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Review Article

A Review on Current Status of Embryo Transfer Technology in Sheep and Goats

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Abstract

Embryo transfer is a procedural biotechnological process which is widely practiced in different species in the world. It is a technique by which embryos are collected from the donor animal and transferred in to the recipient animal. Appropriate donor and recipient ewes and does are selected for embryo production and both animals will be synchronized at the same time. Embryos produced by this technology involves *in vivo* and *in vitro* techniques. In the earlier, oocytes are fertilized inside the donor and matured embryos will be recovered from the donor for transfer. In the later, oocytes are fertilized and matured outside the donor, matured embryos have been undergone the same procedure with the later for transfer. Moreover, the technology involves in the cryopreservation and vitrification techniques to transport the produced embryos in and around the world. Furthermore, embryo transfer needs additional diagnostic tools to evaluate the ovarian response before fertilization and for pregnancy diagnosis after transferring. However, some limitations are identified which could be resulted in as a consequence of the application of this technology. Finally, embryo transfer technology is required to be practiced for genetic, commercial, health and species conservation purposes and further research works will be needed to over-come the limitations.

Keywords: *Cryopreservation; Embryo Production; Goat; In vivo; Invitro; Sheep*

Introduction

Live-stock development is a continuous process that can be obtained via the application of different scientific methods [1]. The important point in the animal breeding model is to multiply animals genetically superior by production and reproduction potentials, disease resistance and with other important factors useful for economic development [2]. In most domestic animals including sheep and goats, the genetic gain is limited on the female side simply due to the naturally determined reproductive efficiency rate rather than the other factors [3]. For example in cattle, only one calf can be produced per year per cow unlike that the average herd bull can sire 15 to 50 calves per year [4]. Similarly, there have annual average reproductive rates of 1.5 for does and 1.2 for ewe and 1:20 ratios of ram or buck [5].

The applications of multiple ovulations, sexed semen production, artificial insemination, in vitro fertilization and other embryo transferring procedures and embryo cryopreservation techniques are the recently useful reproductive technologies [6,7]. Diagnostic procedures are additional techniques, such as ultrasonography and laparoscopy are tools that used for observing the ovarian response to super ovulatory treatments in donor animals as well as for insemination, embryo collection and embryo transfer and pregnancy diagnosis purposes [8].

Reproductive technologies are playing strongly important roles in the production, reproduction managements of animals. Therefore, intervention in the reproduction using multiple ovulation and embryo transfer can minimize the above limitation [2,6]. Embryo transfer following to super ovulation is an effective means of increasing the contribution of superior females to genetic improvement schemes and is also an important procedure of the embryo technology [9]. As result, there have been rapid genetic progress in different breeds and established for meat, milk, and wool and hair production [10].

Embryo transfer is a technique by which embryos are collected from the reproductive tract of female animals (donor) prior to nidation and transferred in to the reproductive tract of other female animal (receiver) to complete their gestation to term [11]. Embryo transfer was first surgically performed and recovered in mice in England by Walter Heape in 1890 as presented by Mapletoft [12]. Since that time, it has been performed commercially in different species of animals in the world. The first male camel calf resulted from interspecies embryo transfer was born in 2008; where as in cattle it has commercialized since 1970 [13].

The development of embryo transfer have been advancing from the application of the in vivo techniques using the animals themselves to the level of intra and interspecies *in vitro* fertilization performed in the laboratory [14]. So far, in the world, in cattle more than 750,000 embryos from superovulated and 450,000 embryos from *in vitro* techniques were produced annually [15,16]. In the history of super ovulation and embryo transfers in sheep and goats which initially were carried out in 1930, and it becomes as one of the widely exercised embryo techniques mainly in North America, and later on continued in Australia and New Zeeland; recently, embryo transfer is used widely in Brazil and Japan as presented by Gibbons and Cueto [10].

In Africa the technology is not used as widely practiced as in the rest regions of the globe [2]. However, in South Africa and Ethiopia, different local sheep and goat breeds have been showing positive response in their reproductive performance after reproductive assisting hormonal treatment for super ovulation. Moreover, there are genetically superior different breeds of ewes and does found in Africa [3,17].

Hence, Ethiopian government is also giving emphasis for the transferable technological skills and useful methods to adopt and applies to increase the reproductive and productivities of the local breed animals [18]. Therefore, the objective of this paper is to understand the currently employed embryo transfer technology in sheep and goats.

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Embryo Production

In the embryo transfer technology, embryos can be produced either using *in vivo* or *in vitro* techniques [19]. Transferrable embryos can be transferred either in fresh or frozen state. The world-wide annual activities of the ET technologies in 2008 had covered the figures indicated in table 1 as presented by Alexander., *et al* [6]. The figure on each species and type of transferred embryos seems small, but in terms of the genetic improvement of the animals, it might be in good position.

Species	Transferable embryo	Transferred embryo		
		Fresh	Frozen	Total
Sheep	18828	4793	433	5226
Goat	3141	824	278	1102

Table 1: *Transferrable and transferred embryos of sheep and goats.*
Source: Alexander., et al. [6]

In Vivo Embryo Production

In vivo embryo production is a technique by which the embryo is originated and developed inside the reproductive tract of the donor sheep or goats [20].

Donor and recipients animal selection

The first thing that should be done in the embryo transfer techniques is selection of the donors and recipient. There are selection criteria that donor ewes and does not have detectable genetic defects, be free from congenital diseases, and have good productive and reproductive performance with pedigree record, and recipients have to be free from congenital diseases, with no malnourishment [9].

Sheep and goats that fulfil the criteria of genetic superiority, reproductive ability and market value of the progeny can be selected as a donor and going synchronization procedures to take place [6]. After selection, both animals have to be treated by assisting reproductive hormone at the same time. Donor must be made ready for insemination and recipient must be made ready for pregnancy to receive the matured embryos [1,10].

Super ovulation

Super ovulation is the releasing of multiple eggs at a single estrus [3]. This technique involves different protocols to bring the donor animal in to estrus to produce more than one ovum at a time [2]. Some researchers [6], suggested that super ovulation program in sheep and goat comprises of the range of 14 to 17 days progesterone exposure. Others [3,21] practiced using the range of 16 to 21 days to use intra-vaginal device such as fluorogeston acetate-FGA or medroxyprogesterone acetate-MPA exposure [21].

In general, according to different research outputs, the intra-vaginal device can stay for a maximum range of 9 to 21 days in the vagina for the purpose of estrus synchronization if the CIDR and PGF2α are used separately; otherwise it can be as short as 6 days to come the animal to heat as reported by Charlotee [5]. In the later Intra vaginal device is followed by 6 to 8 FSH injections given twice to three times a day starting from the last 2 days before the withdrawal of progesterone releasing device [22]; in the former, it has to be followed by 6 FSH injections given twice a day starting from two days after spongy withdrawal resulted in no reduction in conception rate as reported by Ramon-Ugalde., *et a*l [23].

In vivo fertilization

Animals suggested to be super ovulated will be allowed for either natural mating or using artificial insemination after 48 to 50 hours 200 to 400 IU PMSG or/and 50 µg PGF2a or/and 6 or 8 FSH injections [3,6]. The application of artificial insemination includes either trans-cervical or laparoscopic techniques with 60 to 80% pregnancy efficiency rate as described by Charlotee [5]. The frozen or fresh semen has to put in the uterine body or oviduct lumen, 2 cm from the uterus-tubular junction [6,23].

Semen deposited in to the uterine body was found with low pregnancy efficiency rate (20 to 30%) and needed $800*10^6$ sperm cells in sheep and 400 to $600*10^6$ spermatozoa in goats [10]. Semen deposited in the uterine horn using laparoscopic or trans- cervical techniques were resulted in as high pregnancy efficiency rate as 60 to 80%, and needed only $80*10^6$ sperm cells in sheep and $100*10^6$ sperm cells in goats as stated by Charlotee [5].

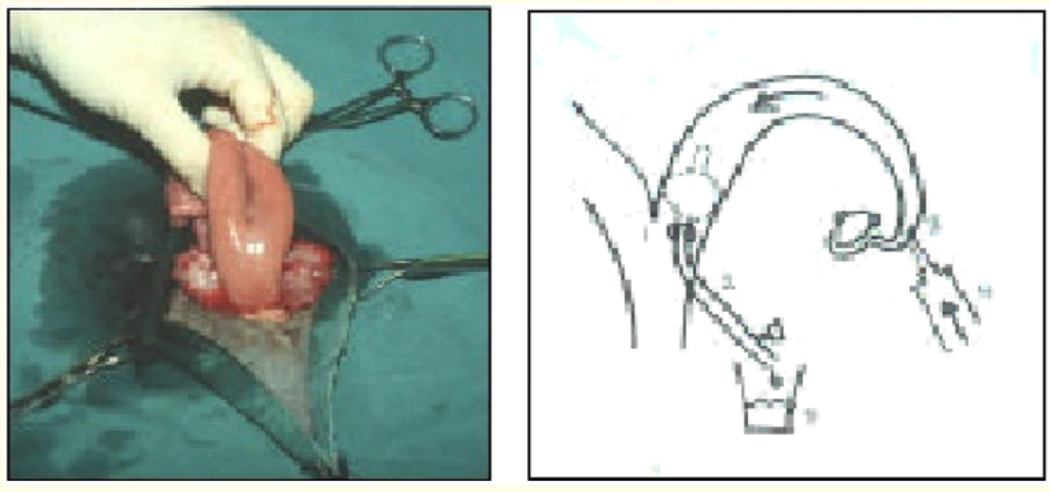
Fertilized eggs run cleavage in 3 to 4 days to have morula stage with 16cell mass, and reach blastocysts stage with 4 to 5 days with more than 16 cells mass. After 5 days, mature embryos can be obtained and recovered for transfer [3].

Embryo recovery and evaluation

Surgical embryo recovery was developed first [12]. It requires local or general anesthesia [24]. Embryos, morulae and blastocysts, have surgically collected from the uterine horn of the donor 4 to 7 day's post inseminated donor as shown in figure 1A [10].

However, flushings can be recovered through Foley catheter into a plastic dish culture containing culture media as indicated in figure 1B. This media pass through a 50 to 70 µm in diameter bacteriological filter [2]. The plastic culture dish examined under microscopic (10 - 40X) the evaluation to be carried out. But it must be evaluated by placing it in 20% serum at 38oC [23]. The collected embryos have to be classified in to 1, 2, 3, and 4 categories with respect to the criteria reported by Chang., *et al* [22].

According to the information in table 2 the first viable two classes have considered for embryo transfer which can be made immediately or can be cryopreserved for future use or distance use as well. The last non-viable two classes have to be discarded [3]. According to research findings, the embryo recovery and viability rates of embryos collected from the oviduct were higher than that of collected from the uterus [3,23].



a b

Figure 1: *Surgical embryo recovery [10].*

Grade	Grade
1	Excellent or good: symmetrical, spherical embryo mass, each blastomeres (cells) are uniform in size, color, and density, 85% of cellular material should be intact, viable embryonic mass, irregularities must be minor
2	Fair: with moderate irregularities in overall shape, size, color, and density of each cell. At least 50% of the cells should be intact and viable embryonic mass.
3	Poor: major irregular in shape, size, color and density embryonic mass
4	Dead or degenerated embryo, oocytes of 1 cell embryos, are nonviable

Table 2: *Classification of microscopically evaluated embryos.*
Source: Genzebu [2].

Non-surgical embryo recovery was not commonly practiced, but it is performed by using the Foley catheter (two way flow catheter) to allow flushing fluids to pass into the uterus and return back to collecting receptacle [10]. Evaluation is carried out in the same manner with embryo collected by surgical technique.

Figure 2: *Embryo evaluation [3].*

In vitro Embryo Production

In vitro embryo production is a technique by which embryo is originated and developed outside the reproductive tract of the donor animal [6]. This technique includes collection and maturation of oocytes, *in vitro* fertilization and embryos culture; cloning by somatic cells nuclear transfer and its modification as: interspecies cell nuclear transfer; nuclear transfer of embryonic stem cells and cloning by embryonic cell nuclear transfer [14,20]. Although this technique is costly and time consuming to perform, there has been a result with better blastocysts survival [10].

Oocytes collection and maturation

Immature oocytes can be collected from the ovaries of life animal by the minimally invasive laparoscopic ovum pick up, LOPU method [25] or by follicular aspiration as cited by Alexander., *et al.* [6], or by ovary slicing from the ovaries of slaughtered female animal. The latter is commonly practiced sources of oocytes for in vitro fertilization and in relative to the follicular aspiration, better quality and higher number of oocytes can be collected in ovary slicing method due to the presence of COC [25].

Ovaries should be transported to the laboratory at 37°C in PO_4^{-3} buffer saline and the collected ova have put in the 2% steer serum in Ham's F10 media [19]. Before LOPU, in sheep short repeated, in goat single shot of gonadotropin, FSH plus eCG, treatments should be used to collect the oocytes [6].

The quality of the oocytes determines to the success of embryo transfer. Because, it depends on the components that will complete the meiosis which is mainly affected by the size of the oocytes. Therefore, collected oocytes must first undergo nuclear and cytoplasm maturations by placing at 38 - 39°C [25]. Maturation of sheep oocytes have to been handed by mature follicular fluid supplements such as FSH and estradiol (ovarian and placental estrogen) with 10% estrous sheep or fetal calf serum in the *in vitro* maturation media [14]. Nevertheless, other media can be used for sheep oocytes maturation.

Similarly, different IVOM media can be used for maturation of goat oocytes. However, oocytes have to be enhanced by the supplements of 100 µM glutathione precursor cysteamine energy sources in IVOM media which have resulted in high blastocyst rate and high survival of blastocyst after embryo transfer [6]. In both cases the presence of cumulus cells has importance for maturation and subsequent fertilization [25].

In vitro fertilization

In vitro fertilization occurs when the collected ovum is fertilized by frozen or fresh sperm cell outside of the reproductive tract of the donor of that ovum [14]. Fertilization of the oocytes is usually carried out after 24 hours maturation in the IVOM media. Surrounding cumulus cells can be removed by gentle pipetting and washing in fertilization medium. Out of the matured groups of oocytes, 40 to 50% of them have to be ready for fertilization in 4 well dishes in 500 µl of synthetic oviductal development fluid covered by 200 µl mineral oil. Of course, this method was tested and succeeded [24].

Motile sperms could be obtained from fresh collected sperm cells or by centrifugation of the frozen-thawed semen in 45% to 90% Percoll gradient at 500g for 10 minutes at 25oC temperature as described by Ricardo., *et al* [25]. Percoll gradient had used for separation of spermatozoa seems to be an effective means of yielding motile sperm from frozen-thawed sperm as cited Alexander., *et al* [6]. Before use, sperm cells have to be supported first. Hence, Heparin or sheep serum can be used to capacitate the sperm cells by Ca^{+2} influxes in to the sperm acrosome [25].

Invitro culture and embryo maturation

Fertilized eggs have to put in *in vitro* culture media for maturation. Different culture media can be used, such as co-culture with somatic cells: tissue culture media TCM199 medium, culture with semi-defined media: sheep oviductal fluid SOF [25], or culture of embryo in *in vivo* systems: oviduct of the same or different species [24]. Cumulus cells are important to IVM success and subsequent fertilization as reported by Ricardo., *et al* [25]. Co-culture media has oviductal cells in TCM199 medium, bicarbonate buffered containing minerals energy

sources (glucose, glutamine) as well as vitamins and other amino acids as stated by Mermillod., *et al.* [19] though results had showed with variable in number and quality. In sheep, SOF medium improves the rate of cleavage compared to TCM199 medium as described by Ricardo., *et al* [25].

Other researchers were also practiced to incubate the fertilized eggs in *in vitro* culture media. An IVCM well can be prepared containing 500 µL of synthetic oviductal fluid developmental media which comprises of 107.7 mM NaCl, 7.16 mM kCl, 1.19 mM K_2PO_4, 1.71 mM $CaCl_2$, 0.49 mM $MgCl_2$, 25.07 mM $NaHCO_3$, 3.3 mM Na lactate, 0.33 mM Na pyruvate, 1.5 mM glucose, 32 mg/ml bovine serum albumin, 100 IU penicillin, 50 µg/ml streptomycin was incubated for 5 days at 38.5°C, 5% CO_2 in air and 90% humidity [3,14]. After 5 days, the embryos cultured in such IVCM have to be evaluated according to the embryo viability evaluating criteria presented by Genzebu [2] and viable ones can be made ready for transfer or storage.

Embryo storage

Matured embryos which are not needed to immediate transfer can be stored by the techniques called cryopreservation and vitrification [10]. Embryo cryopreservation is a technique by which the produced embryo (usually grade 1) has to be stored at low temperature (up to -196°C) for future use [25]. This technique has advantages in commercial embryo transfer program for storage, cheaper to transport embryos, avoids disease transmission and avoids loss of animal during transportation. Embryo cooling rate can be 0.3 to 1°C per minute to store at 5°C; at -30°C straws have to be placed in liquid nitrogen (Jamie., *et al.* 2012). Embryo thawing rate can be 1 to 3°C to thaw till -7°C and warming 0.6°C per minute to be at 37°C [3,10].

Currently a vitrification of embryo cryopreservation is the other use full technique for embryo manipulation [25]. It reduces embryo injuries [3]. It is prepared by the addition of higher concentration of cryoprotectants such as ethylene glycerol, 20% bovine fetal serum, sucrose etc. and very rapid cooling system this has been tested and succeeded in different species. Thus, it is fast and easy in relative to the conventionals low cooling technique [26].

This technique has advantage over the other storage technique in eliminating the formation of ice crystals in the embryo and it enables to have better embryo survival rate [3]. Greater embryo survival has been recorded during thaw when ethylene glycerol (0.5 to 1.5M) in Phosphate-Buffered Saline (PSB) and 20% fetal bovine serum is used rather than glycerol alone or dimethyl sulfoxide placed in 0.2 ml straw [25].

Since the collected embryo is highly susceptible to external environmental hazard, the time between collection and start of freezing has not to be great than 40 minutes. For the frozen embryo has need devitrification in air at 37°C before transferring for 6 seconds [10].

Evaluation of Cryopreserved Embryos

The evaluation of the cryopreserved embryos can be done based on the size, morphology and cleavages of the embryo mass within each blastomeres [2]. Hence, their morphology is evaluated under stereomicroscopic after thawing has done and will be transferred the selected blastocysts [25]. However, some authors suggested that morphological evaluation of thawed embryos was not accurate and the use of direct transfer of embryos were resulted in an improvement of 7 - 8% in offspring born [27]. This fact could be due to that embryo evaluation after embryo warming is dispensable as predictive of success. Therefore, they suggested that, this morphological evaluation system may need other side option that has results with more accurate information [25].

Embryo Transfer

For immediate embryo transfer for fresh embryo, it has to be placed first in the preservative media for at least two hours. For frozen ones, it should be thawed and transferred within 20 to 30 minutes [1,10].

Surgical embryo transfer is more commonly employed in embryo transfer in sheep and goats than the other large animals. It has efficiency rate of 60 to 70% as presented by Chang., *et al* [22]. All embryos have to be preferably transferred in to the uterine horn of a non-pregnant recipient of 6 to 7 days post synchronization [6]. Most of the time a single embryo is put in to the uterine horn, but occasionally, two embryos can be transferred when the recipient has two or more ovulations. It has drawback like post-surgical adhesion can occur in

the donors and recipients, and the percentage of embryo recovery decreases with subsequent operation [3,22].

However, a non-surgical embryo transfer technique can also be used. It is carried out by laparoscopic techniques. Hence, this technique needs skills and experiences to perform. It has found with as high pregnancy efficiency rate as 60% [24]. Moreover, it is advantageous in no reduction in repeated operation as stated by Gibbons and Ceuto [10].

Another non-surgical technique is trans-cervical embryo transfer. Embryos have been recovered through cervical canal and transferred via the same canal in to the uterine body or uterine horns as described by Lima-Verde [28]. This technique has advantage that it reduces post operation adhesion in the recipient; it has drawback of low PER when embryo is put in uterine body.

In poor oocytes donor, embryo transfer should be done only at fresh state to optimal pregnancy efficiency rate. Cryopreserved embryo has found with low viability [10].

Pregnancy Diagnosis

The detailed discussion of pregnancy diagnosis is beyond to the scope of this paper work.

However, it is important to describe few diagnostic tools and the required diagnostic techniques.

Laparoscopic as it was shown in figure 3 and trans-rectal ultrasound assessment as it was indicated in figure 4 are help full tools for observing the ovarian response to super ovulatory treatments in donor animals [29].

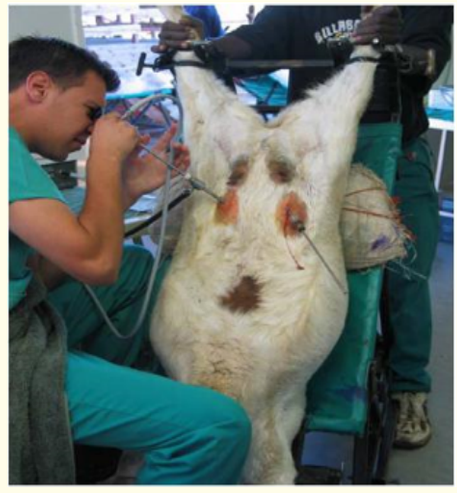


Figure 3: *Laparoscopic evaluation of ovarian response [3].*

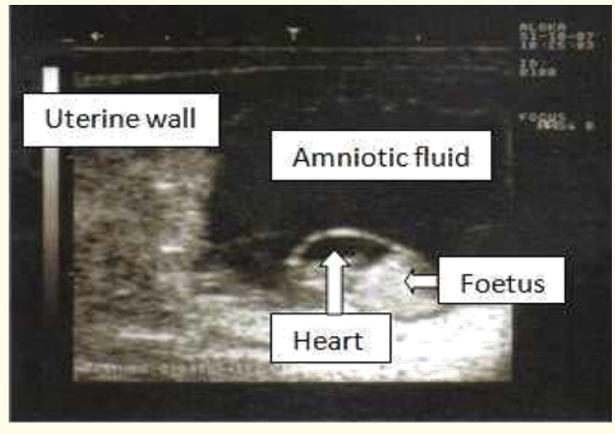


Figure 4: *Trans-rectal probed image at day 28 of gestation [30].*

Ovulatory events are determined by analyzing the progesterone profiles from the RIA results (Delwiche., et al. 2001). This is required when estrus detection seems poor at early pregnancy; diagnosis can be performed by hormonal detection like progesterone detection using Radio immune assay tests, mainly for progesterone detection [23].

The radioimmunological dosing was accomplished by RIA with pre-incubation for all the serums or milk resulted from day 0 till day 25 from mating. The biosensor can detect the progesterone but cannot quantitate the amount. Upon addition of magnetizable immunosorbent the antigen-antibody complex is bound on solid particles which are then separated by either magnetic sedimentation or centrifugation. Counting the radioactivity of solid phase enables a standard curve to be constructed and samples to be quantitated [31].

Since pregnancy diagnosis PG using rectal palpation is difficult in sheep and goats, trans-rectal ultrasonography has used to diagnose the stages and status of fetal developments during late stages pregnancy period, particularly, beyond to 40 days post insemination in sheep and goats.

Limitations of embryo transfer

Nowadays this technology is widely used in all domestic animals [2]. It is becoming as source of profit in the livestock industry. This is due to that it is effective means of increasing the contribution of superior females to genetic improvement schemes and is also an important procedure of the embryo biotechnology [9]. As a result, there have been rapid genetic progress in different breeds and established for meat, milk, and wool and hair production [10]. However, according to some researchers [19,32], the technology had been encountered with some limitations:

- The low success rate of development to the blastocyst stage that takes upto 7 days has found in increasing the cost of each embryo produced.
- Some abnormalities can develop on transferred IVP embryos as reported in the name 'large offspring syndrome'.
- Trans-placental disease transmission may occur during pregnancy.
- In IVP, the low number and quality of oocytes and low viability of frozen-thawed embryos can limit the large scale use of the technology.
- The success or failure of other biotechnologies like multiple ovulation and synchronization can limit the efficiency of embryo transfer.

Conclusion and Recommendations

Nowadays embryo transfer in sheep and goats is less practiced in African in relative to rest of the world. However, this technology is advantageous to shorten the generation interval of superior ewes and does, to increase the number of offspring of the genetically superior donors. Super ovulation procedure helps to ovulate more oocytes than the normal case. Embryo transfer technology can also be useful to commercialize the abattoir ovaries and to utilize genetical inferior recipients. It can be important in prevention and controlling the transboundary disease transmission by producing pathogen free *in vivo* and *invitro* produced embryos. It is also cost effective to transport the cryopreserved or vitrified embryos of superior female genetics at low cost instead of transporting the animals itself. Therefore, it should be practice for genetic, commercial, health and species conservation purposes.

However, embryo transfer is costly, sensitive, time consuming and laborious to perform the work. Therefore, the following points are recommended to perform successful work.

- All techniques should be done in a very well organized and coordinated manner
- Clean environment and hygienic condition are required preconditions
- Costs should be minimized by farther research works.

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