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Bovine Embryo Transfer and Its Application: Arview

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SUMMARY
Since the production of the first calf by embryo transfer in 1951, the method has been progressively improved so that it can now be used for commercial ends. The applications of this method are numerous in the field of research, but its main economic interest is in bovine selection programmes. Selection intensity in donor females can be doubled in elite herds, thus increasing the possibility of producing high yielding bulls and heifers. The embryo transfer technique can also be used for the salvage of genetic material from terminally ill cows or infected herds. International transport of embryos has been made possible by progress in freeze-thaw procedures, but depends upon the resolution of regulatory problems and the training of transfer personnel. Furthermore, embryos are the safest and most cost effective alternatives to move genetics internationally because of their low risk of transmitting diseases. One of most important factors associated with the success and widespread application of this technology is evaluation of the embryos before freezing and/or transfer to a recipient. Embryos are usually classified based on a number code system for their stage of development and for their quality Surgical methods for embryo collection and transfer are increasingly replaced by non-surgical methods, which are much more practical and less expensive in selection programmes.
Keywords: bovine, embryo transfer, donor, recipient, embryo collection

INTRODUCTION

Embryo transfer is a process by which an embryo is collected from a donor female and then transferred into a recipient female where the embryo completes its development. Embryo transfer is profitable for producers of registered purebred animals. Through the use of embryo transfer, a genetically superior female produces more offspring than she could by natural reproduction. The increased number of offspring thus maximizes the donor female's genetic abilities. Embryo transfer is used in several species of domestic animals, namely cows, horses, goats, and sheep. Research indicates its use in certain non-domestic species, such as deer, elk, bison, and wildcats. Interestingly, one country uses embryo transfer to improve the quality of animals used in the sport of camel racing (Stroud, 2010).

Using rabbits, a researcher at the University of Cambridge in 1890 performed the first embryo transfer. It was not until 1951 that others succeeded with the technique in cattle. As an industry, the first transfer of a bovine embryo was reported in 1949 and the first calf from embryo transfer in 1951. Milestones in the development of this technology have been evaluated from the point of view of their significance to our current knowledge of reproduction and to the improvement of animal agriculture. Application of embryo transfer to the cattle industry began in the early 1970s when European dual-purpose breeds of cattle became popular in North America, Australia and New Zealand. Breeders and speculators sought means to circumvent the high costs and lengthy quarantine periods linked to the importation of European breeding stock and to capitalize on premium prices that progeny from these rare dams and sires could command (Betteridge, 2003). Thus, demand for embryo transfer services existed in advance of the ability of veterinarians and reproductive physiologists to supply them. This considerable economic incentive, however, inspired rapid development of practical techniques for superovulation and surgical recovery and transfer of bovine embryos, and the establishment of clinics to sell the technology to the public. Because the value of embryo transfer offspring was based on scarcity, however, the exercise was self-defeating. The inflated market for European dualpurpose breeds in North America collapsed abruptly in 1977 because the numbers of these animals increased markedly as a result of embryo transfer. During this short-lived boom, nevertheless, techniques had been improved and costs reduced. Notable was the development of procedures for non-surgical recovery and transfer and cryopreservation of embryos. With these improvements and a more realistic economic motivation, the industry now plays a useful role in the cattle industries of many countries (Seidel, 2003).

Commercial bovine ET is a well established, mature industry in its fourth decade of existence in many countries. The history of the development of the industry has been previously described. As used henceforth, ET will refer individually or collectively to the collection, handling and transfer of embryos. Research in the field of ET is very expensive and many of the basic procedures were established some years ago. Consequently, little academic research currently is being conducted that is likely to immediately benefit the commercial industry. Because success rates in well-managed cattle herds are generally quite high, most recent improvements involve rather small, albeit important, increments. A valuable approach to understanding how various factors in ET influence success is to analyze well kept, accurate commercial records. A good share of the data presented in this review involves just such an approach (Betteridge, 2000).

Commercial ET programs vary widely in terms of factors that may be beyond the control of practitioners. For example, selection of specific donor cows and service sires are ultimately chosen by the owner. Long term weather problems or storms during the superovulation/recipient synchronization process are beyond the control of anyone and can wreck havoc with ET success. Travel problems sometimes means traveling to a farm a day or two late, which mandates working with older embryos than planned. Probably the single most important variable affecting success in ET is the level of donor and recipient management. Many factors go into cattle management and there are sometimes opportunities for ET practitioners to make a meaningful contribution to improving or changing certain management programs. In some cases, however, change either is not welcome or not possible and then the practitioner must make the best of the situation at hand **(**Stringfellow DA, Givens, MD. 2010).
The objective of this seminar paper is:

- To review the process of embryo transfer in cattle; including flushing, handling, grading, freezing, and transferring embryo.
- To describe the benefit of bovine embryo transfer in cattle productivity and production.
- To insight the potential applications of embryo transfer in bovine.

APPLICATIONS OF EMBRYO TRANSFER IN BOVINE

Embryo transfers in bovine have had many uses over years, especially in research. The widespread use of this technology in cattle breeding schemes, however, is relatively recent. A few of the more common uses of embryo transfer technology in cattle production are as follows (Lucero, 2015).

Genetic Improvement

Genetic progress has generally been considered to be slower with embryo transfer than with conventional artificial insemination (AI), especially on a national herd basis. However, with increased selection intensity and shortened generation intervals, i.e., transferring female offspring, genetic gain can be made on a within-herd basis (Christensen, 1991). This has resulted in the term MOET (multiple ovulation and embryo transfer) (Thomas, 2007). This would be especially worthwhile in improving elite herds, the genetics of which could be spread over a large population using AI. Embryo transfer is now commonly used to produce AI sires from proven donor cows and bulls in AI service. Although economics would not at this time support the use of embryo transfer techniques for anything but seed stock production, the commercial cattle industry can benefit by the use of bulls produced through well-designed MOET programs (Lohuis,1995).

Disease Control

Risk of infectios disease transmition is less by in vivo-produced embryos, providing embryo handling procedures were done correctly (Stringfellow, 2004). Several large studies have now shown that the zona-intact, washed, bovine embryo will not transmit infectious diseases. Consequently, it has been suggested that embryo transfer may be used to salvage genetics in the face of a disease outbreak. For example, this may be a useful alternative in the establishing herds that are free of Bovine Leukosis, as this virus was not transmitted with embryos. Breeders are now using embryo transfer techniques to establish disease free herds to be used strictly for export purposes. However, there is a possibility that embryos from infected donors may have encountered infectious agents whilst in these sites, but this depends to a large extent on the pathogenesis of the agent. Predilection for the genital tract is of special relevance with respect to transmission by embryo transfer. Among the many known genital diseases of livestock brucellosis, bovine genital campylobacteriosis and trichomoniasis and other infections such as infectious bovine rhinotracheitis (IBR) *Haemophils somnus*, Leptospira, Chlamydia and genital Mycoplasma can be transmitted through embryo transfer (Garner *et al*., 1995).

Import and Export

The intercontinental 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 in international trade. This may be the single most important potential application of embryo transfer. Additional benefits of the export of embryos over that of live animals include a wider genetic base from which to select, the retention of genetics within the exporting country and adaptation. The characteristics of embryos as protection by the zona pellucida, minute size, exposure only to a very circumscribed environment, and lack of body systems to host pathogens (e.g. respiratory, digestive, circulatory systems) result in significant barriers to infection. In addition, it is possible to wash, treat, and physically examine the individual embryo, which provides additional, very effective safeguards. Thus, importation of genetic material in the form of embryos is innately safer than importation of post-natal animals or semen (Wrathall *et al*., 2004),

Research

Embryo transfer techniques have proven to be a very useful research tool. In fact, many technical developments in embryo transfer before 1970 were directed toward research purposes rather than for the propagation of superior livestock. Embryo transfer can be applicable in the research on the natural limitations to twin pregnancies, uterine capacity, endocrine control of uterine environment, maternal recognition of pregnancy, embryo-endometrium interactions, and the endocrinology of pregnancy. Studies that were originally planned to answer basic physiological questions are now being used to improve and increase the utilization of embryo transfer. Newer techniques have added an entirely new perspective to the utilization of embryo transfer for research purposes (Betteridge, 2003).

Circumvent Infertility

It is possible to obtain offspring from genetically valuable cows that have become infertile due to injury, disease, or age by means of superovulation and embryo transfer. Although success rates are low, it is possible to recover oocytes from genetically valuable, moribund cows, fertilize them in vitro, transfer them, and obtain offspring (Marahall *et al*., 2002).

SELECTION OF DONOR COWS AND SIRES

Selection of Donor Cows

There are two broad criteria for selecting donor cows for most embryo transfer programmes: (1) genetic superiority, that is animals that contribute to the genetic objectives of the programme, these objective measures are fore example milk production, milk composition, growth rates, calving ease and disease resistance and (2) likelihood of producing large numbers of usable embryos. Although embryo production should be secondary to genetic superiority, it should be considered seriously. Healthy, cycling cattle with a history of high fertility make the most successful donors. Donors at least two months post-partum produce more embryos than those closer to calving. Young cows seem to yield slightly more usable embryos than heifers under some conditions. Lactation in either beef or dairy cows does not decrease response to superovulation provided that cows are cycling and not losing weight. Extremely fat cows make poor donors, both because they do not respond well to superovulation and because their reproductive tracts are more difficult to manipulate. Sick animals usually do not produce many good embryos (Thibier, 2006).

Selection of Sires

Since half of the genes come from the male, it is extremely important to use genetically superior bulls. In fact, 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. Likewise, it is necessary to select fertile bulls and fertile semen which makes it especially important to use high quality semen (David *et al.,* 2016).

General Procedural Steps

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. At this point, they may also be subjected to manipulations, such as splitting and sexing, if necessary and may be cooled or frozen for longer periods of storage. The discussion of donor superovulation, recipient synchronization and embryo transfer must begin with a review of estrous cycle physiology (Hasler, 2003).

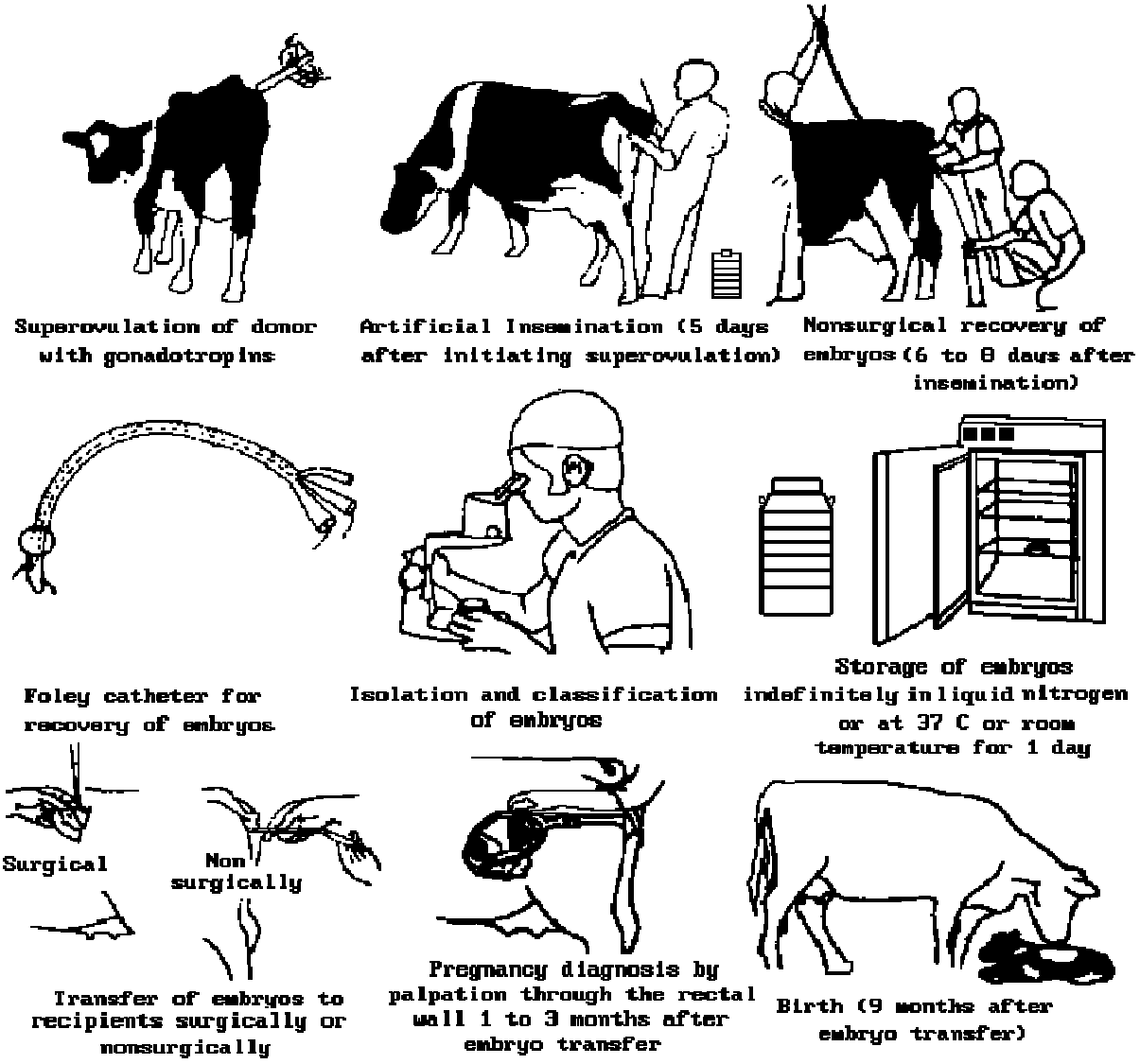

Figure 1 General Procedures of bovine embryo transfer.
*Source: Ross W. (1992): embryo transfer in cattle.

Physiology and Endocrinology of the Normal Bovine Estrous Cycle

The endogenous control of the bovine estrous cycle involves the interrelated secretion of a number of hormones from the hypothalamus, anterior pituitary, ovaries and uterus. These include gonadotrophin releasing hormone (GnRH) from the hypothalamus, follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland, estrogen, progesterone and inhibin from the ovary and prostaglandin F2H (PGF) from the uterus. The primary timing mechanism of the bovine estrous cycle is the demise of the corpus luteum (CL), which occurs about Day 17 - 18 in the normal cycling, non-pregnant cow. The simplest hypothesis for regression of the CL is that the non-pregnant uterus secretes a luteolytic agent into the uterine venous blood. This material is transferred through a local veno-arterial pathway to the ovarian artery whereby it reaches the ovary and causes luteolysis (Adams, 1998).

Prostaglandin F has been proposed as the natural luteolytic agent although definitive proof and details of the mechanism(s) of action are unclear. Regression of the CL results in a rapid fall in serum progesterone concentrations to values less than 1ng/ml. LH pulse frequency increases and follicular growth is further stimulated. The growth and maturation of the preovulatory follicle results in increasing secretion of estradiol, which causes estrogenic changes in the oviduct and uterus, behavioral estrus, and a preovulatory release of LH. The preovulatory LH peak results in resumption of oocyte meiosis, ovulation 24 - 32 h later (the LH peak occurs around the onset of estrus) and luteinization of the ovulated follicle to form a secreting corpus hemorrhagicum. Growth and development of the corpus hemorrhagicum into a fully functional CL results in progestational

changes in the oviduct and uterus that are conducive to embryonic development and establishment of pregnancy. Use of ultrasound to measure luteal volume also has not been predictive of subsequent ET conception rates. Should pregnancy not occur, the cycle will begin again with the demise of the CL on about Day 17 - 18 following ovulation (Spell, *et al*., 2001).

It has now been shown by ultrasonography that follicles in cattle develop in a wave-like fashion (Martinez *et al*., 2000). Bovine estrous cycles are composed of 2 or 3 waves of follicular development. A follicular wave consists of a group of growing antral follicles 3 - 6 mm in diameter from which a dominant follicle is selected while the remaining follicles become subordinate and undergo atresia In both 2- and 3-wave estrous cycles, emergence of the first follicular wave occurs on the day of ovulation (day 0) while the second wave emerges 9 or 10 days after ovulation in 2-wave cycles, and on 8 or 9 days after ovulation in 3-wave cycles, with a third wave emerging on day 15 or 16. Duration of the estrous cycle is approximately 20 days in 2-wave cycles and 23 days in 3-wave cycles. The dominant follicle present at the time of luteolysis will become the ovulatory follicle, and emergence of the next wave is delayed until the ensuing ovulation. The proportions of animals with 2- vs. 3-wave cycles are probably more or less equally distributed, and follicular waves have been reported in heifers before puberty and postpartum cows before the first ovulation. Follicle waves persist in pregnant animals until approximately the last 3 weeks before parturition (Adams, 1998).

Recruitment of follicular waves and selection of a dominant follicle is based on differential responsiveness to FSH and LH. Surges in plasma FSH are responsible for eliciting the emergence of a follicular wave FSH is subsequently suppressed by products of the growing follicles (e.g., estradiol and inhibin). In each wave, the follicle that first acquires LH receptors becomes the dominant follicle while subordinates undergo atresia. Suppression of LH as a consequence of progesterone secretion by the CL causes the dominant follicle to eventually cease its metabolic functions and it begins to regress. This leads to FSH release and emergence of a new follicular wave. Luteal regression allows LH pulse frequency to increase, the dominant follicle increases its growth and dramatically higher concentrations of estradiol result in a positive feedback on the hypothalamo-pituitary axis and a surge of LH followed by ovulation (Colazo *et al.,* 2003).

Estrus synchronization and superovulation are critical components of an embryo transfer program. These techniques involve the manipulation of the basic endocrine patterns outlined above. The key to successful estrus synchronization is obtaining closely synchronized, rapid declines in circulating progesterone to values <1ng/ml and synchronous growth and ovulation of a viable follicle (Baruselli *et al*., 2003).

A superstimulation protocol (named P-36) has been developed in Brazil. In this protocol the progesterone/progestogen-releasing device is removed 36 h after PGF treatment (thus, P-36) and ovulation is induced with Plh (Lutropin-V; Bioniche Animal Health, Belleville, Ont Canada), ad- ministered 12h after progesterone/progestogen releasing device removal (i.e., 48 h after PGF administration). It follows, therefore, that PGF is effective only when a fully developed CL is present (days 7-18 of the cycle) and that withdrawal of exogenous progesterone is only effective if either natural or induced regression of the CL has occurred. If properly implemented, within the physiological constraints of their mechanism of action, current techniques for estrus synchronization are highly successful. However, variation in ovarian follicular wave dynamics makes it difficult to control the time of estrus and ovulation precisel (Nogueira *et al*., 2003).

Superovulation

"Superovulation" refers to the release of many oocytes (eggs) during a single estrus period (Mapletoft, 2003).

Superovulatory Treatments

The objective of superstimulation treatments in the cow is to obtain the maximum number of fertilized and transferable embryos with a high probability of producing pregnancies (Nogueira, 2002). Superovulation is a very inefficient method of obtaining oocytes from bovine ovaries and is likely to be replaced by other approaches within the next decade. However, superovulation results in about ten times more embryos than single ovum recovery. Without superovulation, a usable embryo can be recovered about 60 percent of the time from normal donors by skilled technicians. Under similar conditions, superovulation usually yields an average of six usable embryos, although the variation is astounding. Normally, no embryos are recovered from 20-30 percent of superovulated donors and only one to three embryos are obtained from another 20-30 percent. An ideal response of 5 to 12 embryos is obtained from about one-third of the donors. However, a small percentage of donors yield more than 20 good embryos and, very rarely, more than 50. Variability in ovarian response has been related to differences in superstimulatory treatments such as gonadotrophin preparation, batch of gonadotrophin, duration of treatment, timing of treatment with respect to the estrous cycle, total dose of gonadotrophin and the use of additional hormones in the superstimulation protocol. Additional, perhaps more important sources of variability are factors inherent to the animal and its environment. These factors may include nutritional status, reproductive history, age, season, breed, ovarian status at the time of treatment and the effects of repeated superstimulation. While considerable recent progress has been made in the field of bovine reproductive physiology, factors inherent to the donor animal that affect superovulatory response are only partially understood Superovulation-

inducing treatments are usually initiated between Days 8 and 12 of the estrous cycle (estrus = Day 0) These times were originally based on the theory that a wave of follicles in the ovary was maturing at that time (Adams *et al*., 2002)

The two generally accepted methods of superovulating cattle are based on two different gonadotrophins, although there are many minor variations of these methods. The simplest is to give an intramuscular injection of 1800-3 000IU (usually 2000-2 500IU) of pregnant mare's serum gonadotrophin (PMSG), more correctly designated equine chorionic gonadotrophin (eCG), followed by a luteolytic dose of prostaglandin F2 alpha or an analogue intramuscular injection two to three days later (Pursley *et al*., 1995).

The second method of superovulation is to give eight to ten injections of follicle stimulating hormone (FSH) subcutaneously or intramuscularly. At half-day intervals, intramuscular injection is more reliable under field conditions. As with PMSG, prostaglandin F2 alpha is given 48-72 hours after initiation of treatment with the fifth, sixth, or seventh FSH injection. The most common FSH regimen is 6,6,4,4,2,2,2 and 2 mg at half-day intervals with prostaglandin F2 alpha given with the sixth or seventh FSH injection. About 20 percent more gonadotrophin should be given to cows weighing over 800 kg. Sometimes, higher doses are used for the first two days; others give 5 mg for each injection. There are few studies with adequate numbers of donors per treatment group in which constant and decreasing doses have been compared, so reliable conclusions cannot be drawn regarding efficacy of such regimens (Baruselli, 2006).

Other products that have been used for superovulating cows include equine anterior pituitary extract and human menopausal gonadotrophin (which also contains considerable LH). The former generally is not available commercially and the latter is too expensive for routine use (Steward *et al*., 2002).

Insemination

Because of the release of many ova from multiple follicles, there is a greater need for viable sperm cells to reach the oviducts of the superovulated females. Therefore, many embryo transfer technicians will choose to inseminate the cow several times during and after estrus. One scheme is to inseminate the superovulated cow at 12, 24 and 36 hours after the onset of standing estrus. 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) just in front of the cervix (Baruselli.*et al*,. 2003).

RECOVERY OF EMBRYOS

Non-Surgical Recovery of Embryos

The first step in non-surgical recovery is to palpate the ovaries per rectum to estimate the number of corpora lutea. This is very difficult to do accurately if there is a large response to superovulation, although it is not critical to determine how large this response is. Even in rare cases when only two or three corpora lutea are palpated by skilled personnel, occasionally four or five embryos are recovered. However, it is exceedingly rare to obtain embryos if there are no palpable corpora lutea by day 7. Ultrasonography provides more accurate information about responses than palpation (Kunkel, 2002).

Epidural anaesthesia is recommended for non-surgical recovery procedures. The tailhead should be clipped, then scrubbed with iodine soap and swabbed with 70 percent alcohol to prevent infection of the spinal column. Injecting too much anaesthetic can cause the cow to lose control of the rear legs and fall down in the chute. It is recommended to inject 5 ml of a sterile 2 percent solution of procaine in water using a new 18-gauge needle each time. Good epidural anaesthesia can be monitored by flacidity of the tail. While the epidural anaesthesia is taking effect, the tail should be secured to one side out of the way. The rear end of the cow should be cleaned of mud, manure, loose hair, etc., and then the vulvar area scrubbed thoroughly with iodine soap and rinsed carefully with swabs of 70 percent alcohol Sufficient time should be allowed for the lips of vulva to dry before inserting the recovery instrument to avoid carrying any alcohol into the uterus; disinfectants are extremely toxic to embryos. Recovery procedures are carried out by manipulation per rectum. Because of the epidural anaesthesia, the rectum can balloon easily due to entry of air during removal and reinsertion of the hand. Once air has entered, it is extremely difficult to work effectively. A simple air pump attached to a length of tubing to evacuate air from the rectum is an excellent investment, because ballooning of the rectum occurs occasionally, even with skilled personnel. Even so, the best strategy is to prevent entry of air as much as possible. To collect the embryos non surgically, a small synthetic rubber catheter (Foley catheter) is inserted through the cervix of the donor cow, and a special medium is flushed into and out of the uterus to collect the embryos seven days after estrus (Hasler, 2004)

Non-Surgical recovery of embryos is relatively simple and can be completed in less than an hour without harm to the cow. Apresterilized stylet is placed in the lumen of the catheter to offer rigidity for passage through the cervix into the body of the uterus. When the tip of the catheter is in the body of the uterus, the cuff is slowly filled with approximately 2 ml of normal saline. The catheter is then gently pulled so that the cuff is seated into the internal orffise of the cervix. Additional saline is then added to the cuff to completely seal the

internal orffise of the cervix. AYconnector with inflow and outflow tubes is attached to the catheter. A pair of forceps is attached to each tube to regulate the flow of flushing fluid. The fluid is sequentially added and removed by gravity. The fluid in the uterus is agitated rectally, especially in the upper one third of the uterine horn. The uterus is finally filled with medium to about the size of a 40day pregnancy. One liter of fluid is used per donor. Many embryo transfer technicians use a smaller volume and flush one uterine horn at a time. Each uterine horn is filled and emptied five to ten times with 30 to 200 ml of fluid each time, according to size of the uterus. The embryos are flushed out with this fluid and collected in a filter with the fluid. The pores in the filter are smaller than the embryos, so excess fluid drains out of the filter without losing the embryos. Embryos are separated from the flush media and examined under a microscope to determine their quality and stage of development (George *et al*., 2008).

Surgical Recovery of Embryos

The first successful cattle ET studies obtained from the embryos by a surgical procedure. The donor, which had fasted and been tranquilized, was anaesthetized by intravenous knockdown injection with a halothane/oxygen mixture. This method of recovery 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. An alternate procedure is to occlude the uterine horn at the body of the uterus. Culture medium is introduced through a puncture at the uterotubal 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 (Peregrino *et al*., 2000).

In the early days of commercial bovine embryo transfer, embryos were collected surgically from the cow around day 4 after estrus. Unfertilized oocytes for specialized applications (such as in vitro fertilization) must be collected near the time of ovulation. This is done from the follicles, surface of the ovary, or oviduct. For most applications, embryos are collected any time between fertilization and implantation but usually after they migrate to the uterus. In cattle, embryos for commercial purposes are usually recovered 6 to 9 days after estrus; before this time, nonsurgical recovery is ineffective. After nine days, recovery and pregnancy rates are slightly reduced with surgical transfer of bovine embryos. Surgical recovery can be done in all species and is the method of choice for sheep, goats, and hogs. Techniques vary slightly with the species (Betteridge, 2003).

Reflushing and Prevention of Multiple Pregnancies in Donors

Despite good flushing procedures by experienced technicians, not all embryos are recovered. A few are inaccessible in the oviduct, but some are simply missed. It is particularly frustrating to recover no ova when there is a good ovarian response and no indication of problems with recovery procedures. However, as there were similar situations with surgical recovery of embryos, it is unlikely that the method of recovery is at fault. It is best to give donors a luteolytic dose of prostaglandin F2 alpha, or an analogue, after the flushing procedure. If prostaglandin is not given, donors should be examined ultrasonographically or palpated at 40–50 days after breeding to diagnose potential multiple pregnancy. Even with a luteolytic dose of prostaglandin, an occasional donor remains pregnant. Some recommend two prostaglandin injections at one week intervals. Note also that most donors return to oestrus later than two to five days after prostaglandin, in contrast to non-superovulated cows (George *et al*., 2008).

EMBRYO HANDLING, EVALUATION AND STORAGE

Embryo Handling

Careful handling of embryos between collection and transfer is necessary to prevent the transmission of pathogens. The use of aseptic techniques, sterile solutions, and sterile equipment is essential. In dealing with the handling of embryos in laboratories, where inevitably they come into contact with glassware, petridishes, plastic straws and other equipment, exposure to toxic factors must always be a consideration. (Peregrino *et al*., 2000).

Embryos are located under 10 X magnification with a stereoscopic dissecting microscope after filtering the collection medium through a filter with pores that are approximately 50-70 µm in diameter. Although embryos are usually transferred as soon as possible after collection, it is possible to maintain embryos for several hours at room temperature in holding medium. It is also possible to cool bovine embryos in holding medium and to maintain them in the refrigerator for 2-3 days. As a final alternative, embryos may be frozen for use at a later date. Embryos are normally held in the same or a similar medium to that in which they were collected. Media must be buffered to maintain a pH of 7.2 to 7.6 and have an osmolarity around 300 molar osmolarity. Dulbecco's PBS or more complex media with the Hepes buffer and enriched with FCS and antibiotics are normally used in the field. More complex media with a carbonate buffer generally yield superior results for long term culture of

bovine embryos. As indicated earlier, embryo collection holding and freezing media that are free of animal products have recently become available, avoiding the need for refrigeration and increasing biosecurity (IVIS, 2002).

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	Phosphate or bicarbonate ion (latter must be maintained under 5 percent CO2 atmosphere)

*Source: (George *et al*., 2008).

Embryo Evaluation

Evaluation of bovine embryos must be done at 50 to 100 X magnification, with the embryo in a small culture dish. It is important to be able to recognize the various stages of development and to compare these with the developmental stage that the embryo should be based on the days from estrus. Often a decision as to whether an embryo is worthy of transfer will depend on the availability of recipients. Fair quality embryos should be transferred fresh, if recipients are available. The International Embryo Transfer Society (IETS) considers the export of poor and fair quality embryos to be improper (Savoy *et al*., 1998).

Embryos are classified and evaluated by morphological examination at 50 to 100 X magnification according to the Manual of the International Embryo Transfer Society (Givens *et al*., 2010). The overall diameter of the bovine embryo is 150 to 190 um, including a zona pellucida thickness of 12 to 15 mm. The overall diameter of the embryo remains virtually unchanged from the one cell stage until blastocyst stage. The best predictor of an embryo's viability is its stage of development relative to what it should be on a given day after ovulation. An ideal embryo is compact and spherical; the blastomeres should be of similar size with even color and texture; the cytoplasm should not be granular or vesiculated; the perivitelline space should be clear and contain no cellular debris; the zona pellucida should be uniform; neither cracked nor collapsed and should not contain debris on its surface. Generally the major criteria for quality evaluation include; regularity of shape of the embryo, compactness of the blastomeres (the dividing cells within the boundaries of the embryo), variation in cell size color and texture of the cytoplasm (the fluid within the cell wall), overall diameter of the embryo Presence of extruded cells, thickness and regularity of the zona pellucida (the protective layer of protein and polysaccharides around the single celled embryo) and presence of vesicles (small bubble like structures in the cytoplasm) (Dochi *et al*.,1998).

Quality Evaluation

Excellent: An ideal embryo, spherical, symmetrical and with cells of uniform size, color and texture.
Good: Small imperfections such as a few extruded blastomeres, irregular shape and a few vesicles.
Fair: Problems that are more definite are seen, including presence of extruded blastomeres, vesiculation, and a few degenerated cells.
Poor: Severe problems, numerous extruded blastomeres, degenerated cells, cells of varying sizes, large and numerous vesicles but an apparently viable embryo mass. These are generally not of transferable quality (Mapletof *et al*., 2013).

Embryo Developmental Stage Evalution

Embryos also are evaluated for their stage of development without regard to quality. It is advisable to select the stage of embryo development for the synchrony of the recipient (Stroud, 2012):
Morula: A mass of at least 16 cells. Individual blastomeres are difficult to discern from one another. 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 blastocoele and gives a general appearance of a signet ring. The embryo occupies 70 to 80% of the perivitelline space. Early in 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 evident. The blastocoele is highly prominent, with the embryo occupying most of the perivitelline space. Visual differentiation between the trophoblast and the inner cell mass is possible at this stage of development.
Expanded Blastocyst: The overall diameter of the embryo dramatically increases, with a concurrent thinning of

the zona pellucida to approximately one-third of its original thickness.
Hatched Blastocyst: Embryos recovered at this developmental stage can be undergoing the process of hatching or may have completely shed the zona pellucida. Hatched blastocysts may be spherical with a well defined blastocoele or may be collapsed. Identification of embryos at this stage can be difficult unless it re-expands.
The IETS recommended codes for embryo quality range from "1" to "4" as follows:
Code 1: Excellent or good. Symmetrical and spherical embryo mass with individual blastomeres (cells) that are uniform in size, color and density. This embryo is consistent with its expected stage of development. Irregularities should be relatively minor and at least 85% of the cellular material should be an intact, viable embryo mass. This judgment should be based on the percentage of embryo cells represented by the extruded material in the perivitelline space. The zona pellucida should be smooth and have no concave or flat surfaces that might cause the embryo to adhere to a Petri dish or a straw.
Code 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.
Code 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.
Code 4: Dead or degenerating. Degenerated embryos, oocytes or 1-cell embryos; non-viable. Generaly 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 (Mapletof *et al*., 2013).

Embryo Storage
Procedures such as embryo transfer, in vitro fertilization, sex determination, and cloning depend on maintaining the viability of embryos for hours to days outside of the reproductive tract. For many applications, the storage system must not only maintain the viability of the embryo, but must also support continued development. It may also be desirable to retard growth to a degree approaching suspended animation so development can be synchronized with later events. For example, it may be necessary to store embryos until suitable recipients become available for transfer. Donor embryos can be transferred immediately into recipients, or they can be stored for future use (Sauvé *et al*., 2002).

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 (Atsushi *et al*.,2013).

Long-Term Storage
If embryos are to be transported great distances or suitable recipients are not immediately available, a longterm storage system is essential. Deep-freezing embryos is storage in liquid nitrogen (-196^{o}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 (Atsushi *et al*., 2013).

SELECTION OF RECIPIENT, TRANSFER OF EMBRYO AND POST TRANSFER MANAGEMENT
Selection of Recipient Females
Proper recipient herd management is critical to embryo transfer success. Cows that are reproductively sound, that exhibit calving ease and that have good milking and mothering ability are recipient prospects. They must be on a proper plane of nutrition (body condition score 6 for beef cows and dairy body condition score 3 to 4). These cows also must be on a sound herd health program. Generaly females ideal as reciepents are as follows; (1).Cows 3 to 8 years old make good recipients once they have a good calving record. Cows will be better suited when transferring embryos of relatively high birth weights. In general, cows have more milk than heifers, an important factor to consider when they have to raise an embryo calf, (2).Heifers are good recipients providing they've reached their breeding weight (around 65-70% of mature weight) and are cycling. Avoid embryos with large EPD's for birth weights in heifers, (3).1st calvers are still growing and are under more stress than the rest of the herd due to the fact that they have to raise a calf. For these reasons 1st calvers can be difficult to get pregnant, if they are under an intensive management and receiving enough good quality feed they can be used as recipients .(4).Use fertile animals. Cows with calves at foot that have no history of calving problems, and are

open not because they fail to conceive by AI or natural breeding, make good recipients. Retain animals successfully used previously as recipients. Do not use recipients that have been prepared unsuccessful twice before, (5).Animals with temperament problems should be removed from the program. (6).Select animals that maintain or are gaining body condition, an ideal score of 2.5-3 is preferred at the time of transferring the embryos. (Bova-Tech Ltd, 2012).

Synchronization of the Recipients

To maximize embryo survival in the recipient female following transfer, conditions in the recipient reproductive tract should closely resemble those in the donor. This requires synchronization of the estrous cycles between the donor and the recipients, optimally within one day of each other. Synchronization of the recipients can be done in a similar manner and at the same working time as the donor cows. There are a number of different estrous synchronization protocols (Galina and Orihuela, 2007). Three different estrous synchronization protocols were evaluated. Treatment included an injection of prostaglandin (PGF2α, 25mg i m) on palpation of CL; status of the estrous cycle was evaluated by rectal palpation and fixed time insemination (FTAI) was done 72 h post PGF2α injection. Similar was the second protocol in which, in addition to PGF2α administration Gnadotropin (GnRH; 100µg) was administered 60 h post PGF2α injection and FTAI was done 72 h post PGF2α. For the third protocol, GnRH was administered at the time of A.I. post PGF2α injection. Three different groups of animals were maintained for three different protocols respectively. On comparison it was observed that there was no significant difference ($P>0.05$) in the percentage of pregnant cows when compared between treatments for three different respective protocols; it was observed that, the percentage of pregnant cows resulting on application of third protocol was significantly higher ($P<0.01$) when compared with first and second protocol. The gonadotropin releasing hormone (GnRH) and prostaglandin (PGF2α.) method of estrous synchronization has proven to be very successful in synchronizing estrus in cattle (Lamb *et al*., 2004).

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 seven 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 (Berber *et al*., 2002**)**.

TRANSFER OF BOVINE EMBRYO

Surgical Transfer

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 is located by rectal palpation and the flank ipsilateral to the CL is clipped, washed with soap and water, and sterilized with iodine and alcohol. About 60 ml of 2 percent procaine is given along the line of the planned incision. In everyday practice this seems more reliable than using a paravertebral block. Having scrubbed, the surgeon makes a skin incision about 15 cm long, high on the flank, just anterior to the hip. Muscle layers are separated, and the peritoneum is cut. The surgeon inserts a hand and forearm into the incision, locates the ovary, usually about 25 cm posterior to the incision, and visualizes or palpates the CL. The uterine horn is exteriorized by grasping and stretching with the thumb and forefinger the broad ligament of the uterus, which is located medial to the uterine horn. A puncture wound is made with a blunted needle through the wall of the cranial one-third of the exposed uterine horn,because it is very fragile. Using about 0.1 ml of medium in a small glass pipette (<1.5 mm outside diameter), an assistant draws up the embryo from the storage container. The pipette is then inserted into the lumen of the uterus, and the embryo is expelled. It takes some experience to be confident that the embryo has been deposited in the lumen. The incision is then closed, using two layers of sutures. With practice, the surgery takes about 15 minutes. (Marahall *et al*., 2002).

Non-Surgical Transfe

First, it is necessary to palpate ovaries accurately in order to select the side of ovulation. Pregnancy rates are markedly lowered if embryos are transferred to the uterine horn contralateral to the corpus luteum .Also, recipients should be rejected if no corpus luteum is present or pathology of the reproductive tract is noted. The next step is to pass the embryo transfer device through the cervix. The petridish containing the embryo is placed under the stereoscope to load in a 0.25 ml transfer straw. The straw is then loaded into a transfer gun and covered with a transfer sheath. In a fashion similar to that described for uterine flushing, the transfer gun is introduced into the vagina of recipient. The tip of the transfer gun is guided through the cervix by transrectal manipulation and positioned into the uterine horn. Some authors recommend placement of the embryo in the left uterine horn ipsilateral to the corpus luteum. Heifers present a special challenge because of the small cervix. The best training prior to undertaking non-surgical embryo transfer is experience in artificial insemination. The third step with non-surgical transfer is to be able to insert the tip of the instrument into the desired uterine horn quickly,

smoothly and atraumatically (Trasorras *et al*., 2010).
Nonsurgical transfer is preferable, because it is less expensive, it is quicker and does not involve surgical procedures. This may also obviate the need for veterinary supervision, which is required for surgery in many countries. The most commonly used instrument for non-surgical transfer is the standard Cassou Minseminating gun for French straws, because it is inexpensive and easy to use correctly (Carlos, 2014)

Pregnancy Diagnosis of Reciepents
The first good indicator of pregnancy is failure of the recipients to show oestrus 18–24 days after the pre-transfer oestrus; obviously, the converse, showing oestrus, indicates non-pregnancy, although asmall percentage of pregnant animals are in behavioural oestrus about three weeks after the previous oestrus. Progesterone assay of milk or blood samples 22–24 days after the pre-transfer oestrus is 95 percent accurate in diagnosing non pregnancy and about 80 percent accurate for pregnancy. Development of sensitive automated inline milk progesterone assays should make this technology amenable to commercial application (Fricke *et al*., 2014).

The first reliable pregnancy- specific hormone assays were developed to measure placenta- derived proteins. The first of these measured circulating concentrations is of pregnancy-specific protein B (PSPB) Pregnancy-specific protein is produced by placental giant binucleate cells that form from mononuclear trophoblast cells starting around days 17 to 19 of pregnancy in cattle(Spencer *et al*., 2007). Pregnancy-specific protein B concentrations begin to be reliably detectable in plasma starting at day 24, and by day 28 concentrations are sufficiently elevated to allow their use for a highly reliable test for pregnancy in cattles. Pregnancy diagnosis can usually be diagnosed definitively by palpation per rectum after day 35 of pregnancy (O'Connor, 1994). It is not recommending palpation prior to day 45, both because the conceptus is more fragile at early stages and because the information is not definitive anyway due to occurrence of spontaneous abortion even in the absence of palpation. Thus, it is recommended to palpate per rectum at 45–60 days of gestation and confirm this with another palpation one month later. Cows that show oestrus may be checked earlier than 45 days by palpation or ultrasonography.Of course, ultrasound can also be used at these later stages. At about day 26 of pregnancy in heifers and day 28 in cows, pregnancy can be diagnosed accurately under field conditions by ultrasonography or even earlier in very skilled hands. If they are, in fact, non-pregnant they can be recycled for use as recipients. (Pereira *et al*., 2013).

Managing Pregnant Recipients
Reproductively, a cow performs her best when she is in moderate body condition. Cows that are too fat are the most difficult to get bred. Cows that are too thin often will not cycle so they do not have a chance to breed, and if they do cycle the odds of pregnancy are reduced. One interesting contradiction to thin cows as recipients is that if they are cycling and they have an embryo transferred into them they will get pregnant if they are fed well enough to gain weight for the next 45 days. The key point to successful conception rates is a term we call energy balance. If a cow goes into negative energy balance (weight loss) post transfer, the conception rates decline significantly. Conversely, if a thin female goes into a positive energy balance (weight gain) post transfer, the conception rates are usually acceptable. If a cow breeds back 82 days after she calves she will calve at 12-month intervals. This is a common goal of commercial cattle breeders. It is a lofty, yet achievable goal with recipients if they are well-managed nutritionally. Things that change the balance of energy: (1) difficulty at calving – If a cow needs veterinary assistance at calving she can feel bad for several days and even go off feed. A nursing newborn needs milk and a good cow will make it, but possibly at her own body's expense. She begins to break down her own body fat to make milk for her baby. She is now in a negative energy balance and begins to lose weight. If she retains part of her placenta this weight loss can continue for several months until treated. (2) quality of available forage, hay, silage, or better yet grass from the ground is the single most important constituent of a cow's diet. Depending on where you live, growing conditions can vary significantly from month to month. (3) extended cold/wet or hot/dry periods can take its toll on cows and put them in a negative energy balance. (Stroud, 2010).

Recipients are often overlooked at the feed bunk. However, if we expect them to perform (breed and raise good babies) they must be maintained on a good nutrition program. The last three months of pregnancy are crucial, nutritionally speaking, relative to how soon a cow will breed back after calving. Remember that lactation is the most stressful time in a cow's life – a suckling calf drains her energy reserves quickly. If she is thin at calving and goes directly into heavy milking, she may not cycle (have a heat period) until her calf is weaned. Furthermore if she does cycle while in poor flesh and lactating, her odds of getting pregnant are reduced. If she calves in good flesh, she may cycle and breed back in 60 to 100 days after calving. The main point to be stressed is that nutritional supplement, if necessary, may need to be initiated weeks in advance of anticipated breeding. Don't be near as concerned about trace elements and vitamins are you are with protein and energy supplements. Protein and energy are by far the most limiting nutritional factors concerning fertility in cattle. (Bova-Tech Ltd, 2012).

CONCLUSION AND RECOMMENDATIONS

Commercial embryo transfer in cattle has become a well established industry throughout the world. Although a very small number of offspring are produced on an annual basis, its impact is large because of the quality of animals being produced. Embryo transfer is now being used for real genetic improvement, especially in the dairy industry, and most semen used today comes from bulls produced by embryo transfer. An even greater benefit to embryo transfer is that in vivo-produced bovine embryos can be made specified pathogen-free by washing procedures, making this an ideal procedure for disease control programs or in the international movement of animal genetics. Techniques have improved over the past 60 years so that frozen-thawed embryos can be transferred to suitable recipients as easily and simply as artificial insemination. In vitro embryo production and embryo and semen sexing are also successful, but time and cost limit their widespread use. 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.

Based on the above conclusion the following recommendations are forwarded:

- Taking maximum attention in all segmented process of embryo transfer to reach for success. Management, selection of the donor, recipients and offspring are all important as well as the collection, handling, preservation and transfer or the embryos.
- It should be proven may times that hiring the professional team to provide the ET service with their experience and knwledge will pay dividends towards success of the program.
- Utilize ET technology depends on the profitability and marketability of the offspring produced.
- Improve the use of commercial embryo as amethod of choice for importing breeding stock. Because it comprising low risk on health of national herd; the offspring will be 100 percent of the desired genotype and will adapt more readily to the new environment because of passive immunity acquired from the recipient, Costs of importing embryos are often lower than importing post-natal animals, and it is possible to change the breed of a herd within a single generation and it enables to obtain offspring from genetically valuable cows that have become infertile due to injury, disease or age.

REFFERENCES

Adams, G.P., (1998): Control of ovarian follicular wave dynamics in mature and prepubertal cattle for synchronization and superstimulation. In proceeding of the XX congress of the world association of Buiatrics; Sydney, Australia. 595-605.

Atsushi. I., Yoshito, A., Kanami, T., (2013): Research and Development Group, Zen-noh Embryo Transfer Center, Kamishihoro, Hokkaido. 080-1407.

Baruselli, P.S., (2006): Superovulation and embryo transfer in Bos indicus cattle. Theriogenolgy. 65:77–88.

Baruselli, P.S., Marques, M.O., Carvalho, N.A.T., (2003): Follicular dynamics and pregny rate in embryo recipient (Bos taurus indicus x Bos taurus taurus) treated with "Ovsynch" protocol for fixed time embryo transfer. *Brazilian j vet research and anim sci.*40:96-106.

Berber, R.C., De, A., Madureia, E.H. and Baruselli, P.S., (2002): Comparison of two ovsynch protocols (GnRH versus LH) for fixed timed insemination in buffaloes (bubalus bubalis) Theriogenology 57(5): 1421-1430.

Betteridge, K. J., (2000): Reflections on the golden anniversary of the first embryo transfer to produce a calf. Theriogenology.53:3-10.

Betteridge, K.J., (2003): A history of farm animal embryo transfer and some association techniques. *Anim reprod Sci*.79:203-244.

Bo, G.A., Baruselli, P.S., Martinez, M.F., (2003): Pattern and manipulation of follicular development in Bos indicus cattle. *Anim Reprod. Sci*. 78:307–26.

Bó, G.A., Mapletoft, R.J., (2013): Evaluation and classification of bovine embryo. *Anm.Reprod*., v.10. 3:344-348.

Bova-Tech Ltd., (2012): Embryo trasplnts, *info@bova-tech.com* URL.

Brad Stroud., (2010): recipient cow selection & management. Weatherford, *brad@stroudet.com.* URL.

Carlos. R.F., (2014): Embryo transfer in cattle.the merik veterinary manual.

Christensen, L.G., (1991): Use of embryo transfer in future cattle breeding schemes. Theriogenology. 35:141-156.

Colazo, M.G., Kastelic, J.P., Mapletoft, R.J., (2003): Effects of estradiol cypionate (ECP) on ovarian follicular dynamics, synchrony of ovulation, and fertility in CIDR-based, fixed-time AI programs in beef heifers. Theriogenology. 60:855–65.

David & Shelley Hamilton., (2016): Hamco cattle co. 18thAnnual angus bull sale. Glenboro, Manitoba Canada.

Dochi, O., Yamamoto, Y., Saga, H., Yoshiba, N., Kano, N., Maeda, H., Miyata, K., Yamauchi, A., Tominaga, K., Nakashima, T., Inohane, S., (1998): Direct transfer of bovine embryos frozen-thawed in the presence of propylene glicol or ethylene glicol under on-farm conditions in an integrated embryo transfer program. Theriogenology. 49:1051-1058.

Fricke, P.M., Carvalho, P.D., Giordano, J.O., Valenza, A., Lopes, G., Amundson, M.C., (2014): Expression and detection of estrus in dairy cows: the role of new technologies. *Animal, suppl*. 1:134-143.

Galina, C.S. and Orihuela, A., (2007): The detection of estrus in cattle raised under tropical conditions : What we know and what we need to know Hormones and Behaviour 52: 32-38.

Garner, M.G., Lack, M.B., (1995): Modelling the potential impact of exotic diseases on regional Australia. *Aust. vet. J*. 72, 81-87.

George, E., Seidel, J.R. and Sarah Moore Seidel, (2008): Training manual for embryo transfer in cattle (FAO). Animal reproduction laboratory.

Hasler, J. F., (2004): Factors Influencing the Success of Embryo Transfer in Cattle. 23rd World Buiatrics Congress, Québec, Canada.

Hasler, J.F., (2003): The current status and future of commercial embryo transfer in cattle. Anim Reprod Sci.79:245-264.

IVIS,(International Veterinary Information Service).(2006): Reviews in Veterinary Medicine.

Kunkel, J. R., (2002): "Embryo Transfer." Dairy Integrated Reproductive Management (IRM). *Available:http://www.wvu.edu/~exten/infores/pubs/livepoul/dirm26.pdf*. URL

Lamb, G. C., Cartmil, J.A.I. and Stevenson, J. S., (2004): Effectiveness of select synch Gonadotropin-releasing hormone and Prostaglandin$F_{2\alpha}$ for synchronizing estrus in replacement beef heifers.

Lohuis, M.M., (1995): Potential benefits of bovine embryo-manipulation technologies to genetic improvement programs. Theriogenology. 43: 51-60.

Lucero, M., (2015): Artificial insemination and embryo transfer. new Mexico youth ranch management camp beef day 4-H agriculture agent.

Manual of the IETS., (1998): Third Edition. DA Stringfellow & SM Seidel (eds). Savoy, I.L: IETS.

Mapletoft, R.J., Steward, K.B., Adams, G.P., (2002): Recent advances in the superovulation in cattle. *Reprod Nutr*. Dev.42:601–11.

Marahall, D.M. and Minyard, J.A., (2002): Embryo transfer in beef cattle. College of Agriculture & Biological sciences / South Dakota State University / USDA.

Martinez, M.F., Adams, G.P., Kasteli, J.P., Bergfelt, D.R., Mapletoft, R.J., (2000): Induction of follicular wave emergence for estrus synchronization and artificial insemination in heifers. Theriogenology.54:757–69.

Nogueira, M.F.G., Barros, B.J.P., Teixeira, A.B., Trinca, L.A., D'Occhio, M.J., Barros, C.M., (2002): Embryo recovery and pregnancy rates after the delay of ovulation and fixed-time insemination in superstimulated beef cows. Theriogenology.57:1625–34.

Nogueira, M.F.G., Barros, C.M., (2003): Timing of ovulation in nelore cows superstimulated with P36 protocol. *Acta. Sci. Vet*.31:509.

O'Connor, M.L., (1994): Alternative methods for determining pregnancy status. In proceedings of the National Reproduction Symposium, Pittsburgh, PA. Dallas, TX: Texas agricultural extension service. 23-32.

Peregrino, G., (2000): Technical aspects of the recovery, handling and transfer of embryos. National headquarters and gene pool Philippine Carabao center muñoz, nueva ecija 3120 Philippines.

Pursley, J.R., Mee, M.O., Wiltbank, M.C., (1995): Synchronization of ovulation in dairy cows using PGF2a and GnRH. Theriogenology.44:915–23.

Ross, W., (1992): Embryo transfer in cattle. Maffra Secondary College.

Sauvé, R., (2002): Embryo Transfer. Available: *http://www.embryobec.com/TEang.html.* URL

Spell, A. R., Beal, W.A., Corah, L.R. and Lam, G.C., (2001): Evaluating recipient and embryo factors that affect pregnancy rates of embryo transfer in beef cattle. Theriogenology. 56:287-297.

Spencer, T.E., Johnson, G.A., Bazer, F.W., Burghardt, R.C., (2007): Fetal maternalinteractions during the establishment of pregnancy in ruminants. *Soc Reprod Fertil Suppl,* 64:379-396.

Stringfellow, D.A., Givens, M.D., (2010): Manual of the International Embryo Transfer Society (IETS). 4th ed. Champaign, IL: IETS.

Stringfellow, D.A., Givens, M.D., Waldrop, J.G., (2004): Biosecurity issues associated with current and emerging embryo technologies. *Reprod Fert. Dev.* 16:93-102.

Stroud, B., IETS., (2012): Statistics and Data Retrieval Committee Report. The year 2011 worldwide statistics of embryo transfer in domestic farm animals. *Embryo transfer newsl*. 30:16-26.

Thibier, M., (2006): Data retrieval committee annual report. embryo transfer newsletter 24(4):12-18.

Thomas, H.S., (2007): Embryo transfer can maximize best genetics. *Cattle today, inc.*

Trasorras, V.,Chaves, M.G., Neild, D., Gambarotta, M., Aba, M., Agüero, A., (2010): Embryotransfertechnique: factorsaffectingtheviabilityoftecorpusluteuminllamas.*Anim.Reprod.Sci*.121:279–285.

Wrathall, A.E., Simmons, H.A., Bowles, D.J., (2004): Biosecurity strategies for conserving valuable livestock genetic resources. *Reprod Fert. Dev*. 16:103-112.

York Animal Hospital Bellevue., (2016): Ohio Powered by *Lifelearn WebDVM®4 Veterinary Websites.URL.*