Available online at www.worldscientificnews.com

World Scientific News

An International Scientific Journal

WSN 127(3) (2019) 191-211 EISSN 2392-2192

Review on Growth and Development of Multiple Ovulation and Embryo Transfer Technology in Cattle

Habtie Arega Kidie

Ethiopian Institute of Agricultural Research, Pawe Agricultural Research Center, Po. Box-25 Pawe, Ethiopia

E-mail address: habtiearegahb@gmail.com

ABSTRACT

The study was conducted in the world multiple ovulation and embryo transfer technology in cattle. In animal husbandry application of artificial insemination, estrus synchronization, multiple ovulation and embryo transfer used for improving animal breeding and genetics. Embryo is an egg that has already been fertilized by a sperm cell. Embryo transfer is a process by which an embryo is collected from a donor female and then transferred in to a recipient female which serves as surrogate mothers for the reminder of pregnancy. The International Embryo Transfer Society data retrieval committee presents the embryo transfer activity participated countries was 39 and 50 in 2014 and 2015 respectively. There are two methods of production of embryos from donor females; in vivo and in vitro fertilization. The number of embryos collected by in-vivo in 2015 was 660,221, this result highest of 614,464 recorded in 2014. The value is increased by 7.45% from this contribution of North America (64.66%), Europe (22.4%) and South America (11.09%). Embryos collected by in-vitro fertilization were recorded 592,450 and 671,161in 2014 and 2015 respectively. According to the data United States and Canada are the leading embryo exporter countries, each exporting over 10,000 bovine in-vivo embryos each year followed by Australia and Argentina. It is expected South America, Africa and some Asia countries export only limited amounts of embryos, mainly due to risk of transmission diseases. I recommended that care will be taken during selection of donor and recipients, transfer procedures of embryos as well as in recipient management will be needed to enhance the efficiency by reducing risk of transmitting genetic disease via embryo transfer and the member state will be recorded appropriate data and send to international embryo transferring society for compiled.

***Keywords*:** Embryo, Embryo transfer, multiple ovulations, In-vivo, In-vitro, Artificial Insemination

(Received 03 April 2019; Accepted 19 April 2019; Date of Publication 20 April 2019)

1. INTRODUCTION

Multiple ovulation and embryo transfer are one of the reproductive technologies which is important to increase animal production (Faizah et al., 2018). In animal husbandry application of various bio-technological tools like AI, estrus synchronization for timed AI, multiple ovulation and embryo transfer, rumen microbial manipulation and modern breeding technique may be of great use for improving animal species in near future (Mondal et al., 2014). Embryo transfer has become the most power full animal scientist and breeders to improve genetic construction of their animal herds and increase quickly elite animal numbers which have recently gained considerable popularity with seed stock dairy and beef producers. The most modern applicable embryo transfer technology was developed in the 1970s. Nowadays, embryo transfer technology is considered to be the principal technique which is very much necessary for achieving success in various assisted reproductive technologies, especially in case of in-vitro fertilization and animal cloning (Kennady et al., 2018).

Embryo is an egg that has already been fertilized by a sperm cell. Embryo transfer is a process by which an embryo is collected from a donor female and then transferred in to a recipient female which serves as surrogate mothers for the reminder of pregnancy. A cow normally produces only one egg per estrus cycle (which lasts 21 days) and the gestation period is 40 weeks. On average, a cow produces only 2-3 calves in her lifetime. Thus, without intervention, the rate at which a particularly desirable cow can be used to improve the genetic status of a herd is slow. Smith (1988) introduced the concept of MOET and demonstrated how well-designed MOET programs could led to increased selection intensity and reduced generation intervals, resulting in improving genetic gains. Embryo transfer is now commonly used to produce artificial insemination sires from high proven cows and bulls.

Worldwide, close to 500,000 bovine embryos are produced and transferred each year. The transfer of bovine embryos to day commonly involves estrus synchronization and superovulation of a donor animal, insemination of the donor animal, and collection of embryos from the donor approximately 7 days after estrus, then transferred to recipients fresh or frozen and transferred at a later date. Initially, all collections and transfer were performed surgically through mid-ventral exposure of the uterus and ovaries. However, non-surgical embryo recovery and transfer techniques were developed in the mid-1970's (Hasler, 2003)

Recent advances in techniques for embryo transplantation are revolutionizing the rate of genetic improvement. The essential stages are: donor cows of good pedigree animals are treated with hormones (FSH and LH) to increase the number of eggs released at ovulation - multiple ovulation (MO), then the cows are artificially inseminated using semen from a proven bull. After 6-7 days the embryos are flushed out non-surgically, using a catheter placed into the uterus. This is possible because, in cattle, there is a delay in more embryos becoming implanted in the uterine wall. On average, 4-7 embryos are collected. The embryos may then be implanted into recipient cows whose estrus cycle is at the correct receptive stage-usually as the result of hormonal manipulation. Embryos may be frozen and stored, using techniques similar to those applied to semen.

The commercial embryo transfer industry in North America developed in the early 1970's with the introduction of continental breeds of cattle Betteridge (2003). The use of embryo transfer technology in cattle breeding has continued to increase (especially within the dairy industry) over the past 30 years with the movement toward genetic improvement as opposed to the production of desirable phenotypes Smith (1988). In Canada, approximately 70% of the

embryo transfer work is now being done on dairy cattle, and approximately 15,000 embryos are being frozen annually for export (Canadian Embryo Transfer Association Statistics, www.ceta.ca). Throughout the world over the past year, more than 100,000 donor cows were super stimulated and more than 500,000 bovine embryos were transferred Thibier (2005). This technology is influencing the direction of cattle breeding industries; the numbers are small but the impact is high. Commercial cattle breeders must recognize that they can benefit from well-designed embryo transfer programs providing selection criteria are appropriate for their environment and individual breeding objectives (Hasler, 2003).

In the last three decades, embryo transfer has developed into a specific advanced biotechnology which has gone through three major changes, the first with embryo derived from donors (in-vivo) by superovulation, non-surgical recovery and transfer, especially in cattle embryos, the second with in-vitro embryo production by ovum pick up with in-vitro fertilization (OPU-IVF) and the third including further in-vitro developed techniques, especially innovated embryo micromanipulation technique, which can promote us to perform embryo cloning involved somatic cells and embryonic stem cells, pre-implantation genetic diagnosis(PGD), transgenic animal production etc. At the same time, commercial animal embryo-transfer has become a large international business (Betteridge, 2006).

Significant progress has been made in methods of recovery, storing and implanting cattle embryos (including hormone applications) in several countries of the world. It has been initiated and about 10% genetic gains are achieved in cows. Embryo transfer has the potential to bring about genetic improvement twice as fast as AI alone. The objective of this review is to understand the current status of the MOET technology in cattle.

2. PRINCIPLES AND APLLICATIONS OF MULTIPLE OVULATION AND EMBRYO TRANSFER (MOET)

2. 1. Historical background and application of MOET

The first transfer of a bovine embryo was reported in 1949 (Umbaugh, 1949), and the first calf from embryo transfer in 1951 (Willett et al., 1951). Milestones in the development of this technology have been evaluated from their significance to our current knowledge of reproduction and 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.

Embryo transfer in cattle has recently gained considerable popularity with seed stock dairy and beef producers. Most of the applicable embryo transfer technology was developed in the 1970s and 1980s; however, the history of the concept goes back much farther. Embryo transfer was first performed and recorded by Walter (1890). He transferred two Angora rabbit embryos into a gestating Belgian doe. She went on to produce a mixed litter of Belgian and Angora bunnies. Embryo transfer in food animals began in the 1930s with sheep and goats, but it was not until the 1950s that successful embryo transfers were reported in cattle and pigs by Jim Rowson at Cambridge, England.

The first commercial embryo transfers in this country were done in the early 1970s. Initially, embryos were recovered from valuable donors and transferred to recipient animals using surgical procedures. It was not until non-surgical methods were developed in the late 1970s that embryo transfer grew in popularity.

Table 1. First young born after transfer of frozen - thawed embryos.

Year	Species	Researchers
1971	Mouse	Whittinghamet al.
1973	Cow	Wilmut and Rowson
1974	Rabbit	Bank and Maure
1974	Sheep	Willadsen
1975	Rat	Whittingham
1976	Goat	Bilton and Moore
1982	Horse	Yamamotoet a
1984	Human	Zeilmakeret al
1985	Hamster	Ridha and Dukelow
1988	Cat	Dresseret al
1989	Pig	Hayashiet al
1989	Rhesus monkey	Wolfet al.

Source: Gordon (2004).

2. 2. Common uses of embryo transfer technology

2. 2. 1. 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. 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

2. 2. 2. Planned mattings

AI has permitted the widespread dissemination of a male's genetic potential. Embryo transfer has also been used to rapidly expand a limited gene pool. The dramatic rise of the embryo transfer industry in North America is a direct result of the introduction of European breeds of cattle, which were then in short supply.

2. 2. 3. Genetic testing for mendelian recessive traits

A common use of embryo transfer procedures is to genetically test AI sires for deleterious heredity traits (Johnson *et al*., 1980). Some AI organizations keep known carriers of certain genetic defects on hand to serve as donors in testing new sires. Embryos are transferred into unrelated recipients and pregnancy may be terminated at various stages to examine fetuses for presence or absence of the defect. Depending on the heritability of the defect, generally eight to ten non-affected fetuses are sufficient to declare a bull free of that trait. Another alternative is to mate the bull in question to seven or eight of his super ovulated daughters. Offspring will then represent all recessive traits that a bull may carry. A less attractive alternative would be to naturally mate the bull in question to 40-50 of his daughters.

2. 2. 4. Twinning in cattle

Compared to other domestic animals, beef production is somewhat less efficient because not all cows produce a calf each year. However, it has been estimated that unit beef production can be increased by 60% in intensively managed herds through twinning Approximately 70% of nutrient intake by a beef cow is utilized for her own maintenance, whereas only about 30% is for growth and maintenance of the calf during pregnancy and lactation. Thus, it seems desirable to take advantage of the efficiency of gestation and lactation. Genetic selection for twinning in cattle has been largely unsuccessful and gonadotropin treatments to induce twinning have also been unreliable. Embryo transfer does provide a very real alternative in the production of twins.

The most limiting factor at this time is the cost of transfer which continues to exceed the average price for calves to be raised for meat. It must be remembered that recipients carrying twins will require extra nutrition and management, especially around calving. Furthermore, recipients must be sufficiently large to carry twins and produce enough milk to feed twins.

2. 2. 5. Disease control

Risk of infectious disease transmission is less by in-vivo-produced embryos, providing embryo handling procedures were done correctly 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.

2. 2. 6. 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. 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. This is particularly true of tropical and subtropical climates where the embryo would have the opportunity to adapt both in the uterus and then suckling a recipient indigenous to the area.

2. 2. 7. Salvage of reproductive function

Embryo transfer procedures have been useful in the diagnosis, treatment and salvage of reproductive function in so-called infertile cows. Although it is recommended that the cause of the infertility not be of genetic origin, this is often difficult to determine. The market place should sort this out if a genetic problem were inadvertently propagated through embryo transfer.

2. 2. 8. Research

Embryo transfer techniques have proven to be a very useful research tool. In fact, many technical developments in embryo transfer prior to 1970 were directed toward research purposes rather than for the propagation of superior livestock. These studies included natural limitations to twin pregnancies, uterine capacity, and endocrine control of uterine environment, maternal recognition of pregnancy, embryo-endometrium interactions, and the endocrinology of pregnancy.

2. 3. Application of embryo transfer

2. 3. 1. Why consider embryo transfer in cattle

The reproductive potential of each normal newborn calf is enormous. There are an estimated 150,000 potential "eggs" or ova in the female and countless billions of sperm produced by each male. By natural breeding, only a fraction of the reproductive potential of an outstanding individual could be realized. The average herd bull will sire 15 to 50 calves per year and the average cow will have one calf per year. With artificial insemination, it is possible to exploit the vast numbers of sperm produced by a genetically superior bull; however the reproductive potential of the female has been largely unutilized. She will produce an average of eight to 10 calves in her entire lifetime under normal management programs. Like artificial insemination has done for the bull, embryo transfer is a technique that can greatly increase the number of offspring that a genetically important cow can produce.

2. 3. 2. How is embryo transfer performed on cattle?

Virtually all commercial embryo transfer has done today uses nonsurgical recovery of the embryos rather than surgical techniques. The process involves several steps and considerable time as well as variable expense. The term MOET, multiple ovulation and embryo transfer was coined by Nicholas and Smith (1983) to consider embryo transfer and related technology in the context of optimizing genetic improvement of cattle. Most MOET schemes require one or a few large nucleus herds. The resulting genetic improvement would be disseminated to the general population by embryo transfer, artificial insemination, or more practically by young bulls to be used in natural breeding.

2. 4. Current status of cattle embryo transfer technology

The embryo transfer technology grew rapidly in the late 1970s, both in terms of the number of practitioners and in the number of donors. These technologies have resulted in new methods for producing embryos, for improving embryo quality, for long-term storage of embryos and Source of embryo oocytes, and for screening of embryos for important genes.

2. 4. 1. Participant countries on Embryo collection

The International Embryo Transfer Society (IETS) Data Retrieval Committee presents the 25th annual report on the data collected globally during 2016 for embryo transfer (ET) activity in 2015. The participant country increased from 39 in 2014 to 50 countries in 2015.

Table 2. Number and proportion of countries submitting data.

Region	No countries in Region	No. Countries submitting data	% Countries in Region	No. Countries submitting data	% Countries in region
		2014		2015	
Africa	57	3	5.26	2	3.51
Asia	53	0	0.00	4	7.55
Europe	45	27	60.00	32	71.11
North America	3	3	100.00	3	100.00
Oceania	23	2	8.70	2	8.70
South America	44	4	9.09	7	15.91
GLOBALLY	**225**	**39**	**17.33**	**50**	**22.22**

Source: (IETS, 2015)

Challenges of embryo transfer technology

Collecting quality data continues to be problematic despite the recent development of the secure IETS database system for the following reasons:

- Lack of trust by ET practitioners in ensuring the confidentiality of the source of the ET data collected
- The effort required by individual ET practitioners to collect ET activity data, especially those with small teams that have little contact with their national ET industry or the IETS
- The lack of interest by many countries not submitting ET data despite the evidence of the benefits.
- The ability to find national ET data collectors recognized by colleagues as being impartial
- The reluctance of some countries/regions to adapt their ET data collection procedures to suit the IETS format

2. 4. 2. Global data on embryo collection and transfer

2. 4. 2. 1. Sources of embryo collection

There are two procedures presently available for production of embryos from donor females. One consists of superovulation, followed by AI and then flushing of the uterus to gather the embryos in-vivo derived (IVD) and *in-vitro* fertilization (IVF) consists of recovery

of eggs from the ovaries of the female then maturing and fertilizing them outside the body until they are ready for implantation into foster females. IVF facilitates recovery of a large number of embryos from a single female at a reduced cost thus making ET techniques economically feasible on a larger scale. In-vitro embryo collection is using ovum pickup (OPC) and abattoir.

A. In-vivo fertilization

The bovine embryo transfer industry has recorded its highest ever number of collections and transfers globally. The number of in-vivo derived (IVD) bovine embryos collected in 2015 was 660,221, this result highest of 614,464 recorded in 2014. The value is increased by 7.45%.

Table 3. Comparison of IVD embryo ET activity

Region	BOVINE IN-VIVO DERIVED EMBRYO COLLECTION								
	Flushes	Transferrable embryos	No embryos per collection	% of global embryo production	Flushes	Transferrable embryos	No embryos per collection	% of global embryo production	% change transferrable embryos from 2014
	2014				2015				
Africa	794	5,782	7.28	0.94	711	5,534	7.78	0.84	-4.29
Asia	0	0	0.00	0.00	14,689	105,685	7.19	16.01	0.00
Europe	22,408	137,998	6.16	22.46	20,497	127,980	6.24	19.38	-7.26
North America	58,934	397,306	6.74	64.66	53,536	360,020	6.72	54.53	-9.38
Oceania	1,326	5,224	3.94	0.85	2,353	11,187	4.75	1.69	114.15
South America	11,204	68,154	6.08	11.09	8,953	49,815	5.56	7.55	-26.91
Grand Total	**94,666**	**614,464**	**6.49**	**100.00**	**100,739**	**660,221**	**6.55**	**100.00**	**7.45**

Source: (IETS, 2015)

Regionally, the number of IVD bovine embryo collected leading by North America (64.66%), Europe (22.4%) and South America (11.09%).

B. In-vitro fertilized (IVF)

The first IVF followed by birth of offspring was achieved in the rabbit. The first calf after IVF was born during 1981. Here, unfertilized eggs are fertilized in the laboratory and cultured

for a few days until they have developed into early embryos. These are then transplanted, using a special long syringe, into the uterus of the recipient cows that are at the correct receptive stage of the oestrus cycle. The technique has been greatly improved, now. Obviously, it is possible to choose the egg and semen from high quality parents. The recovery of eggs from the oviduct requires surgery. The eggs to be used may be fully mature ones, recovered after ovulation, from the oviduct of a super ovulated cow.

Table 4. Collection and transfer of bovine OPU IVP embryos by regions.

REGIONS	OVUM PICK-UP FOR BOVINE IN-VITRO PRODUCED EMBRYOS									
	COLLECTION			TRANSFERS		COLLECTION			TRANSFERS	
	DONORS	OOCYTES	EMBRYOS	FRESH EMBRYOS	FROZEN EMBRYOS	DONORS	OOCYTES	EMBRYOS	FRESH EMBRYOS	FROZEN EMBRYOS
	2014					2015				
Africa	1359	20976	5081	1202	170	1113	21494	3733	162	21
Asia	0	0	0	0	0	3177	59224	9438	3250	1164
Europe	9710	83785	15693	10980	2957	9092	73397	13780	9799	4703
North America	43452	812468	206139	71263	21667	35980	683717	212046	65844	32027
Oceania	3250	30549	6486	2044	3171	1646	17533	3892	1825	2491
South America	71327	861100	356960	211177	40096	60696	1205840	369820	224066	58821
Grand Total	129098	1808878	590359	296666	68061	111704	2061205	612709	304946	99227

Source: (IETS, 2015)

Table 5. Collection and transfer of bovine abattoir-derived IVP embryos by regions.

REGIONS	ABATTOIR DERIVED OOCYTES FOR BOVINE IN-VITRO PRODUCED EMBRYOS									
	COLLECTION			TRANSFERS		COLLECTION			TRANSFERS	
	DONORS	OOCYTES	EMBRYOS	FRESH EMBRYOS	FROZEN EMBRYOS	DONORS	OOCYTES	EMBRYOS	FRESH EMBRYOS	FROZEN EMBRYOS
	2014					2015				
Africa	0	0	0	0	0	156	2033	235	0	0
Asia	0	0	0	0	0	35335	714783	56740	11485	11445
Europe	1335	37414	1369	0	35	65	2472	434	0	0
North America	13	258	187	193	0	5	9117	1037	273	418
Oceania	9	146	16	260	0	7	142	16	8	8
South America	118	2025	519	410	0	0	0	0	0	0
Grand Total	1475	39843	2091	863	35	35568	728547	58462	11766	11871

Source: (IETS, 2015)

The global production of bovine IVF embryos is exceeded the half-million mark with a total of 592,450 IVF embryos produced 590,359 by ovum pick-up (OPU) method and 2091 by collection of ovaries at abattoirs in 2014 increased by 671,161 IVF embryos produced 612,709 by ovum pick-up (OPU) method and 58,452 by collection of ovaries at abattoirs in 2015. This is a significant jump of 13.3%. Production of OPU transferrable embryos grew significantly from 590,359 embryos to 612,709 embryos, a significant increase of 3.8% and abattoirs 2091to 58452 this increased by 270% in two successive years 2014 and 2015 respectively.

2. 4. 3. Embryo marketing

A major concern is the lack of ET data for exports from most European and Asian countries. The collection, handling, processing and transfer of livestock embryos has, over the past 30 plus years of commercial ET activity, has consistently proved to be very safe and to have negligible risk of disease transmission. The risk is negligible even with fresh embryos, often washed only up to three times and collected from animals of unknown health status before transfer to other animals within the same country. It is clear that given the very high level of safety of embryo transfer in livestock, conditions for international trade in livestock embryos should be one of trust rather than mistrust, as currently evidenced by the onerous and unnecessary biosecurity conditions imposed by many countries for importation of livestock embryos. The time is rapidly coming when countries will need to be prepared for international trade in fresh or chilled micro-manipulated (DNA tested) IVF embryos. Such trade will require a high level of trust in the ET industry as evidence by a streamlined and rapid border post processing of the imported consignments to ensure the survival of such embryos at the time of transfer to recipients.

The techniques employed to ensure that frozen embryos are free of pathogens include the use of specific pathogen-free donors, the washing and trypsin treatment of embryos, or a combination of these methods. Health certification procedures for international trade generally require embryos to be examined under the microscope over all surfaces to ensure that there is no material adhering to the zona pellucida cattle embryos are apparently less sticky than those of sheep and pigs. According to the standards agreed by the IETS, should be free from bacterial and viral infections; in the preparation and pre-freeze treatment of embryos, it is essential that the integrity of the zona pellucida be preserved (Gordon 2004).

Table 6. Countries known to export embryos.

Animal species	Country	Exported
Bovine IVD	South Africa	659
	Canada	12758
	United states	15896
	Australia	2426
	New Zealand	28
	Argentina	2946
Bovine IVF OPU	Canada	122
	Dominican Republic	590

Source: (IETS, 2015)

Export data remains very problematic, with very little reporting, especially from Europe. According to the data received, United States and Canada are the major exporters, each

exporting over 10,000 bovine IVD embryos each year. They are followed by Australia and Argentina. It is expected South America, Africa and to some extent Asia export only limited amounts of embryos, mainly because of concerns with risk of transmission of exotic diseases. It is not known if the EU member states keep any record of intra-EU trade or trade with third countries but the AETE have been approached to see if this can be addressed. It is believed Japan does not permit export of bovine embryos in order to protect their Wagyu industry. As yet, there is very little international trade in IVF embryos and many countries are reluctant to commit resources to developing import protocols for IVF embryos, especially from South America.

2. 5. General procedures of bovine embryo transfer

2. 5. 1. Selection of donor cows

The first step in embryo transfer is the selection of the donor cow. According to FAO (1991), there are two broad criteria for selecting donors for most embryo transfer programs: (1) genetic superiority, those animals that contribute to the genetic objectives of the programmed milk production, milk composition, growth rates, calving ease and disease resistance and (2) likelihood of producing large numbers of usable embryos**.** Healthy, cycling cattle with a history of high fertility make the most successful donors. Donors at least two months post-partum produce more embryos than those closer to calving. Young cows seem to yield slightly more usable embryos than heifers under some conditions. The donor should be maintained at the level of nutrition appropriate for her size and level of milk production. Both the very obese cow and the thin cow will have reduced fertility, so it is important that the donor cow be in an appropriate body condition score at the time of embryo transfer (Selk, 2013). 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.

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).

2. 5. 2. Superovulation of donor cow

A cow normally produces only one egg per estrous cycle (which lasts 21 days) and the gestation period is 40 weeks. On average a cow produces only 2-3 calves in her lifetime. Thus, without intervention, the rate at which a particularly desirable cow can be used to improve the genetic status of a herd is slow. However, recent advances in techniques for embryo transplantation are revolutionizing the rate of genetic improvement. Superovulation is the release of multiple eggs at a single estrus. Cows or heifers properly treated can release as many as 10 or more viable egg cells at one estrus. Approximately 85% of all normal fertile donors will respond to superovulation treatment with an average of five transferable embryos. Some cows are repeatedly treated at 60-day intervals with a slight decrease in embryo numbers over time (Selk, 2013). The basic principle of superovulation is to stimulate extensive follicular

development through the use of a hormone preparation, which is given intramuscularly or subcutaneously, with follicle stimulating hormone (FSH) activity. Commercially available preparations of FSH are injected twice daily for four days at the middle or near the end of a normal estrous cycle, while a functional corpus luteum (CL) is on the ovary. A prostaglandin injection is given on the third day of the treatment schedule which will cause CL regression and a heat or estrus to occur approximately 48 to 60 hours later

Many factors may influence how donors respond to superovulation and generate a high number of fertilized good to excellent quality embryos. Outside of genetics, nutrition probably is the single greatest factor that influences the response of donor cows to superovulation. It is important to ensure that cows are maintained on a positive plain of nutrition and are fed a diet that meets maintenance requirements (Lamb, 2011).

Data provided by both the American and Canadian Embryo Transfer Associations indicate that mean embryo yields per donor are in the range of 5-7 and basically have not changed for many years. The embryo means below are a composite average of individual means of both beef (6.6) and dairy cattle (5.7) flushed by the certified members of AETA. Increased understanding of the processes of oocyte growth and maturation is essential to improving the efficiency of superovulation (Merton, 2003).

2. 5. 3. Insemination of the cow

The time when the donor is first observed in standing oestrus is the reference point for insemination treatment. Because of the release of many ova from the multiple follicles on the ovary, there is a greater than normal need to be certain that viable sperm cells reach the oviducts of the super ovulated females, the estrus donor is inseminated, usually with at least two straws of semen 12h apart, and 7 days later the uterus is flushed to recover the embryos (Sinclair et al., 2000). Using high quality semen with a high percentage of normal, motile cells is a very critical step in any embryo transfer program. The correct site for semen placement is in the body of the uterus. This is a small target (1/2 to 1 inch) that is just in front of the cervix. There seems to be a tendency for inseminators to pass the rod too deep and deposit the semen into one of the uterine horns, thereby reducing fertility if ovulations are taking place at the opposite ovary (Selk, 2013).

2. 5. 4. Recovery of embryo

2. 5. 4. 1. Surgical embryo collection method

Early collection techniques involved either slaughtering the females and excising the oviducts, or surgically removing the oviducts from live females at 72 hours post ovulation so that the embryos could be recovered by flushing (Duran *et al*., 1998). A surgical method was developed first. This is done by performing a laparotomy (flank or midline abdominal incision) to expose the reproductive tract. A clamp or the thumb and forefinger can be used to block the distal one third of the uterine horn, so that fluid injected into that segment can be forced through the oviduct with a gentle milking action and collected at the infundibulum. Culture medium is introduced through a puncture at the utero-tubal junction or through the oviduct until the uterus is turgid. The uterus is then punctured with a blunt needle attached to a flexible catheter. The pressure will cause the medium to gush through the catheter, with enough turbulence to carry the embryos into a collection tube. These procedures allow for the recovery of a high percentage of embryos. However, because of the surgical trauma and resulting adhesions they can be

repeated only a few times. Adhesions make it difficult, if not impossible, to expose the reproductive tract repeatedly, and limit surgical interventions to a maximum of around Three. In cattle, embryos for commercial purposes are usually recovered 6 to 9 days after estrus

2. 5. 4. 2. Non-surgical embryo collection method

Non-surgical techniques are preferred as they are not damaging to the reproductive tract, are repeatable and can be performed on the farm. 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. In cattle, embryos are collected normally on days 6 to 8 (average day 7) after the onset of the estrus induced by Superovulation

2. 5. 5. Embryo handling, evaluation and storage

2. 5. 5. 1. 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, petri-dishes, plastic straws and other equipment, exposure to toxic factors must always be a consideration. Once an embryo is identified in the searching dish, it is immediately transferred to a small Petri dish (35 × 10 mm) containing fresh, filtered (0.22–0.45μ pore size), sterile medium. As a holding medium, generally phosphate buffered saline (PBS) containing penicillin plus 10–20% heat inactivated serum is used.

2. 5. 5. 2. Evaluating embryos

Quality evaluation

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 blasto meres (cells) that are uniform in size, color and density. This embryo is consistent with its expected stage of development. 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. Generally embryos of excellent and good quality, at the developmental stages of compact morula to blastocyst yield the highest pregnancy rates, even after freezing. Fair and poor quality embryos yield poor pregnancy rates after freezing and should be transferred fresh. It is advisable

to select the stage of the embryo for the synchrony of the recipient. It would also seem that fair and poor quality embryos are most likely to survive transfer if they are placed in the most synchronous recipients (Mapletof *et al*., 2013).

2. 5. 5. 3. Embryo storage

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 long-term storage system is essential. Deep-freezing embryos is storage in liquid nitrogen (-196oc) 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).

2. 5. 6. Selection of recipient females

Recipients must have a proven reproductive performance, free of congenital or infectious diseases to obtain high conception rates and have a sturdy body size to avoid problems of dystocia (Larson, *et al*., 2010). Recipient cows were animals not pregnant and with more than 90 days postpartum, fewer than five calving, and without any gross pathological features in genital tract.

Only animals with a CL detected by rectal palpation were selected. Generally females ideal as recipients are as follows: Cows 3 to 8 years old make good recipients once they have a good calving record, Heifers are good recipients providing they have reached their breeding weight (around 65-70% of mature weight) and are cycling, Use fertile animals, Animals are docile and body condition, an ideal score of 2.5-3 is preferred at the time of transferring the embryos. Any routine treatment should take place at least 3 weeks prior to transfer; changes in the feeding regimen should be prohibited for 3–4 weeks before and after transfer. Recipients should be located where they can be easily and quietly handled on the day of transfer (Gorden, 2004).

2. 5. 7. Donor - recipient synchrony

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. 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).

Table 7. Embryo-recipient synchrony and pregnancy rates

Oestrus synchrony category(h)	Number of embryo transfers	Pregnancy rate(Mean ± SEM)
-12 to -24	37	51.4±8.2
0 to 12	67	58.2 ±6.1
0	9	66.7 ±16.6
0 to +12	78	61.5 ±5.6
+12 to +24	37	48.6 ±8.2
0 to ±12	126	62.7 ± 4.4b
±12 to ±24	102	50 ± 4.9a

Source (Gorden, 2004).

2. 5. 8. Transfer of bovine embryo

2. 5. 8. 1. Surgical embryo 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 (corpus luteum) is located by rectal palpation and the flank ipsi lateral to the CL is clipped, washed with soap and water, and sterilized with iodine and alcohol. About 60 ml of 2% procaine is given along the line of the planned incision.

2. 5. 8. 2. Non- surgical embryo transfer

Work in Ireland in the mid-1970s and else-where showed that it is possible to establish pregnancies by a non-surgical procedure involving the use of the standard Cassou inseminating instrument. The embryo is loaded, held in a small volume of medium (e.g. phosphate-buffered saline (PBS) supplemented with 15% bovine serum), into a plastic straw (usually 0.25 ml

capacity). At transfer, the straw is inserted into the inseminating instrument ('gun') in the usual way and the same procedure followed as for AI, the main difference being that the embryo is deposited around the mid-horn position (ipsilateral horn); before carrying out the transfer, the recipient animal is given an epidural anaesthetic and tranquillizer. During the past quarter-century several variants of the standard transfer instrument have been marketed, with appropriate modifications to ensure that the embryo is deposited safely in the uterus (Gorden, 2004).

2. 5. 9. Pregnancy diagnosis of recipients

The first good indicator of pregnancy is failure of the recipients to show oestrus 18–24 days after the pre-transfer oestrus. Progesterone assay of milk or blood samples 22–24 days after the pre-transfer oestrus is 95% accurate in diagnosing non pregnancy and about 80% accurate for pregnancy. Development of sensitive automated inline milk progesterone assays should make this technology amenable to commercial application (Fricke et al., 2014). The first reliable pregnancy- specific hormone assays were developed to measure placenta- derived proteins. This protein starting around days 17 to 19 of pregnancy in cattle (Spencer et al., 2007). Also begin to be reliably detectable in plasma starting at day 24, and by day 28. Palpation recommended at 45–60 days per rectum and at about day 26 of pregnancy in heifers and day 28 in cows, pregnancy can be diagnosed accurately under field conditions by ultrasonography. Finally the cow must manage properly on the bases of nutrition, health.

2. 6. Application of bovine embryo transfer in developing countries

After artificial insemination and oestrus synchronization, embryo transfer is the third most commonly used biotechnology (Cowan, 2010). Embryo transfer from one mother to a surrogate mother makes it possible to produce several livestock progenies from a superior female. However, embryo transfer is still not widely used despite its potential benefits. In developing countries this is mainly due to absence of the necessary facilities and infrastructure. An evaluation of country reports (FAO, 2007) shows that only five of the African countries providing information (Ivory coast, Kenya, Madagascar, Zambia and Zimbabwe) use of ET also been independently reported in south Africa (Greyling *et al*., 2002) The first successful embryo transfer in Ethiopia, resulted in the birth of a Holstein-jersey calf a the Adami Tulu Animal Research Center in the beginning of May, 2010 and five more calves had born. In April 2010, eighty frozen embryos that were imported the previous August were implanted in the native cows (Lonny, 2010). Embryo transfer increases reproductive rate of selected females, reduces disease transfer and facilitates the development of rare and economically important genetic stocks as well as the production of several closely related and genetically similar individuals that are important in livestock breeding research.

2. 7. Challenges of embryo transfer in developing countries

The constraints and limitation of biotechnology in animal production in developing countries are due to factors such as the poor conditions of the human population in such countries that include poverty, malnutrition, disease, poor hygiene and unemployment (Madan, 2003).The major constraints of animal biotechnology includes: insufficient access to land and other productive resources, unfavourable terms of trade for food products, especially for animal products, lack of database on livestock and animal owners in most of the developing world,

uniqueness of animal breeds in developing world, lack of trained scientists, technicians and field-workers, absence of coordination between industry, universities and institutions for technology transfer, expensive technology to be purchased from developed world, high cost of technological inputs, poor bio-safety measures of biotechnology developed in developing countries, negligible investment in animal biotechnology in Africa, lack of clear policy and commitment from the government, disregards for indigenous knowledge and local agricultural resources management.

3. SUMMARY

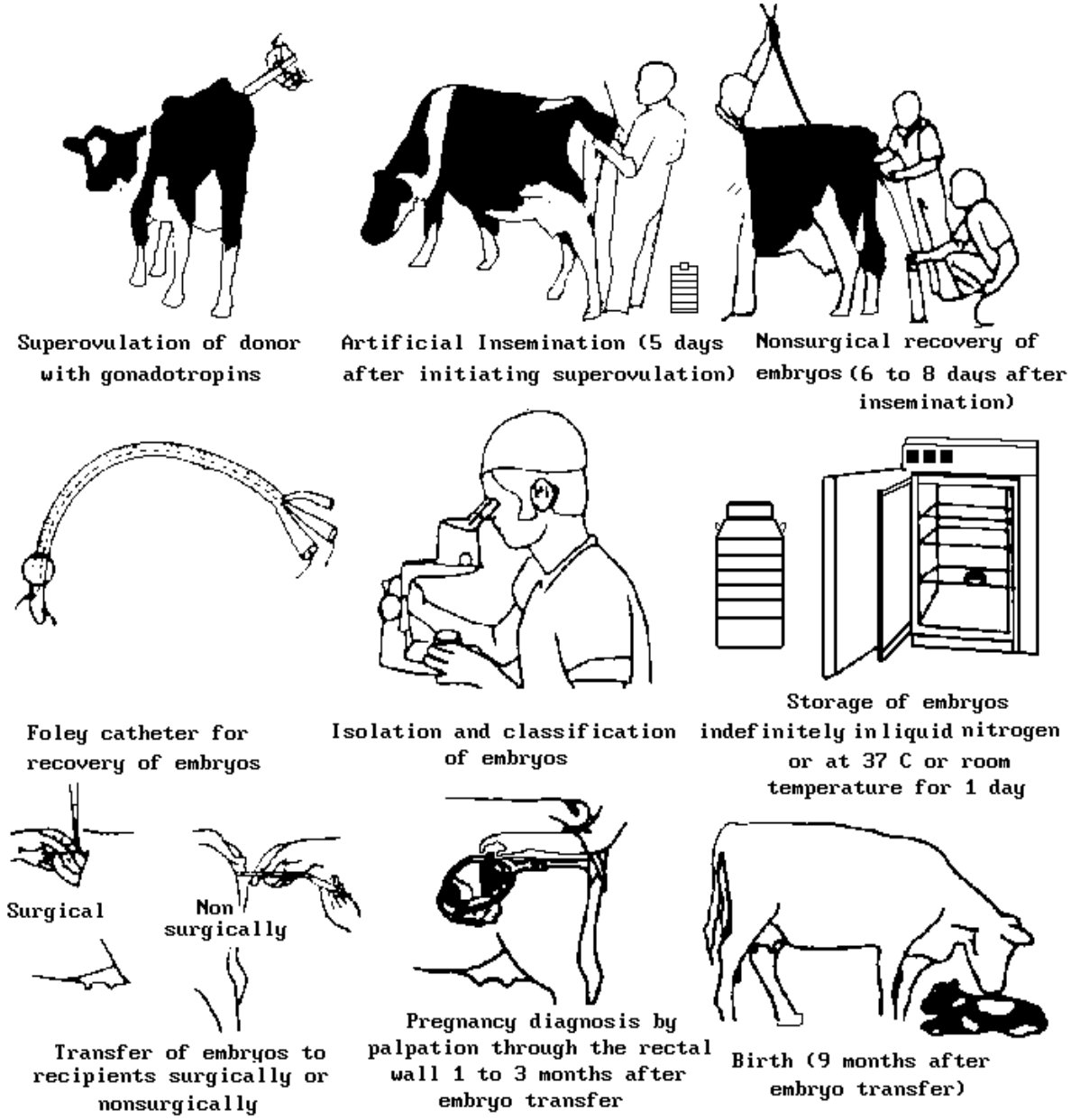

Fig. 1. General steps of MOET technology

4. CONCLUSSION AND RECOMMENDATION

4. 1. Conclusions

In animal husbandry, embryo transfer has become the most powerful tool for animal scientists and breeders to improve genetic make of their animal herds and increase quickly elite animals. Embryo transferring is a technique by which embryos are collected from a donor female and are transferred to recipient females. It could send female and male genetics worldwide in a cryopreservation tank without the hassle and complications of exporting live animals and the associated risk.

The success and economics of cattle embryo transfer programed is dependent on several factors. Using high quality semen with a high percentage of normal, motile cells is a very critical step in any embryo transfer program, the transfer must be to a recipient in the same stage of cycle as the donor, recipients must have a proven reproductive performance, free of congenital or infectious diseases to obtain high conception rates and have a sturdy body size to avoid problems of dystocia.

Embryo transfer techniques can operate in surgical and non-surgical. In cattle non-surgical embryo recovery procedures combined with non-surgical transfer techniques become available. Embryos can be transferred immediately upon recovery and evaluation or may be stored frozen in liquid nitrogen and transferred at a later date. The freezing and thawing process also is also very intricate and usually results in an approximate 10 - 20% reduction in pregnancy rates from those observed with fresh embryos

4. 2. Recommendations

Care will be taken during selection of donor and recipients, transfer procedures of embryos as well as in recipient management will be needed to enhance the efficiency by reducing risk of transmitting genetic disease via embryo transfer. The member state will be recorded appropriate data and send to international embryo transferring society for compiled.

References

[1] Betteridge KJ. (2003). A history of farm animal embryo transfer and some associated techniques. *Journal of Animal Reproduction Science* 79: 203-244

[2] Betteridge, K.J. (2006). A history of farm animal embryo transfer and some associated techniques. *Animal Reproduction Science, 79,* 203- 244

[3] Johan P C Greyling, M van der Nest, L. M. J Schwalbach, T Muller. Superovulation and embryo transfer in South African Boer and Indigenous feral goats. *Small Ruminant Research* January 2002, 43(1): 45-51. DOI: 10.1016/S0921-4488(01)00257-7

[4] Faizah H.M.S, Richard F., Meena P., Stanley K.L., Amriana H., Alhassany A., Yadav S.B., Marie L., Crouch B., and Saipul B.A.R., 2018. Multiple Ovulation Embryo Transfer (MOET) In Dairy Cattle in Gatton; *Malaysian journal of veterinary research* 9(2) pp. 109-116

[5] Hasler JF. (2003). The current status and future of commercial embryo transfer in cattle. *Animal Reproduction Science* 79: 245-264

[6] Hasler, J. F. (2001). Factors affecting frozen and fresh embryo transfer pregnancy rates in cattle. *Theriogenology* 56: 1401-1415

[7] IETS (International Embryo Transfer Society) 2015. Statistics and Data Retrieval Committee Report the year world-wide statistics of embryo transfer in domestic farm animals.

[8] Kennady Vijayalakshmy, J Manimegalai, Ranjeet Verma and Vikas chaudhiry, 2018. Embryo transfer technology in animals: An overview. *Journal of Entomology and Zoology Studies* 6(5), pp. 2215-2218

[9] Larson J., G. Lamb, B. Fennel, S. Bird, A. Martin and J. Rodgers. (2010). Embryo production in super ovulated Angus cows inseminated four times with sexed-sorted or conventional, frozen-thawed semen. *Theriogonology,* 73(5): 698-703

[10] Madan, M., (2003). Opportunities and constraints for using gene-based technologies in animal agriculture in developing countries and possible role of international donor agencies in promoting R&D in this field. In: FAO/IAEA international symposium on applications of gene based technologies for improving animal production and health in developing countries, Vienna, Austria, 6 -10 October. *Theriogenology* 73(5): 698-703

[11] Mapletoft R.J., (2013): History and perspectives on bovine embryo transfer. *Animal Reproduction,* 10 (3): 168-173

[12] Mondal, M., Baruah, K.K., and Rajkhowa, C. (2014). Application of Biotechnology for Augmentation of Productivity in Mithun (Bos frontalis). *Global Journal of Animal Science Scientific Research* 2(4): 357-364

[13] Thibier M. (2005). Significant increases in transfers of both in vivo derived and in vitro produced embryos in cattle and contrasted trends in other species in 2004. *IETS Newsletter* 23(4): 11-17

[14] Wrathall AE, Brown KF, Sayers AR, Wells GA, Simmons MM, Farrelly SS, Bellerby P, Squirrell J, Spencer YI, Wells M, Stack MJ, Bastiman B, Pullar D, Scatcherd J, Heasman L, Parker J, Hannam DA, Helliwell DW, Chree A, Fraser H. Studies on embryo transfer from cattle clinically affected by bovine spongiform encephalopathy (BSE). *Vet Rec* 2002; 150: 365-378

[15] Bousquet D, Twagiramungu H, Morin N, Brisson C, Carboneau G, Durocher J. In vitro embryo production in the cow: an effective alternative to the conventional embryo production approach. *Theriogenology* 1999;51:59-70

[16] Hagemann LJ, Beaumont SE, Berg M, Donnison MJ, Ledgard A, Peterson AJ, Schurmann A, Tervit HR. Development during IVP of bovine oocytes from dissected follicles: interactive effects of estrous cycle stage, follicle size and atresia. *Mol Reprod Dev* 1999; 53: 451-458

[17] Garcia A, Salaheddine M. Effects of repeated ultrasound-guided transvaginal follicular aspiration on bovine oocyte recovery and subsequent follicular development. *Theriogenology* 1998; 50: 575-585

[18] Goodhand KL, Watt RG, Staines ME, Hutchinson JSM, Broadbent PJ. In vivo oocyte recovery and in vitro embryo production from bovine donors aspirated at different frequencies or following FSH treatment. *Theriogenology* 1999, 51: 951-961

[19] Duby RT, Damiani P, Looney CR, Fissore RS, Robl JM. Prebuberal calves as oocyte donors: promises and problems. *Theriogenology* 1996; 45: 121-130

[20] Armstrong DT. Effects of maternal age on oocyte developmental competence. *Theriogenology* 2001; 55: 1303-1322

[21] Vajta G, Kuwayama M, Holm P, Booth PJ, Jacobsen H, Greve T, Callesen H. A new way to avoid cryoinjuries of mammalian ova and embryos: the OPS vitrification. *Mol Reprod Dev* 1998; 51: 53-58

[22] Young LE, Sinclair KD, Wilmut I. Large offspring syndrome in cattle and sheep. *Rev Reprod* 1998; 3: 155-163

[23] Numabe T, Oikawa T, Kikuchi T, Horiuchi T. Birth weight and birth rate of heavy calves conceived by transfer of in vitro or in vivo produced bovine embryos. *Anim Reprod Sci* 2000; 64: 13-20

[24] Farin PW, Crosier AE, Farin CE. Influence of in vitro systems on embryo survival and fetal development in cattle. *Theriogenology* 2001; 55: 151-170

[25] Van Wagtendonk-de Leeuw AM, Mullaart E, de Roos APW, Merton JS, den Daas JHG, de Ruigh L. Effects of different reproduction techniques: AI, MOET or IVP, on health and welfare of bovine offspring. *Theriogenology* 2000, 53: 575-597

[26] Sangild PT, Schmidt M, Jacobsen H, Fowden AL, Forhead A, Avery B, Greve T. Blood chemistry, nutrient metabolism, and organ weights in fetal and newborn calves derived from in-vitro produced bovine embryos. *Biol Reprod* 2000; 62: 1495-1504

[27] Wilmut, I, Schnieke, A.E., Mcwhir, J., Kind, A.D. and Campbell, K.H.S. (1997). Viable offspring derived from fetal and adult mammalian cells. *Nature,* 385, 810-813

[28] Wells, D.N., Misica, P.M. and Tervit, R.H. (1999). Production of cloned calves following nuclear transfer with cultured adult mural granulosa cells. *Biol. Reprod.* 60, 996-1005

[29] Galli, C., Duchi, R., Moor, R.M. and Lazzari, G. (1999). Mammalian leukocytes contain all the genetic information necessary for the development of a new individual. *Cloning* 1: 161-170

[30] Vajta G, Lewis IM, Hyttel P, Thouas GA, Trounson AO. Somatic cell cloning without micromanipulators. *Cloning* 2001; 3: 89-95