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Review on Bovine Embryo Transfer and its Contribution for Genetic Improvement of Dairy Cattle

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Abstract: The review aims to look at the role Embryo transfer in genetic improvement of dairy cow of local breed in most developing countries, huge in number but low in production and productivity. Embryo transfer may be a multi-step process involving the event and selection from genetically superior females (Donors) of preimplantation embryos and therefore the subsequent transfer of the harvested embryos into reproductively healthy females (Recipients) so as to develop pregnancies and produce live offspring. Currently, in vitro embryo development (IVP) is additionally one among the foremost essential component of bovine embryo transfer technology for genetic enhancement so as to extend the efficiency of livestock. within the field of genetic enhancement, intended marriage, genetic testing of twinning in animals, disease control, circumventing infertility, import and export, still as for research purposes, the applications of this approach are numerous. Advances are applied in research on in vitro fertilization, twin or multiple developments, sexing, embryo genetic stock manipulation and other techniques. But the applying of ET technology is not applicable in most developing countries like Ethiopia, while there is almost two thirds of the livestock population found in this country. This can be due to numerous limitations and also the lack of feedback required for the technology. The government of the developing country should therefore invest within the equipment and other inputs needed for embryo transfer technology to spice up the genetics of local livestock so as to balance the expansion of the human population and therefore the demands of livestock products.

Key words: Bovine · Donor · Embryo Transfer · Recipient

INTRODUCTION

Embryo transfer may be a procedure by which an embryo is obtained from a female donor and so transferred to a female recipient where its production is completed by the embryo and is lucrative for producers of registered purebred animals. A genetically superior female produces more offspring through the utilization of embryo transfer than she could by natural reproduction. The increased number of offspring thus maximizes the genetic abilities of the donor females [1]. It is a multi-step process involving the event and selection from genetically superior females (called donors) of preimplantation embryos and also the subsequent transfer of the harvested embryos into reproductively healthy females (called recipients) so as to develop pregnancies and produce live offspring. Developing countries have almost two-thirds of the world's livestock population, but only about one-quarter to one-third of the world's meat and one-fifth of the world's milk output [2].

The largest livestock population in Africa is believed to be in Ethiopia and this livestock sector has contributed a considerable portion to the country's economy. It is one in every of the world's largest livestock populations, with a population of 59.5 million cattle, placing the state first in Africa and 6th within the world [3]. However, because of their low capacity and poor technical knowledge on a part of milk owners, their contribution to overall production has shown low productivity [4]. Because of the favourable climate of the country, which inspires the utilization of improved, high-yielding animal breeds and provides a comparatively disease-free environment for livestock growth, the country has great potential for the event of milk production.

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Genetic enhancement by crossbreeding has been implemented within the last four decades by growth and research projects. Major components of the development projects were the distribution of crossbred heifers, the supply of insemination service and also the setup of bull service stations [5]. AI is that the first and most often used ART in Ethiopia, like most developing countries. It is been around for nearly fifty years. OSMAI was run because the second reproductive and management technology. Embryo transfer (ET) has also begun at the Debrezeit research facility, but thanks to numerous constraints, the technology has not been extended [6]. This review aimed to present the transfer of bovine embryos and their application within the improvement of cattle reproductive efficiency.

Implementation of Embryo Transfer

Genetic Improvement: With embryo transfer, genetic advancement has generally been considered to be slower than with traditional AI (AI), especially on the idea of national herds. However, genetic advantage may be made on a within-herd basis with increased selection strength and shortened generation intervals, i.e., transferring female calf. The “MOET” multiple ovulation and embryo transfer [7]. This might be particularly useful in enhancing elite herds, the genetics of which may well be dispersed using AI over an outsized population. Embryo transfer is now widely used for the creation of AI sires within the AI service from established donor cows and bulls. "Now nucleus herds are being established in many countries round the world and heifer offspring are subjected to" Juvenile MOET, "while male are chosen during this way for the following generation of AI bulls, it is been estimated that genetic gains will be doubled [8].

Genetic Testing: The genetic testing of AI sires for deleterious inheritance traits is a typical use of embryo transfer procedures [9]. In evaluating new sires, some AI organizations keep known carriers of such genetic defects on hand to act as donors. In order to test foetuses for the presence or absence of the defect, embryos are passed into unrelated recipients and pregnancy may be terminated at different times. Generally, eight to ten non-affected foetuses are adequate to declare a bull free of the feature, depending on the heritability of the defect. Another choice is to mate seven or eight of his superovulated daughters with the bull in question. All recessive characteristics that a bull can bear will then be reflected by Offspring. A less desirable solution would be to pair 40-50 of his daughters naturally with the bull in question.

The success of MOET programs has now led to genetic testing of AI sires using this technology. A program has been established by the Canadian Association of Animal Breeders for the development and testing of the next generation of AI sires. Selected donor cows were super stimulated and the most highly established bulls available were inseminated. Male offspring have been put on hold while female offspring have been put into development. Bulls were then confirmed by production records of their sisters rather than records of their daughters. It was possible to genetically test a bull in 3.5 years with this method, compared to 5.5 years using conventional progeny testing [10].

Circumvent Infertility: By means of super ovulation and embryo transfer, embryos may be obtained from genetically useful cows that have become infertile due to reproductive injury, disease, or age [11]. But only around one-third of those reached with good, fertile donors are success rates. There should be no propagation of infertile heifers and cows with genetically induced sub-fertility. While success rates are poor, oocytes can be recovered from genetically valuable, moribund cows, fertilized, transferred and obtained *in vitro*. Before applying them in the field, this approach requires only systematic optimization [12].

Twinning in Cattle: Beef production is much less effective compared to other domestic animals, since not all cows produce a calf per year. However, it has been estimated that the yield of unit beef can be increased by 60 percent in intensively controlled herds by twinning about 70 percent of a beef cow's nutritional intake for its own maintenance, while only about 30 percent is used for calf growth and maintenance during pregnancy and lactation. Thus, taking advantage of the productivity of gestation and lactation seems attractive. Genetic selection in cattle for twinning has been largely ineffective and therapies with gonadotropin to induce twinning have also been inaccurate. In the development of twins, embryo transfer offers a very real option.

At this time, the most restricting factor is the transfer cost that tends to surpass the average price for beef to be raised for calves. It should be remembered that recipients carrying twins need additional nutrition and management, particularly special nutrition.

Disease Control: None of the infectious diseases studied in the bovine species were transmitted by embryos that were developed *in vivo*. Several major studies have now

shown that the Zona-intact bovine embryo cannot transmit infectious diseases, provided embryo handling procedures have been performed correctly. Consequently, in the face of a disease epidemic, embryo transfer has been proposed to be used to save genetics. For example, in creating herds that are free of Bovine Leucosis, this could be a useful alternative, since this virus was not transmitted with embryos [13].

Import and Export: It cost several thousand dollars to transport a live animal on an intercontinental basis, while an entire herd can be shipped in the form of frozen embryos for less than the price of a single plane fare. The decreased chance of transmission of infectious diseases, however, is the overwhelming advantage of using embryos in foreign trade [14]. A large genetic base from which to pick, the conservation of genetic within the exporting country and adaptation are further advantage of the export of embryos over that of live animals. Finally, as staff within the importing country must be able to thaw and transfer embryos effectively, as they do with semen today, the international movement of embryos is heavily dependent on technology transfer [15].

Research: Techniques for embryo transfer have proved to be a very useful method for science. In fact, before 1970, many technological advances in embryo transfer aimed at research purposes rather than the propagation of superior livestock. In research on the natural limits of twin pregnancies, uterine ability and endocrine regulation of the uterine environment, maternal pregnancy identification, embryo-endometrial interactions and pregnancy endocrinology, embryo transfer may be used. Studies initially designed to address basic physiological questions are now being used to enhance and maximize embryo transfer utilization. In the use of embryo transfer for research purposes, newer methods have added a whole new viewpoint [16].

***In vivo* Embryo Production Procedure**

Selection of Donor Cows: For most embryo transfer programs, there are two general requirements for the choice of donor cows: (1) genetic dominance, i.e. animals contributing to the program 's genetic objectives; such objective metrics include milk quality, milk composition, growth rates, calving ease and disease resistance; and (2) the probability of generating large numbers of usable embryos. While the event of embryos should be secondary to genetic dominance, it should be seriously considered. The foremost active donors involve healthy, cycling cattle with a history of high fertility. Donors develop more embryos a minimum of two months post-partum than those closest to calving. Under certain circumstances, young cows appear to supply significantly more accessible embryos than heifers. Lactation does not decrease the response to superovulation in either beef or dairy cows, providing cows are cycling and do not slim. Extremely fat cows are weak donors because they not respond well to superovulation and since it is harder to manage their reproductive tracts. Many healthy embryos are typically not developed by sick animals [17].

Selection of Sires: Given that 1/2 the genes come from the male, genetically superior bulls are extremely essential to use. In fact, it is generally more essential to pick out the male than to pick the female donor since males will typically be raised to many females and may be chosen more correctly than females. Fertile bulls and fertile semen must even be picked, which makes it particularly important to use top quality semen [18].

Superovulation of Donor Cows: Superovulation refers to the discharge during one oestrous cycle of several oocytes (eggs) [19]. The goal of super stimulation treatments in cows is to get the utmost number of highly likely pregnancy-producing fertilized and transferable embryos [20]. Superovulation could be a very inefficient thanks to extract oocytes from bovine ovaries and is probably going to be substituted over the subsequent decade by other methods. Superovulation, however, ends up in about ten times more embryos than the regeneration of one ovum. A functional embryo is retrieved about 60 percent of the time from average donors by qualified technicians without superovulation. Superovulation normally produces a mean of six functional embryos under identical circumstances, but the variation is incredible. No embryos are usually retrieved from 20-30 percent of superovulated donors and from another 20-30 percent, only 1 to a few embryos is obtained. From about one third of the donors, a perfect response of 5 to 12 embryos is obtained. A limited proportion of donors, however, produce quite 20 healthy embryos and, very rarely, over 50. Differences in super stimulatory therapies like the preparation of gonadotropin, the batch of gonadotropin, the length of treatment, the timing of treatment with relevancy the oestrous cycle, the overall dose of gonadotropin and also the use of additional hormones within the super stimulation protocol were related to variability in ovarian response. Factors inherent within the animal and its environment are secondary, even more important, causes of variability. Nutritional status, reproductive history , age, season, breed, ovarian status

at the time of treatment and therefore the effects of repeated super-stimulation is also included in these variables. While significant recent progress has been made within the field of bovine reproductive physiology, factors intrinsic to the donor animal that influence the superovulatory response are only partially understood. Superovulation-inducing treatments are typically initiated between days 8 and 12 of the estruos cycle (oestrus = day 0) these periods were originally supported the hypothesis that a follicle wave within the ova was initiated. The two widely accepted cattle superovulation techniques are supported two distinct gonadotropins, although there are several minor variations in these techniques. The best is to present a more correctly designated equine chorionic gonadotropin (eCG) injection of 1800-3 000IU (usually 2000-2 500IU) pregnant mare serum gonadotropin (PMSG), among a luteolytic dose of prostaglandin F2 alpha or an analogue shot two to 3 days later [21].

The second style of superovulation is to subcutaneously or intramuscularly give eight to 10 injections of follicle stimulating hormone (FSH). At half-day intervals, under field conditions, shot is more practical. Like PMSG, 48-72 hours after initiation of treatment with the fifth, sixth, or seventh FSH injection, prostaglandin F2 alpha is given. At half-day intervals with prostaglandin F2 alpha administered with the sixth or seventh FSH injection, the foremost typical FSH regimen is 6, 6, 4, 4, 2, 2, 2 and 2 mg. Cows weighing over 800 kg can receive about 20 percent more gonadotropin. Higher doses for the primary two days are often used; others give 5 mg for every injection. There are few studies with ample donor numbers per treatment group during which constant and declining doses are compared, so it is difficult to draw accurate conclusions about the effectiveness of such regimens [22]. Equine anterior pituitary gland extract and human menopausal gonadotropin (which also contains significant LH) are other drugs that are used for superovulating cows. The previous is generally not commercially available and therefore the latter is simply too costly for normal use [23].

More recent findings indicate that superovulation protocols may be conducted frequently and with an interval of care of as less as 33 days without affecting embryo recoveries using the present research aimed toward evaluating superovulation in cattle at short intervals employing a porcine follicle stimulating hormone (FSH) prostaglandin implant [24].

Heat Detection and Insemination of Donor: Estruos identification, where this can be achieved exactly, is one of the major achievements of ET. The duration of the oestrous cycle should be normal on a regular basis, since it can have a detrimental effect on the superovulation process if it is irregular. It is easier to perform oestrous detection on two consecutive oestrous cycles and it is normally performed in the morning and afternoon. When conducting oestrous detection, an abnormality of the oestrous cycle, such as silent heat event, should be avoided. Oestrous synchronization should be performed correctly in both donor and recipient as this will help the achievement of the ET program. Also, the key aspect that should be taken into account is visual observation [25]. There is a greater need for viable sperm cells to enter the oviducts of the superovulated females due to the release of several ova's from multiple follicles. Therefore, before and after oestrous, several embryo transfer technicians will option to inseminate the cow many times. At 12, 24 and 36 hours after the start of the standing oestrous, one scheme is to inseminate the superovulated calf. It is a very important move in any embryo transfer program to use high quality semen with a high percentage of natural, motile cells. The proper location for the positioning of semen is in the uterine body. This is just in front of the cervix, a tiny goal (1/2 to 1 inch) [26].

Surgical Recovery of Embryos: The first active cattle ET experiments were obtained by surgical procedure from the embryos. By intravenous knockdown injection with a halothaneoxygen mixture, the donor, who had fasted and calmed, was anaesthetized. This healing procedure is achieved by conducting a laparotomy to reveal the reproductive tract (flank or midline abdominal incision). To obstruct the distal one-third of the uterine horn, a clamp or the thumb and forefinger may be used to force fluid injected into that segment through the oviduct with a gentle milking motion and collect it at the infundibulum. The occlusion of the uterine horn in the uterine body is an alternative treatment. The medium of culture is inserted at the uterotubal junction through a puncture or through the oviduct until the uterus is turgid. With a blunt needle attached to a flexible catheter, the uterus is then punctured. The strain, with enough instability to bring the embryos into a collection tube, will cause the medium to gush through the catheter. These procedures allow for a high percentage of embryos to be recovered. However, they can only be replicated a couple of times because of the surgical trauma and associated adhesions [27].

In the early days of commercial bovine embryo transfer, around day 4 after oestrus, embryos were harvested surgically from the cow. Near the time of ovulation, unfertilized oocytes for advanced applications (such as in vitro fertilization) must be obtained. This is

achieved from the follicles, ovary surface, or oviduct. Embryos are obtained at any time between fertilization and implantation for most applications, but usually after migrating to the uterus. In cattle, embryos normally recover 6 to 9 days after oestrus for commercial purposes; nonsurgical recovery is unsuccessful before this point. With the surgical transfer of bovine embryos, regeneration and pregnancy rates was significantly decreased after nine days. In both animals, surgical recovery may be performed and is the method of choice for sheep, goats and hogs. Techniques differ slightly with the organisms [16].

Non-Surgical Recovery of Embryos: To estimate the amount of corpora lutae, the first step in non-surgical recovery is to palpate the ovaries per rectum. If there is a large response to superovulation, this is very hard to do correctly, but calculating how large this response is not important. Four or five embryos are rarely retrieved, except in exceptional cases where only two or three corpora lutae are palpated by trained staff. However, if there is no palpable corpora lutae by day 7, it is extremely uncommon to receive embryos. Ultrasonography offers details about responses that are more precise than palpation [28].

For non-surgical rehabilitation operations, epidural anaesthesia is advised. To avoid spinal column infection, the tail head should be trimmed, then scrubbed with iodine soap and swabbed with 70 percent alcohol. The cow can lose control of the rear legs by injecting too much anaesthetic and fall down in the chute. Using a new 18-gauge needle, it is recommended to inject 5 ml of a sterile 2 percent solution of procaine into water each time. Healthy epidural anaesthesia can be tracked by tail flaccidity. The tail should be secured to one side, out of the way, as the epidural anaesthesia takes place. Dust, manure, loose hair, etc. should be washed from the rear end of the cow and then the vulvar region thoroughly scrubbed with iodine soap and properly rinsed with 70 percent alcohol swabs. A reasonable period of time should be provided for the vulvar lips to dry until inserting the recovery tool to prevent bringing any alcohol into the uterus; disinfectants are highly toxic to embryos. Recovery procedures are carried out by rectal manipulation. Due to epidural anaesthesia, during removal and reinsertion of the hand, the rectum can easily balloon due to air entry. It is incredibly difficult to operate efficiently once air has come in. A simple air pump attached to a tubing length to remove air from the rectum is an outstanding investment, as rectum ballooning occurs occasionally, even with trained staff. Even then, the safest way is to avoid as much oxygen as possible from entering the oxygen. A tiny silicone rubber catheter (Foley catheter) is inserted through the cervix of the donor cow to extract the embryos non-surgically and a special medium is flushed into and out of the uterus to extract the embryos seven days after oestrus [29].

Non-Surgical embryo recovery is reasonably easy and can be done without harm to the cow in less than an hour. In order to give rigidity for passage through the cervix into the body of the uterus, the pre sterilized stylet is inserted in the lumen of the catheter. The cuff is progressively filled with roughly 2 ml of regular saline while the tip of the catheter is in the body of the uterus. The catheter is then pulled softly into the inner orifice of the cervix so that the cuff is seated. To fully seal the internal orifice of the cervix, additional saline is then applied to the cuff. Attached to the catheter is a Y-connector with inflow and outflow tubes. To control the flow of flushing fluid, a pair of forceps is connected to each drain. The fluid is applied and extracted by gravity sequentially. The fluid in the uterus, especially in the upper one-third of the uterine horn, is stirred rectally. Finally, the uterus is loaded with medium to about 40-day pregnancy sizes. Per donor, one litre of fluid is used. Most technicians for embryo transfer use a smaller volume and flush one uterine horn at a time. Depending on the size of the uterus, each uterine horn is filled and drained five to ten times with 30 to 200 ml of fluid each time. With this fluid, the embryos are flushed out and harvested with the fluid in a filter. In the filter, the pores are smaller than the embryos, but without losing the embryos, excess fluid drains out of the cell. In order to assess their consistency and stage of development, embryos are isolated from flush media and studied under a microscope [30].

Invitro Embryo Production Procedure

Oocyte Collection: The collection of oocytes is normally achieved by follicle separation, dissection, aspiration or follicle slicing or oviductal flushing. The recovery technique will vary depending on whether pre- or postovulatory oocytes are required and whether collection is to be made on single or repeated occasions from slaughtered animals or from live cattle. It may be a matter of extracting the ovaries by ovariectomy or accessing the ovaries by laparoscopy or ultrasound guidance while dealing with live animals [31, 32].

Oocyte Recovery: Abattoir Ovaries: From ovaries harvested at the slaughterhouse, oocytes can be readily retrieved. In order to maintain oocyte developmental competence, ovaries should be transported to the

laboratory within 1 h in Dulbecco's phosphate buffered saline (PBS) at temperatures between 25 and 35°C. It is understood that cell metabolic activities are slowed down or completely stopped at low temperatures, while cellular autolysis can occur at elevated temperatures in the ovaries during a long period of transport. In several species, several authors have reported that ovary storage at 38°C for several hours decreased the rate of blastocyst development. Low-temperature ovary storage in cattle does not appear to affect the maturation of oocytes and the ability of oocytes to grow into blastocysts. However, at low temperatures, porcine oocytes are more susceptible.

Oocytes may be extracted from antral follicles either by aspiration or by slicing after washing the ovaries in a fresh medium. In the first case, a 5 mL syringe containing Tissue Culture Medium-199 (TCM-199) with Earle's salts and bicarbonate combined with Hepes, heparin, antibiotics (most widely used: penicillin / streptomycin solution or gentamycin) and polyvinyl alcohol (PVA) is used to aspirate antral follicles > 2 mm in diameter using a needle (usually 19-20 gauge). In the other scenario, using the micro-blade and the follicle material released in the same medium mentioned above, the ovaries are cut. Cumulus-oocyte complexes (COCs) are then examined under a stereomicroscope and selected for *in vitro* maturation within the follicle oocytes, surrounded by many layers of somatic cells, the cumulus cells.

Ovum-Pick-Up (OPU) Technique: The Ovum-Pick-Up (OPU) is a procedure that enables the aspiration of the follicles from live animals after the endoscopic or ultrasound visualization of the ovaries and thus the collection of the oocytes. OPU / IVP's current technology aims to extract oocytes from preselected genetically superior living donors, followed by in vitro maturation, fertilization and culture until the morula or blastocyst stage has been reached by embryos. This technique makes it possible to repeatedly create embryos of particular value from live animals and is a significant alternative to superovulation. Many studies have been conducted after the initial production of the OPU technique to maximize the output of the oocytes harvested and thus the subsequent yield of the embryo. The first experiments were aimed at improving the surgery and based on technological changes (geometry of the needles, vacuum pressure, etc.). At the same time, biological factors were also studied, such as the donor animal itself, hormonal pre-stimulation, timing and frequency of OPU and the experience of OPU operators. Hormonal pre-stimulation prior to OPU using gonadotropins was the most important change with respect to improved oocyte yield and quality and thus subsequent embryo development. Multiple treatments with exogenous gonadotropins prior to follicular aspiration have been shown to boost the amount of oocytes recovered in both bovine and small ruminants and to increase the embryo development of processed oocytes compared to non-stimulated donors. However, systematic experience has shown that follicular dimension and health, hormonal profile, interval between last exogenous gonadotrophin stimulation and follicle aspiration and donor therapy can affect the quality of recovered oocytes.

***In vitro* Embryo Maturation:** *In vitro* oocyte maturation can be triggered by extracting the fully grown (competent) oocyte from its follicular environment; *in vitro* maturation (IVM) culture systems, this phenomenon is used. Oocytes are matured in TCM199 supplemented with 10% serum for 24 hours in the presence of gonadotrophins at 38.5°C at 5 % CO2. Different techniques such as staining the oocytes (M-II stage), identification of the extruded first polar body in the perivitelline space and degree of cumulus cell mass expansion evaluate the maturation rate in vitro of oocytes.

Sperm Capacitation: In the epididymis, the spermatozoa do not necessarily move; they become motile only after ejaculation or when dissolved in an acceptable medium. Bull sperm slow down and resume their migration only when ovulation occurs after ejaculation and when they enter the isthmus of the reproductive tract of the cow; they finally reach the ampulla region of the oviduct, where fertilization takes place. Sperm must undergo a further substantial maturation process, called capacitation, in addition to acquiring the capacity to step forward.

***In vitro* Fertilization:** Efficient *in vitro* fertilization (IVF) of cattle requires sufficient preparation of the two gametes, as well as favourable conditions for cultivation. Many of the fundamental mammalian fertilization events have been explained and the molecular mechanisms behind these events have been recognized [33]. In vitro matured oocytes are co-incubated in the fertilizing medium {Brackett and Oliphant (BO) medium or Tyrode's modified medium} with frozen in vitro capacitated spermatozoa for 24-48 hrs at 38.5°C at 5 % CO2.

***In vitro* Culturing:** Mechanically denuded by pipetting, presumptive zygotes were cultivated in four-well dishes in Synthetic Oviduct Fluid (SOF) supplemented with 5 % (v / v) ECS, 40 µL / mL BME (Amino Acids Solution 50, Sigma-Aldrich, Germany) and 10 µL/1 mL MEM

(Non-essential Amino Acid Solution 100, Sigma-Aldrich) coated with mineral oil at 39°C in a maximum humidified atmosphere of 5 % CO2, 5% CO2 and 5% CO2, respectively. On day 3 (IVF = day 0) and day 7, respectively, cleavage and blastocyst rates were reported.

The cleavage rates in experiment 1 are based on oocytes recovered in 14 OPU sessions (8 and 6 for experimental periods 1 and 2, respectively). In 9 (3 and 6) sessions, blastocyst rates are dependent on oocytes. This is because a proportion of oocytes and developing embryos have been sent to other parts of this project where structural and proteomic studies have been conducted. The outcomes of these studies will be separately published. The cleavage and blastocyst rates in experiment 2 are based on 4 IVF experiments for this purpose. The total number of COCs used for the measurement of cleavage and blastocyst concentrations per age group and experiment.

Embryo Handling, Evaluation and Storage

Embryo Handling: To prevent the transmission of pathogens, careful handling of embryos between selection and transfer is important. It is important to use aseptic procedures, sterile solutions and sterile equipment. Exposure to harmful factors must always be a concern when dealing with embryo handling in laboratories, where they eventually come into contact with glassware, petri dishes, plastic straws and other devices [27].

After filtering the collection medium through a filter with pores that are around 50-70 µm in diameter, embryos are situated under 10 X magnification with a stereoscopic dissecting microscope. While embryos are generally moved as soon as possible after selection, it is possible to keep embryos at room temperature for several hours in a medium holding position. Bovine embryos may also be cooled in a holding medium and stored for 2-3 days in the refrigerator. As a final option, at a later date, embryos can be frozen for use. Embryos are typically preserved in the same medium or in a medium close to that in which they were gathered. To maintain a pH of 7.2 to 7.6 and have an osmolality of about 300 molar osmolality, the media must be buffered. Dulbecco's PBS or more complex media are usually used in the field with the Hepes buffer and enriched with FCS and antibiotics. For the long-term culture of bovine embryos, more complex media with a carbonate buffer usually produce superior results. Embryo collection keeping and freezing media free of animal products have recently become available, eliminating the need for cooling and increasing biosecurity, as suggested earlier [34].

Table 1: Recommended culture conditions for bovine embryo

PH	7.2-7.6
Osmolality	270-310 mOsM/kg
Humidity	100 percent
Temperature	Room temperature (15-25°C) or 37°C in incubator
Buffer	Dulbecco'sPBS or bicarbonate ion

Source: [30].

Embryo Evaluation: Assessment of bovine embryos must be performed at a magnification of 50 to 100 Times, with the embryo in a small culture dish. It is important that the different stages of development can be identified and contrasted with the stage of development at which the embryo should be entered on the days of oestrus. The availability of recipients will also depend on a decision as to whether an embryo is worthy of transmission. Embryos of reasonable quality should be freshly transferred if recipients are accessible. The International Embryo Transfer Society (IETS) finds inappropriate exports of embryos of low and equal quality [35].

According to the Manual of the International Embryo Transfer Society, embryos are identified and analyzed by morphological inspection at 50 to 100 X magnification [36]. The total diameter of the bovine embryo is 150 to 190 um, with a thickness of 12 to 15 mm for the zona pellucida. From the single cell stage until the blastocyst stage, the total diameter of the embryo remains largely unchanged. The best measure of the viability of an embryo is its development stage compared to what it should be after ovulation on a given day. An ideal embryo is compact and spherical; with even colour and texture, the blastomeres should be of equal size; the cytoplasm should not be granular or vesiculated; the space of the perivitelline should be smooth and contain no cellular debris; the zone pellucida should be uniform; neither fractured nor collapsed and should not contain surface debris. The key requirements for quality evaluation usually include: the regularity of the embryo's form, the compactness of the blastomeres (the dividing cells within the embryo's boundaries), the difference in the colour of the cell size and the texture of the cytoplasm (the fluid within the cell wall), the overall diameter of the embryo The presence of extruded cells, the thickness and regularity of the zone pellucid (the protective l of the cell wall), the overall diameter of the embryo[37].

Quality Evaluation: The IETS has suggested embryo quality codes ranging from "1" to "4" as follows:

Code 1: Outstanding or fine. With individual blastomeres (cells) that are uniform in size, colour and density, symmetric and spherical embryo mass. This embryo is in line with its planned developmental stage. Zona pellucida should be smooth and have no concave or flat surfaces that can cause a Petri dish or a straw to cling to the embryo.

Code 2: Fair. Moderate defects in the general form of the mass or size of the embryo, the colour and the density of each cell. An intact, viable embryo mass should be at least 50 percent of the cellular content.

Code 3: Poor mean the presence of significant anomalies in the shape of the mass of the embryo or in the scale, colour and the density of each cell. An intact, viable embryo mass should be at least 25 percent of the cellular content.

Code 4: Dead or degenerating. Degenerated, non-viable embryos, oocytes or 1-cell embryos. Generally, embryos of outstanding and decent quality show the highest pregnancy rates, even after freezing, at the developmental stages of the compact morula to blastocyst. Embryos of good and bad quality produce poor pregnancy rates after freezing and should be freshly moved. It is advisable to pick the embryo stage for the recipient's synchrony. If they are put in the most synchronous recipients, it would also appear that reasonable and low quality embryos are most likely to survive transfer [38].

Embryo Developmental Stage Evaluation: Embryos are evaluated for his or her stage of development without relation to quality. It is advisable to pick out the stage of embryo development for the synchrony of the recipient [39].

Morula: A mass of a minimum of 16 cells. Individual blastomeres are difficult to discern from each other. The cellular mass of the embryo occupies most of the perivitelline space.

Compact Morula: Individual blastomeres have coalesced, forming a compact mass. The embryo mass occupies 60 to 70% of the perivitelline space.

Early Blastocyst: An embryo that has formed a fluid-filled cavity or blastocoels and provides a general appearance of a seal ring. The embryo occupies 70 to 80% of the perivitelline space. Early during this stage of development, the embryo may appear of questionable quality.

Blastocyst: Pronounced differentiation of the outer trophoblast layer and of the darker, more compact inner cell mass is clear. The blastocoele is extremely prominent, with the embryo occupying most of the perivitelline space. Visual differentiation between the trophoblast and therefore the inner cell mass is feasible at this stage of development.

Expanded Blastocyst: The diameter of the embryo dramatically increases, with a concurrent thinning of the zone to approximately one-third of its original thickness.

Hatched Blastocyst: Embryos recovered at this developmental stage will be undergoing the method of hatching or may have completely shed the zone. Hatched blastocyst is also spherical with a well-defined blastocoele or could also be collapsed. Identification of embryos at this stage is difficult unless it re-expands.

Embryo Storage: The storage system must not only preserve the viability of the embryo for several applications, but must also promote continued development. So as for get to be compatible with later events, it should even be beneficial to retard growth to a degree approaching physiological state. For example, before appropriate recipients become available for transfer, it's going to be necessary to store embryos. It is possible to transfer donor embryos directly into recipients or to preserve them for future use [40].*Short-term storage*

For direct transfer from the donor to the recipient, embryos are kept at temperature for in the future. Embryos must be preserved at 4°C in PBS, medium 199, or medium L15 for periods of 24 to 72 hours, each supplemented with 50 percent FBS. For preserving the viability of the embryo between donor and recipient, most media and culture systems are adequate [41].

Long-Term Storage: A long-term storage system is important if embryos are to be transported distant or suitable recipients don't seem to be immediately available. For an unspecified period of your time, deep-freezing embryos are preserved in cryogen (-196oc). Long-term freezing storage typically ends up in damage to 30 percent to 50 percent of the embryos deposited. Damage is usually caused within the formative cell by frost snow

formation. Although the typical survival rate of frozen-thawed embryos is about 65 percent, storing embryos in long-term storage is profitable [41].

Recipient Selection, Synchronization and Embryo Transfering

Recipient Selection: To achieve high pregnancy rates and have a decent body size to forestall dystocia problems, recipients must have proven reproductive efficiency, freed from congenital or infectious diseases [42]. The recipient cows were non-pregnant animals with a postpartum span of quite 90 days, but five calves and no gross pathological features within the genital tract.

Only animals were selected with a CL that was identified by rectal palpation. Generally, females are ideal as recipients: Cows 3 to eight years old observe recipients once they need an honest calving record, Heifers are good recipients providing they need achieved their breeding weight (about 65-70% of mature weight) and are cycling, Use fertile animals, Animals are docile and body condition, a perfect score of two.5-3 is desired at the time of transfer Every regular therapy should happen a minimum of 3 weeks before transfer; improvements to the feeding regimen should be avoided for 3-4 weeks before and after transfer. Recipients should be placed where, on the day of transition, they will be handled quickly and quietly [43].

Synchronization: Conditions within the recipient's reproductive tract should closely mimic those within the donor so as to optimize embryo survival within the recipient female after transfer. This includes coordination between the donor and therefore the recipients of the oestrous cycles, optimally within someday of every other. Recipient synchronization are often performed in a very similar way and at the identical working time because the donor cows. There are a range of protocols for oestrous synchronization [44]. Various oestrous synchronisation protocols are tested. Treatment included prostaglandin injection (PGF2 alpha, 25 mg i.m.) on CL palpation and warmth sign observation, the second regimen within which gonadotropin (GnRH; 100 µg) was administered additionally to PGF2 alpha 7 days before PGF2 alpha injection and warmth sign detection after PGF2 alpha injection. The gonadotropin-releasing hormone (GnRH) and prostaglandin (PGF2alp) oestrous synchronization system has been shown to be very efficient in cattle oestrus synchronization [45].

The key point for recipient cow oestrous synchronization is that the timing must match the donor cow's insemination period specified when the transition happens, the donor and also the recipients have an identical uterine environment seven days apart. Synchronizing items are more efficient for female who are already cycling with recipients. "The valuable beneficiaries wouldn't be" Anoestrus "or non-cycling cows that are too thin or too short in postpartum days [46].

Surgical Transfer: Embryos can be transferred to cows under general anaesthesia through mid-line abdominal incision, but it is much more realistic via flank incision. By rectal palpation, the CL is located and the flank ipsilateral to the CL is clipped, washed with soap and water and iodine and alcohol sterilized. This appears more accurate in daily practice than using a paravertebral block. The surgeon makes a skin incision approximately 15 cm long, high on the flank, just anterior to the hip, after scrubbing. There are divided muscle layers and the peritoneum is sliced. The uterine horn is externalized by gripping the broad uterine ligament, which is situated medial to the uterine horn and extending it with the thumb and forefinger. One third of the exposed uterine horn is made of a puncture wound with a blunted needle through the cranial wall, so it is very delicate. An assistant draws the embryo from the storage container using approximately 0.1 ml of medium in a small glass pipette (< 1.5 mm outside diameter). Then, the pipette is inserted into the uterine lumen and the embryo is removed. To be sure that the embryo has been deposited in the lumen needs some practice. The incision, using two layers of sutures, is then covered. The surgery takes about 15 minutes with preparation[47].

Non-Surgical Transfer: First, in order to pick the ovulation side, it is important to correctly palpate the ovaries. If embryos are moved to the uterine horn contralateral to the corpus luteum, pregnancy rates are markedly decreased. Recipients may also be refused if no corpus luteum is present or reproductive tract pathology is noted. Passing the embryo transfer system via the cervix is the next step. In a 0.25 ml transfer straw, the petri dish containing the embryo is put under the stereoscope for loading. Then the straw is placed into a transfer gun and protected with a sheath for transfer. The transfer gun is inserted into the vagina of the receiver in a manner

similar to that defined for uterine flushing. Transrectal manipulation directs the tip of the transfer gun through the cervix and brings it through the uterine horn. Some scholars suggest that the embryo be inserted ipsilateral into the corpus luteum in the left uterine horn. Because of the tiny cervix, heifers present a particular challenge. Experience with artificial insemination is the perfect preparation before attempting non-surgical embryo transfer. The third stage of non-surgical transfer is to be able to rapidly, smoothly and traumatically insert the tip of the instrument into the target uterine horn [48].

Since it is less costly, non-surgical transfer is preferable; it is easier and does not require surgical procedures. This can also obviate the need for veterinary supervision, which in many countries is needed for surgery. The basic Cassou Inseminating gun for French straws is the most widely used method for non-surgical transfer, as it is inexpensive and easy to use properly [49].

Pregnancy Diagnosis of Recipients: Failure of receivers to show oestrus 18-24 days after pre-transfer oestrus is the first successful pregnancy indicator; clearly, the reverse, showing oestrus, suggests non-pregnancy, although a small percentage of pregnant animals are around three weeks after the previous oestrus in behavioural oestrus. 22-24 days after the pre-transfer oestrus, the progesterone assay of milk or blood samples is 95 percent effective in diagnosing non-pregnancy and about 80 percent effective for pregnancy. This technology should be commercially applicable in the production of responsive automated inline milk progesterone assays [50].

To measure placenta-derived proteins, the first accurate pregnancy-specific hormone assays have been created. Pregnancy-specific protein B (PSPB) is the first of these measured circulating concentrations. Pregnancy-specific protein is developed by placental giant bi nucleate cells that develop from mononuclear trophoblast cells beginning around days 17 to 19 of cattle pregnancy [51]. Pregnancy-specific protein B concentrations begin to be reliably detectable in plasma from day 24 and are sufficiently elevated by day 28 to enable them to be used for a highly accurate pregnancy test in cattle.

Diagnosis of pregnancy can typically be definitively identified after day 35 of pregnancy by palpation per rectum [52]. Until day 45, palpation is not recommended, both because the idea is more delicate at the early stages and because the evidence is not conclusive due to the incidence of spontaneous abortion, even in the absence of palpation. At 45-60 days of gestation, it is also advised to palpate per rectum and confirm this with another palpation one month later. Cows that display oestrus may be checked by palpation or ultrasonography prior to 45 days. Of course, in these later phases, ultrasound may also be used. Pregnancy can be correctly diagnosed at approximately day 26 of pregnancy in heifers and day 28 in cows under field conditions by ultrasonography or even earlier in very professional hands. They can be recycled for use as recipients if they are, in fact, non-pregnant [53].

Managing Pregnant Recipients: Recipients at the bunk are frequently ignored. However, if we would like them to perform (breed and lift healthy babies), a decent feeding program must be maintained. The last three months of pregnancy are crucial, nutritionally speaking, relative to how soon a cow will breed back after calving. Bear in mind that lactation is that the most difficult time within the lifetime of a cow. A suckling calf rapidly consumes its energy reserves. She doesn't cycle (have a heat period) until her calf is weaned if she is lean at calving and goes into heavy milking directly. Additionally, her chances of becoming pregnant are decreased if she does cycle when in bad flesh and lactating. She may cycle and breed back in 60 to 100 days after calving if she calves in good flesh. The key point to be emphasized is that dietary supplements should have to be implemented weeks prior to of the planned breeding, if necessary. You are not near pondering trace elements and vitamins with protein and energy supplements. Protein and energy are out and away the foremost restrictive dietary variables for livestock fertility [54].

CONCLUSION

For real genetic improvement, embryo transfer is now getting used, especially within the dairy industry and most semen used today comes from bulls provided by embryo transfer. A good greater advantage of embryo transfer is that by washing procedures, defined pathogen-free bovine embryos generated *IN VIVO* is made, making this a perfect technique for disease control programs or within the international movement of animal genetics. The event of in vitro embryos and also the sexing of embryos and semen also are successful, but time and price restrict their widespread use. A mixture of embryo

transfer using proven cows inseminated with semen from proven bulls, followed by industry-wide AI appears to be the foremost common use of bovine embryo transfer within the near future. In most of developing countries including Ethiopia Bovine embryo transfer technology is not widely applied as a tool of animal genetic improvement because of different constraints. Therefore, the manufacturers, embryologists, Veterinarians and other Herd Management Members of the team must be mindful of short-term and long-term variables. to steer to a successful embryo transfer program and to possess adequate manufacturing process, selection of Donors and recipients and embryo transfer procedures before, additionally as in recipient management, it will be essential to a successful and economical transfer of embryos are often Method for improving fertility in herds. Stakeholders like, research centers and University workers must collaborate with one another so as to hold the sustainability of the program's development.

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