: 52-74.
7. Lonergan, P., 2007. State-the-art embryo technologies in cattle. *Social Reproduction and Fertilization Suppliers ;***64**:315–325.
8. Lamb, C., 2012. Factors affecting pregnancy rates in an IVF embryo transfer program. Joint Proceedings of the AETA and the CETA Pp. 3136.
9. Lopes, R. F., F. P, Forell, T. D, Oliveira and J. L. Rodrigues, 2001. Splitting and biopsy for bovine embryo sexing under field condition.*Theriogenology*; **56**: 1383-1392.
10. Mapletoft, R., 2012. Perspectives on Bovine Embryo Transfer. *WCDS Advances in Dairy Technology*;**24**: 83-93. University of Saskatchewan, Canada.
11. Betteridge, K., 2003. A history of farm animal embryo transfer and some associated techniques. *AnimalReproduction Science*; **79**:203-244.
12. Gordon, I., 2004. Reproductive Technologies in Farm Animals. CABI Publishing CAB International Wallingford, Oxford shire OX10 8DE, UK.
13. David, A. and S. Hamilton, 2016. Hamco cattle co. 18th Annual Angus bull sale. Glenboro, Manitoba Canada.
14. Selk, G., 2013. Embryo Transfer in Cattle DASNR 102 Agriculture Hall, Oklahoma State University Stillwater, OK 74078, ANSI-3158, *http://osufacts.okstate.edu*
15. Çizmeci, SÜ. and M, Güler, 2018. Superovulation in cows: A review. *International Journal of VeterinaryScience ;***7**(2): 65-68. *www.ijvets.com*.
16. Tekeli, T., 2010. Embryo Transfer. In: Alaçam E (Editor). Obstetrics and Infertility in Domestic Animals, 7th edition, Medisan, Ankara, Pp: 81-97.
17. Rincker, C. E., 2013. Drafting Embryo Transfer Contracts for Cattle Producers. Texas Agriculture Law Conference, Lubbock, Texas.
18. *https://doi.org/10.1016/j.cvfa.2016.01.008*
19. Jahnke, M., J. West and C. Youngs, 2014. Evaluation of in vivo derived embryos. In Hopper RM, editor.*Bovine reproduction*; Hoboken (NJ),Wiley and Sons. Pp735.
20. Jahnke, M., J, West and C. Youngs, 2017. Evaluation of in vivo derived bovine embryos. Iowa state university, Ames, Iowa, USA.
21. George, E., J, Seidel and M, Sarah, 2005. Training manual for embryo transfer in cattle (FAO) *Animal production and Health* ;Pp: 77.
22. Jordana, S. L., A. T, Estefanía, S. U, Cristina, H, Meriem, C, Sebastian, R, Dimitrios and C, Pilar, 2020. Reproductive Outcomes and Endocrine Profile in Artificially Inseminated versus Embryo Transferred Cows; **10:** 1359. *doi:10.3390/ani10081359. www.mdpi.com/journal/animals.*
23. Chowdhury, M.M.R., X, Lianguang, K, Rami, P, Bun-Young, M, Ayman, J, Myeong-Don, A, Fahmida, I, Jong, L, Hyun-Tae and K, Il-Keun, 2019, Invitro production of sex preselected cattle embryos using a monoclonal antibody raise against bull sperm epitopes. *Animal Reproduction Science;* **205**: 156–164.

24. Mitchell, J.R. and A. Doak, 2004. The artificial insemination and embryo transfer of dairy and beef cattle. **3**: 243-327.
25. Ongubo, M.N., H. A, Rachuonyo, F. N, Lusweti, D. K, Kios, J.K, Kitilit, K, Musee, W.K, Tonui, I. K, Lokwaleput and G. O, Oliech, 2015. Factors affecting conception rates in cattle following embryo transfer. *Uganda Journal of Agricultural Sciences;* **16** (1): 19 – 27.
26. Baruselli, P. and D, Moreno, 2002. The control of follicular wave development for selfappointed embryo transfer programs in cattle. *Theriogenology*; **57**:53-72.
27. Mebratu, B., H, Fesseha and G. E, Eyob, 2020. Embryo Transfer in Cattle Production and Its Principle and Applications, *International Journal of Pharmacalogyand Biomedi*cal *Research*; **7**(1): 40-54. *doi: http://dx.doi.org/10.18782/2394-3726.1083*
28. Baruselli, P. S., R. M, Ferreira, J. N, Sales, L. U, Gimenes, M. F, Sá Filho, C. M, Martins, C. A, Rodrigues and G. A, Bó, 2011. Timed embryo transfer programs for management of donor and recipient cattle. *Theriogenology*; **76**:1583–1593.*doi:10.1016/j.theriogenology.2011.06.006*
29. George, E., J, Seidel and M, Sarah, 2008. Training manual for embryo transfer in cattle (FAO).*Animal reproduction laboratory*; Pp: 79.
30. Mapletoft, R., 2013. History and perspectives on bovine embryo transfer.*Animal Reproduction* ;**10**(3):168-173.
31. Arav, A., 2014. Cryopreservation of oocytes and embryos. *Theriogenology*; 81:96-102.
32. Leibo, S.P. and T. B, Pool, 2011. The principal variables of cryopreservation. Solutions, temperatures, and rate changes, *Fertilization and Sterility*; **2**(96):269-76.
33. Atsushi I., A, Yoshito and T, Kanami, 2013. Research and Development Group, Zennoh Embryo Transfer. Center Kamishihoro, Hokkaido. 080-1407.
34. Dhavalkumar, P., H, Nilufar, P, Gaurangkumar, CH, Ashvinkumar, M, Mahesh, B, Nikita, J, Natvarbhai and P, Pankaj, 2018. Implication of Embryo Transfer Technology in Livestock Productivity. *International Journal of Current Microbiology and Applied Science;* **7**: 1498-1510.
35. Ribeiro, E., W, Galvao, J, Thatcher and J, Santos, 2012. Economic aspect of applying reproductive technologies to dairy. *Herd Animal Reproductive*; **9**:370-380.
36. Mapletoft, R., 2006. Bovine embryo transfer. In: I.V.I.S. (ed.): IVIS Reviews in veterinary medicine, Saskatchewan, Canada.
37. Stringfellow, D., Givens, M and J, Waldrop, 2004. Biosecurity issues associated with current and emerging embryo technologies. *Reproductive Fertilization Development*, 16, 93-102.
38. Stringfellow, D.A. and M. D, Givens, 2000. Epidemiologic concerns relative to in vivo and in vitro production of livestock embryos. *Animal Production Science*; **6061**:629-642.
39. Wrathall, A.E., H. A, Simmons, D. J, Bowles and S, Jones, 2004. Biosecurity strategies for conserving valuable livestock genetic resources. *Reproduction Fertilization Development; 16:103*-112.
40. Ball, P. and A, Peters, 2004. Reproduction in cattle. 2nd edition.
41. Stroud, B., 2012. The year 2011 worldwide statistics of embryo transfer in domestic farm animals. *IETS Newslet*; **50**: 16-25.
42. IETS (International Embryo Transfer Society), 2015. Statistics and Data Retrieval Committee Report the year world-wide statistics of embryo transfer in domestic farm animals.

43. Viana, JHM., 2020. Statistics of embryo production and transfer in domestic farm animals. IETS Data Retrieval Committee: *Embryo Technology Newsletter*; **38**: Pp 1-15.
44. Degefa, T., 2016. Ovarian follicular dynamics, super-ovulatory response, and in vivo embryo production potential of Boran (Bosindicus) and Boran Holstein cross cattle in Ethiopia. PhD Thesis, Addis Ababa University, Faculty of Veterinary Medicine, Bishoftu, Ethiopia.

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