Review on Advances in improving quality of bovine embryo transfer

Habtamu addis, Abebe mequnent, Temsegen sendekie

University of Gondar College of veterinary medicine and animal science, Department of veterinary clinical, Gondar, Ethiopia, P.O. Box:196
Email: yohansaddis68@gmail.com

Summary: Embryo transfer is a breeding techniques where by a sexually mature female (donor) is injected with exogenous hormones to produce more ova which are then fertilize in vivo, remove prior to their implantation and transferred to the reproductive tract of a surrogate mother (recipient). This seminar paper is prepared to review recent advances in improving bovine embryo quality, give highlight on the general embryo transfer technology and review the noble cryopreservation techniques. At present embryo transfer is mostly utilized for genetic improvement, twining, importation and/or exportation of genetic material, disease control and applied research in animal production. Cryopreservation is a process where embryos, cells, whole tissues, or any other substances susceptible to damage caused by chemical reactivity or time are preserved by cooling to sub-zero temperatures. Cryopreservation methods seek to reach low temperatures without causing additional damage caused by the formation of ice during freezing. There are several ways that we can improve the quality of bovine embryo, among this artificial insemination, embryo culture media in vitro production of embryos represent techniques aimed at a better control of animal reproduction thereby embryo must be preserved by appropriate preservative methods before transferring to recipients and attentions should be concentrated on the culture media that the embryo grows.
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Introduction

As a result of the increased world population, demand for animal products has been grown several folds. In view of this, livestock production has also dramatically transformed allowing for efficiency and improvement in productivity. Significant improvements in livestock productivity in the developed countries were achieved through the application of different biotechnologies, followed by the extensive uptake of these new technologies'. Among the most recent advances are made in the area of artificial insemination, embryo transfer, invitro fertilization, cloning, transgenic and genomics. While some of these applications are still achieved under continuous research scale ups, techniques like embryo transfer have achieved remarkable success particularly in the diary industry (Gardner, 1994). Embryo transfer refers to a step in the process of assisted reproduction in which one or several embryos are placed into the uterus of a female with the intent to establish a pregnancy. In animal husbandry, embryo transfer has become the most powerful tool for animal scientists and breeders to improve genetic construction of their animal herds and increase quickly e-late animal numbers which have recently gained considerable popularity with seed stock dairy and beef producers (Betteridge, 2000).

An essential part of livestock production, research, species preservation, and human reproduction is the ability to collect and store embryos for extended periods of time, which can later be used to produce healthy offspring. Storage is made possible by freezing embryos at extremely low temperatures in cryoprotectant solutions that reduce damage due to ice formation. This technology is referred to as cryopreservation and has made significant advances in recent years. The first recorded instances of embryo transfer did not include storage of embryos for any length of time. These first primitive embryo transfers were surgical procedures involving the direct transfer of the embryo from donor to recipient speared on the tip of a needle(Wrathal *et al.*, 2004).

Further advances in embryo transfer technology have led to improved freezing that allows storage for almost indefinite periods of time. Typically embryos are frozen in cryoprotectant solutions which reduce damage caused by ice crystal formation. This process allows these embryos to later be thawed and transferred to recipients as viable embryos (Vajta and Kuwayama, 2006).

Embryo quality is an important determinant of the success of practical embryo transfer. Accurate evaluation of embryo quality before embryo transfer contributes to improvements in rate of pregnancy. Successful development to the morula and blastocyst stage is influenced by oocyte morphology, medium composition, embryo handling, and exposure to changes in temperature and atmosphere Conditions of

oocyte maturation and fertilization also affect the ability of embryos to develop to the later pre - implantation stages. Therefore, successful assisted production of high quality embryo and an accurate method for assessment of embryo quality (Gardner, 1994).

2. Litereture Review

2.1. General principle of embryo transfer in cattle

The principle of ET and related process has been reported to be simple however; it is also a technology that requires expertise in many areas. it includes several consecutive stage such as the selection of genetically superior donors, selection of recipients (usually of low genetic but high ability), hormonal treatment of the donor for the induction of super ovulation, synchronization of the donor and recipient, fertilization of the donor, embryo recovery from the donors, evaluation and storage of embryo recovered and embryo transfer to the recipient by either surgical or non surgical method which would complete the pregnancy and give birth to calf. The procedure of embryo transfer is very crucial and great attention and time should be given to this step. The ultimate goal of a successful embryo transfer is to deliver the embryos atraumatically to the uterine fundus in a location where implantation is maximized (Gilbert and Epel, 2009).

2.1.1 Application of embryo transfer technology

Over the years, techniques associated with ET have has many uses, especially in research. The widespread use of this technology in animal breeding schemes, however, is relatively recent. Genetic engineering and related new technologies will only increase its utilization For example; several research laboratories are presently using IVF techniques to study the fertilizing capacity of sperm (Christensen, 1991).

On the other hand application of ET has been useful in the control of disease transmission. None of the infectious disease studied in the bovine species have been transmitted by in vivo- produced embryos, provided embryo handling procedures were correct. Several studies have now shown that bovine embryo will not transmit infectious disease consequently, it has been suggested that ET be used to salvage genetics in the face of a disease outbreak. Breeders are now using embryo transfer techniques to establish disease free herds to be used strictly for export purpose (fellow, 1998).

Another area of application of ET is transport of high value animal across different countries. The international transport of a live animal may cost 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. However, the reduced risk of infectious disease transmission is the overwhelming benefit for using embryos for international trade. This may be the single most important potential application of embryo transfer (Wrathal *et al.*, 2004).

2.1.2. Selection synchronization and super ovulation of donor

Strict selection criteria should ensure genetic superiority and a high level of success, thereby making the procedure more economical. In some cases, the sole criterion for selection is scand ET is used to increase number of animal available. In the selection of donors the objective measures of genetic superiority can be, for example in diary breed, milk production milk composition, growth rate, calving case, and donor selection may involve a previous history of success in ET. In beef herds, when selecting genetically superior donors the objective trait such as calving ease, milk production, weaning and yearling weight and carcass value should be considered (Daris, 1998).

For successful transfer of embryo the recipient must be in good health and its cycle well for successful transfer of embryo the recipient must be in good health and its cycle well synchronized with that of the donor. Careful attention to estrus detection, handling, and nutrition of the recipient are essential parts of any ET program. Regardless of the method of synchronization used, timing and critical attention to estrus detection are important. Recipients synchronized with PGF must be treated 12 to 24 hour before donor cows because PGF –induced estrus will occur in recipients in 60 to 72 hours and super ovulated donors in 36 to 48 hours the success of estrus synchronization program in bovine is dependent on an understanding of three general areas:1) estrus cycle physiology in the cow,2) pharmacological agents and their effect on the cows estrus cycle, and3)herd management factors that reduce anestrous and increase conception rates (Bo *et al.*, 2005).

Another important component of the ET program is super ovulation which is the release of multiple eggs at a single estrus. Cows or heifers that are properly treated can release as many as 10 or more viable egg cells during one estrus. The basic principle of super ovulation is to stimulate extensive follicular development through the use of a hormone preparation given intramuscularly or subcutaneously. Three different types of Gonadotropin have been used to induce super ovulation in the cow; Gonadotropin from extracts of porcine or other domestic animal pituitaries, equine chorionic Gonadotropin (eCG) and human menopausal Gonadotropin (hMG). eCG has a longer biological half life in the cow than either FSH or hMG; consequently a single injection of 2000-3000 IU will induce so, while FSH and hMG require

multiple injection treatment regimens for optimum effect (Noakes *et al*., 2002).

2.1.3. Embryo collection, evaluation and handling

The first method developed to collects embryos was a surgical method which allowed the recovery of high percentage of embryo however, because of surgical trauma and the resulting adhesions, it was not possible to repeatedly perform the procedure many times. More recently a non surgical technique of recovery was developed. This involves the use of aloes catheter that will allow flushing fluids to pass in to the uterus and at the same time fluids to be returned from the uterus to a collecting receptacle (Betteridge, 2000).

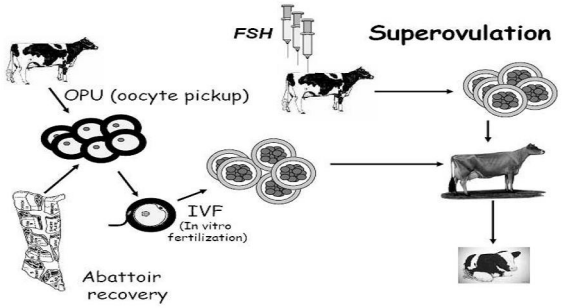
Collection is performed at the most flexible stages of the embryo (usually day 7 of estrus cycle) for both freezing and fresh transfer in bovine. Antibiotics are added to protect the embryo from bacterial contaminates acquired during flushing and to avoid transferring pathogens to the recipient. The success rate of ET depends on great deal on an accurate evaluation of embryo recovered. Certain deviations from normal development in 6 to 8 day old embryo can be detected with the light microscope. Attention should be given to the compactness of the cell, regularity of shape, variation in the size, color, and texture of cytoplasm, presence of vesicles, presence of extruded cells normal embryo size, regulation of the zona pelucida, presence of cellular debris and proper stage of development for the age (Smith, 2004).

Although embryos are usually transferred as soon as possible after collection, it is also possible to maintain embryos for several hours at room temperature in holding medium. It is also possible cool bovine embryos in holding medium and to maintain them in the refrigerator for 2-3 days.

. Embryo may be frozen for use at alter date. Initially, the commercial embryo transfer industry primarily utilized simple media such as phosphate buffered saline (PBS) (Arthur *et al*., 2003).

2.1.4. Embryo transfer

Embryo transfer refers to the collection of embryo from donor animal and its placement in to the oviduct or uterus of recipient animals. Initially, embryo transfer in bovine was done surgically, whereas most are done today using non-surgical methods. Non surgical embryo transfer techniques utilized today involve the use of an artificial insemination pipette and more recently, specialized embryo transferred pipettes. After confirming synchrony of estrus, the recipient is restrained and the rectum is evacuated of feces. At the same time, the presence and side of a functional CL is confirmed. Care is taken to prevent ballooning of the rectum with air. An epidural anesthesia is administered and the vulva is washed with water (no soap) and dried with a paper towel. The embryo is loaded in 0.25 ml straw between at least two air bubbles and the straw is loaded in in the embryo transfer pipette. care must be taken to ensure that the straw engages thesheath tightly so as to avoid leakage(Betteridge, 2000).



(Betteeridge, 2000).

Figure 1. Different schemes for producing embryos for embryo transfer programs in cattle

2.2. The techniques of cryopreservation

The development of effective methods of freezing embryos has made embryo transfer a much more efficient technology. For many years, embryos were frozen very successfully in glycerol, but because glycerol penetrated cell membranes rather slowly, it also had to be removed slowly after thawing. This necessitated microscopic examination and time for dilutions in the Laboratory. Recently, the use of highly permeating cryoprotectants such as ethylene glycol has allowed the direct transfer of bovine embryos. With this approach, the embryo straw is thawed in a water-bath, and its contents are deposited directly into the uterus of the recipient, much like AI. There is no need of a microscope or complicated dilution procedures. Recently new technology has improved the chances of an embryo developing after thawing. Cryopreservation is impossible without some inherent damage to the embryo. The extent of this damage depends on the size and shape of embryos, permeability of membranes, and overall condition of the embryo at the time of collection (Vajta and Kuwayama, 2006).

2.2.1. Slow freezing

Classic cryopreservation, commonly referred to as slow cooling, involves controlled cooling rates and the presence of low concentrations of cryoprotectants. Embryos are cooled at 0.3- 0.5^0c which slowly dehydrates the cells rendering the cytoplasm a glassy solid. The dehydration process prevents intracellular ice from forming thus limiting damage. Effective slow cooling takes place at relatively low cryoprotectant concentrations, approximately 1.0 molality. At these low concentrations, the toxicity to the embryos is minimal (Kasai, 1996).

In slow-freezing, cells in a medium are cooled to below freezing point. At some stage, ice masses containing pure crystalline water will form. What remains between the growing ice masses is the so-called unfrozen fraction, in which all cells and all solutes are confined. The concentrations of sugars, salts and cryoprotectant (e.g. glycerol) increase, while the volume of the unfrozen fraction decreases. The increase in osmotic strength causes an efflux of water from the cells. Slow cooling is needed in order to allow sufficient efflux of water to minimize the chance of intracellular ice formation (Moussa *et al*., 2005).

2.2.2. Verifications

Advances in verification appear to be the most promising with respect to efficiently preserving viable embryos. These techniques not only require less sophisticated equipment than older methods but also are less labor intensive and time consuming. Also in certain species verification has dramatically increased efficiency and even helped make cryopreservation a reality in species whose embryos were previously extremely difficult to freeze (Seidel, 2006).

Freezing and thawing procedures are time consuming and require the use of biological freezers and a microscope. These steps can be replaced by a relatively simple procedure called verification. High concentrations of cryoprotectants are used and the embryo in its cryoprotectant solution is placed directly into liquid nitrogen. Because of the high concentration of cryoprotectants, ice crystals do not form; the frozen solution forms a glass. As ice crystal formation is one of the most damaging processes in freezing, verification has much to offer in the cryopreservation of oocytes and IVF embryos. However, its greatest advantage is its simplicity in application. Verification procedures are now widely used experimentally and it is only a matter of time before they find commercial application (Wagtendonk, 1996).

2.2.3. Cryoprotectant

Cryoprotectants such as glycerol in concentrations ranging from 1.0 to 2.0 molality are required to ensure embryo survival after freezing. It is thought that cryoprotectants act by reducing the amount of ice present at any temperature during freezing, thereby moderating the changes in solute concentration. Recommended criteria for a cryoprotectant include high solubility, low toxicity at high concentrations, and a low molecular weight both for easier permeation and to exert a maximum colligative effect(Palasz. and Mapletoft 1996).

Glycerol has been most often used for the protection of embryos during freezing but more recently, more permeating cryoprotectants such as ethylene glycol, have been preferred because they can be used with "direct transfer" i.e., transfer into a recipient without prior removal of the cryoprotectant. During the addition and dilution of a permeating cryoprotectant, the cell undergoes osmotic changes resulting in swelling or contraction. Consequently, if the addition or particularly the dilution is carried out inappropriately, the viability of cells can be affected (Wagtendonk, 1996).

2.3. Advance in improving quality of bovine embryo

Production of competent embryos is the ultimate goal of ARTs to maximize the probability of implantation and ensuring embryonic/fetal development. Successful development to the morula and blastocyst stage is influenced by oocyte morphology, medium composition, embryo handling, and exposure to changes in temperature and atmosphere Conditions of oocyte maturation and fertilization also affect the ability of embryos to develop to the later pre implantation stages (Holm, 1998).

2.3.1. Donor and recipient selection

Reasons for wanting to do embryo transfer on a given animal are more often economic than genetic. As optimal results will reduce costs, making the procedure much more economical, donor selection may involve a previous history of success in embryo transfer. In addition, it has been suggested that the potential donor animal be at its prime reproductive age, that it has a previous history of a high level of fertility and that it has demonstrated superiority in traits of economic importance. Strict selection criteria will not only ensure genetic superiority, but should also ensure a high level of success thereby making the procedure more economical (Seidel, 2006).

Good nutritional and health status, as well as adequate management, are important factors to ensure the success of invitro embryo production (IVEP) programs. The use of virgin heifers as recipients is usually indicated as the best option for achieving higher pregnancy rates. The option of using cows with suckling calves should consider nutrition and health conditions, so that estrous cycles can restart soon after calving, in time to respond to synchronization protocols). If an adequate nutritional and health status has been established, the use of cows may effectively present certain advantages compared to heifers. Another interesting aspect is that cows have been exposed more to pathogens. Thus, they may have greater disease resistance and better quality colostrum. Finally, the frequency of dystocia tends to be higher in heifers than in cows (Pontes *et al*, 2009).

2.3.2. Semen quality and success in AI

Regarding the quality of semen used in AI programs, it has been reported that differences in Fertility level could be attributed to variations in sperm qualitative characteristics. The Success of bovine AI programs largely depends on the use of

good quality semen. When only High fertility bulls are used; better conception rates are achieved, which reduces costs of reproductive programs. Individual bulls may differ in their ability to fertilize oocytes and/or to develop to blastocyst stages after in vitro fertilization (IVF) procedures. In addition, different sires and/or batches may differ in the individual response to induction of in vitro sperm cap citation methods, and in the response to acrosomal maintenance after invitro incubation (Wei and Fukui 1999).

Semen handling (and/or semen thawing protocol) might also be an important factor influencing in semen quality and, therefore, in AI results. Because the size of breeding herds continues to increase and the use of estrus synchronization (as well as the fixed-time artificial insemination protocols) becomes more frequent worldwide, there are increasing probabilities that several cows will be inseminated on the same day. Hence, several inseminators have used the practice of thawing, simultaneously, more than one straw of semen in the same thawing-bath unit to increase the convenience of semen handling (Sudano *et al*., 2011).

The standard recommendations for cryopreserved bovine semen are (unless otherwise specified by the manufacturer): 1) to thaw no more straws than can be deposited in the female within 15 minutes between thawing and insemination, in a water-bath at 35°C for a minimum of 45 seconds, always maintaining thermal homeostasis during this interval; 2) Prevent direct straw to straw contact during the thaw process; 3) Implement appropriate thermal and hygienic protection procedures to maintain thermal homeostasis and cleanliness during gun assembly and transport to the cow (Takahashi *et al.,* 1993).

In Ethiopia, AI was introduced in 1938 in Asmara (the current capital city of Eritrea). In 1967, an independent service was started in the Arsi Region, Chilalo Awraja under the Swedish International Development Agency (SIDA). The present national artificial insemination technology for cattle has been introduced at the farm level in the country over 35 years (Sudano *et al*., 2011).

The National Artificial Insemination Center (NAIC) was established in 1984 to coordinate the overall AI operation at national level. The efficiency of the service in the country, however, has remained at a very low level due to infrastructure, managerial, and financial constraints, as well as poor heat detection, improper timing of insemination and embryonic death. Cattle breeding are mostly uncontrolled in Ethiopia making genetic improvement difficult and an appropriate bull selection criteria have not yet been established, applied and controlled. Although artificial insemination, the most commonly used and valuable biotechnology has been in operation in Ethiopia for over 30 years, the efficiency and impact of the operation has not been well-documented. Reproductive problems related to crossbreed dairy cows under farmers' conditions are immense. It is widely believed that the AI service in the country has not been successful to improve reproductive performance of dairy industry from the previous little studies; it has been found that AI service is weak and even declining due to inconsistent service in the smallholder livestock production systems of the Ethiopian highlands (Dekeba *et al*., 2006).

2.3.3. Quality of oocyte

The influence of the oocyte quality on the developmental potential of the embryo has been recognized in the cow more clearly that in any other species. When mammalian oocytes, including those from the cow, are removed from their follicles, they have the ability to spontaneously resume meiosis. Meiotic resumption can be visualized under the microscope by the first polar body extrusion or with specific dyes to stain the metaphase. The capacity to cleave is almost automatic and is an intrinsic potential within the fully grown oocytes of large mammals because it can occur in absence of fertilization by a simple activation stimulus (electrical current, ethanol). The quality of oocyte can be determined more specifically by the evaluation of the characteristics of, oocyte cytoplasm, polar body, perivitelline space, zona pellucida, and meiotic spindle at the same time (Wang and Sun, 2001).

Table*:* The criteria showing the quality of oocyte

Criterion	Parameters
Cumulus-oocyte complex	Compactness and thickness of the cumulus investment, brightness of the cytoplasm
Cytoplasm	Granularity, coloration, regions of organelle clustering
Polar body	Shape (round or ovoid), size (large or small), surface (smooth or rough), cytoplasm (intact or fragmented)
Zona pellucida	Thickness, structure
Perivitelinis space	Size (normal or increased), the presence or absence of grain
Meiotic spindle	Location and refraction

Source: (Wang and Sun, 2001).

2.3.4. Embryo culture media

Embryo culture is a component of in vitro fertilisation where in resultant embryos are allowed to grow for some time in an artificial medium before being inserted into the uterus (Kasai, 1996).

Glutathione contain culture media

Developmental competence of oocytes is strictly dependent on their (nuclear, cytoplasmic, and

membrane) maturation. The super oxide dismutase (SOD) treatment was apparently effective in protecting oocytes from superoxide radicals during maturation. The ineffective addition of GSH might be explained by the fact that the cumulus cells surrounding the oocytes are GSH rich, as already shown in the hamster. This should sufficiently protect oocytes from free radical damage. Hydrogen peroxide production was detected in oocytes and with increased levels in zygotes, but not in the cumulus oophorus. The presence of GSH, even in the control group, could be sufficient to protect oocytes from oxidative stress and the cysteine could support a good level of GSH synthesis during in vitro maturation to decrease chances of finding expected differences between untreated and treated groups (Takahashi *et al.,* 1993).

Effect of SOD and GSH during IVC Protection of embryos from oxidative stress may be a prerequisite for development in vitro because of extreme sensitivity to superoxide anions. GSH showed a beneficial effect on development, and the greatest influence was with addition on day 6 post inseminations. One of the effects of GSH is reduction of H_20_2 acting as a substrate of GSH peroxidase; In vitro development is usually retarded; thus, day 6 post insemination, corresponding to the time that embryos progress from 8-16 cells to the morula stage, is a phase in which they could be more sensitive to the oxidative stress (Costa *et al*., 2013)..

Proportions of oocytes developing to morula and blastocyst stages were not increased by the presence of SOD in maturation and culture medium, and SOD was deleterious when present during the insemination interval. Interestingly, cleaved oocytes after IVM with an appropriate concentration of SOD demonstrated greater viability in vitro. The addition of GSH for culture significantly improved the development of bovine embryos. The efficacy of exogenous GSH in enhancing development emphasizes a possible extracellular role since intracellular concentrations of GSH are generally adequate and GSH is not readily transported into cells (Takahashi *et al.,* 1993).

Serum free culture

Bovine embryos cultured without serum had more cryo resistance, particularly when embryos were vitrified. Culture in the absence of serum results in less lipid accumulation in embryos. A further finding is that embryos with fewer lipids, produced without serum in the medium, also cryopreserve more successfully that lipid rich embryos produced in the presence of serum. The relationship between the lipid droplets (LD) accumulated in the embryo and its impaired ability to survive cryopreservation has been clearly demonstrated. Bovine embryos cultured in serum containing media, abnormally accumulated lipids into their cytoplasm. The presence of serum, and no other media components, was the primary cause of most developmental differences observed between in vitro produced embryos. In embryos, the maturation of mitochondria during IVC is associated with increases in metabolism reflected in the oxygen consumption and CO_2 production and it appears to be related to depletion of stored products (cytoplasmic lipids) (Abe *et al*., 2002).

On the other hand, the presence of large amounts of lipid droplets in embryos developed in serum supplemented media probably damages cellular mechanisms responsible for repairing plasma membranes after cryopreservation. Moreover, the addition of serum could promote the incorporation of saturated fatty acids into the plasma membrane, since in adult ruminants these fatty acids are predominant in serum. This incorporation could probably induce changes in membrane composition, making it more rigid, and unable to withstand cryopreservation (Mucci *et al.,* 2006).

Embryos developed in serum containing media have a higher proportion of tropho ectoderm cells compared with embryos produced in serum-free media. Tropho ectoderm cells are crucial for blastocoels re-expansion and maintenance after cryopreservation, and the higher lipid contents in tropho ectoderm than the ICM, could make T cells particularly damaged during cryopreservation (Rizos, 2001).

Invitro bovine culture media with thyroid hormone

Recent studies showed that supplementing *in vitro* bovine culture media with thyroid hormones (THs) was significantly beneficial effect in terms of developmental rates and overall embryo quality. It has recently been shown that THs have a beneficial effect on bovine oocyte and embryo quality, as well as developmental rates *in vitro.* Costa and colleagues reported that addition of thyroid hormone T3 to oocyte maturation medium increased the quality of oocytes and rate of development to the blastocyst stage). Further they reported that the THRs α and β were detectable in granulosa cells but only THR α was detected in oocytes (Costa *et al*., 2013).

The present review has demonstrated a clear positive effect of TH supplementation on the early bovine embryo development *invitro*, with the blastocyst formation and hatching rates significantly increased after exposure to THs. The supplementation of IVC media alone was able to produce these capacity were also improved. Collectively, these results provide evidence that TH supplementation would likely help optimize currently used *invitro* embryo production media, enhance efficiency of Assisted Reproduction Technology (ARTs) and help combat the obstacles obviating the applications of assisted reproductive

methods in many mammalian species, including humans (Sutton *et al*., 2004).

2.3.5. Invitro production of embryo

Although each ovary contains hundreds of thousands of oocytes (eggs) at birth, many thousands undergo atresia and are lost, starting before birth. This tremendous loss of genetic material could be salvaged by harvesting oocytes from the ovary and using IVP techniques bovine IVP is now a reasonably efficient procedure; transvaginal ultrasound-guided oocyte aspiration at frequent intervals, in combination with in-vitro fertilization (IVF) has proved its worth in improving the yield of embryos from designated (Hasler *et al*., 2003).

For the set up of the techniques as well as for the large scale production of average genetic merit embryos, large quantities of material can be obtained at low cost by collecting ovaries at slaughterhouses. The needle used as well as the aspiration vacuum is important factors in determining the number and quality of the oocytes collected. After aspiration or slicing, the collected fluid is screened under a stereomicroscope to select the oocytes. The oocytes are surrounded by several layers of somatic cells (cumulus oophorus). These cells establish contacts together as well as with the oocyte to allow cell communication through gap junctions, which is necessary for efficient maturation of the oocyte (Van *et al*., 2002).

In vitro maturation is probably the most critical part of the whole process of in vitro embryo production. Ruminant oocytes are usually matured at 39°C under a 5% CO2 in a humidified atmosphere. The optimal maturation time (more than 90% of the oocytes at the metaphase II stage) are 22-24h. Due to the high lipid content of oocytes cytoplasm, it is not possible to follow the progression of their nuclear status during culture. Additionally, the presence of the cumulus cells is required during IVM for efficient cytoplasmic maturation and these cells mask the oocyte. Consequently, the only visible sign of oocyte maturation during IVM is the expansion of the cumulus cells. These cells produce hyaluronic acid which is secreted and polymerized in the extracellular matrix, leading to the increase of intercellular space (Kasai, 1996).

The media used for IVF are Tyrode derived saline supplemented with bovine serum albumin (BSA), lactate and private and with a capacitating agent. Sperm cap citation occurs physiologically in the female genital tract. This phenomenon involves modification of spermatozoa membrane as well as changes in its mobility properties that lead to spermatozoa fertilization ability. Many protocols support sperm cap citation in vitro. In cattle, heparin is widely used for this purpose, usually without pre treatment. Frozen semen is generally used and a selection step is required to enrich the sperm suspension in living and motile spermatozoa. In the swim-up method, spermatozoa are deposit at the bottom of a culture medium containing tube and allowed to swim in the medium. The top fraction, containing motile sperms is then collected after a given incubation time (Cognie *et al*., 2004).

2.3.6. Determination of embryo quality

Morphological evaluation is the most common and simplest method to grade embryos. The age of the embryo, stage of development and morphology of the embryo can all be assessed by visualization with a stereomicroscope. Embryos progress through several stages. The international invitrro embryo transfer society (IETS) has categorized the embryonic stages of development. Code 1 is an unfertilized oocyte or a 1-cell embryo. Code 2 describes embryos that are 2 to 16- cells (Day 2 to 5). Code 3 is an early morula (Day 5 to 6). Code 4 is a morula (Day 6). Code 5 identifies an early blast cyst (Day 7). Code 6 is a blast cyst (Day 7 to 8). Code 7 describes an expanded blast cyst (Day 8 to 9). Code 8 is a hatched blast cyst (Day 9). Code 9 depicts expanding hatched blast cysts (Day 9 to 10). The rate of development can also be used to assess embryo viability (Merton *et al*., 2002)

Embryos are examined for the presence of cellular debris, degree of compaction, blastomere color and texture, density and evenness of cleavage. Poor quality embryos are less likely to result in a pregnancy than higher quality embryos. According to the IETS, embryos are scored with one of 4 codes. Code 1 consists of excellent or good embryos. Irregularities should be relatively minor, and at least 85% of the cellular material should be an intact, viable embryonic mass. Code 2 embryos are fair. They have "moderate irregularities in overall shape of the embryonic mass or in size, color and density of individual cells. At least 50% of the cellular material should be an intact, viable embryonic mass." Poor embryos are code 3. These embryos have "major irregularities in shape of the embryonic mass or in size, color and density of individual cells. At least 25% of the cellular material should be an intact, viable embryonic mass." Code 4 constitutes dead or degenerating embryos, oocytes or 1- cell embryos (String fellow, 1998).

3. Conclusion And Recommendations

Embryo Transfer is one of the biotechnologies which have considerable impact on farm animal breeding programs in developed countries. It consists of several steps each of which is critical; therefore, failure in any portion of the procedure will probably result in overall failure. A combination of embryo transfer using proven cows inseminated with semen

from proven bulls, followed by industry-wide artificial insemination appears to be the most common use of bovine embryo transfer in the near future. Super ovulation and embryo technology have become a crucial part of cattle breeding in most parts of the world. In addition, the underlying review has clarified various features of endocrinology, culture conditions and manipulations which may affect oocyte and embryo quality, and thus help to explain reduced fertility in cattle. It greatly facilitates the transfer of genetic material between countries, both for individual animal of high genetic material and to induce exotic breeds. Apart from revolutionizing the animal production system, it contributes a lot in understanding of application of ET program in different manners. The science and practice of artificial embryo production (in vitro production and cloning) have given researchers valuable insight into the importance of the early embryonic period for later foetal and neonatal development. Successful assisted reproduction in animal requires culture techniques for production of high quality embryos and an accurate method for assessment of embryo quality. Based on the above conclusion the following recommendations are forwarded:

- Embryo must be preserved by appropriate preservative methods before transferring to recipients.
- The more advanced technologies must be adopted to ensure quality of bovine embryo for effective preservation and transfer of embryo.
- During the in-vitro production of embryo attentions should be concentrated on the culture media that the embryo grows.

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Corresponding Author:
Habtamu Addis
Department of veterinary clinical medicine
College of veterinary medicine and animal science
Tewodros campus, university of Gondar
Gondar, Ethiopia p.o. Box:196
Telephone: +251921281124
Email: yohansaddis68@gmail.com

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