Multiple Ovulation and Embryo Transfer in Kenya: A Review of the Success Rates and Lessons Learnt

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Abstract

There is a high demand for high producing dairy cows in Kenya and in the East African region. Milk plays an important role in human nutrition especially provision of cheaper much needed proteins, minerals and other nutrients. It is also a source of income to many households and the people employed within the dairy farms. The livestock sector contributes 10-12% of the gross domestic product (GDP), which represents 47% of the Agricultural GDP, in Kenya. Dairy farming contributes over half of these through milk production and sale of breeding stock. Over 80% of all milk is produced by small scale farmers in rural areas who depend on large scale breeders to provide affordable replacement heifers. The use of assisted reproductive techniques including artificial insemination, sexed semen, multiple ovulation and embryo transfer and invitro embryo production has propelled many countries to achieve sustainable production of milk and replacement heifers. Commercial cattle embryo transfer in the world was established during the early 1970s. It is difficult to have adequate and high quality replacement heifers due to low reproductive capacity and inadequate number of high quality breeding stock in Kenya. Attempts to bridge this gap with newer biotechnologies including multiple ovulation and embryo transfer (MOET) have had variable success rates. This has led to higher cost of production thus discouraging the adoption of such high value technologies. The aim of the review was to evaluate the MOET technology since its introduction to Kenya in the 80s. The super ovulations resulted in variable embryo production with a range of 0 to 13 transferable embryos and successful transfer rates between 0 to 67%. Lack of an appropriate MOET protocol, poor choice of donors and recipients, poor technique and lack of finance were among the factors that have contributed to the observed variations. Reproductive efficiency of the top producing cows through MOET may provide a solution to the high demand which has driven the prices of breeding cattle way above the ordinary milk producer.

Keywords: *Biotechnology, Dairy Cows, Multiple Ovulation, Embryo Transfer, Superovulation, Cattle*

Introduction

The demand for livestock and livestock products is the fastest growing in the world with increasing trends at 114% in demand of meat and 133% for milk. The demand is clearly more than the current supply. To improve on food security it is essential to double livestock production in the developing world by 2020 (Okeyo *et al.*, 2009). Developing countries have nearly two thirds of the world livestock population but produce only about a quarter to a third of the world‘s meat and fifth the milk. Low output in the developing regions is due to both low off take rates and low yields per animal (Rege, 2009). Kenya is faced with the challenge to rapidly increase agricultural productivity to help feed its growing population without depleting the natural resources base.

The livestock sector contributes 10-12% of the gross domestic product (GDP), which represents 47% of the Agricultural GDP, in Kenya (Mwangi & Omore, 2004; Kabubo-Mariara, 2009; Kios *et al.*, 2011). Dairy farming contributes nearly half of these through milk production and sale of breeding stock. Milk plays an important role in human nutrition especially provision of cheaper much needed proteins, minerals and other nutrients. It is also a source of income to many households and the people employed within the dairy farms (Kitilit *et al.*, 2007; Kabubo-Mariara, 2009). Milk producers provide raw material to the milk processing industry which support many people directly through employment and indirectly through sale of processed milk. There are also many middlemen and traders involved in informal milk and dairy cattle trade. The sale of heifers provides the extra income to a milk producer and it's crucial in the sustainability of the dairy industry.

Modern biotechnology approach is regarded as a means to meet the objectives through addressing the production constraints of small scale or resource-poor farmers who contribute more than 70% of the food produced in the country (Rege, 2009). New technologies can help achieve productivity increases but needs to be transferred to the producers to improve on the impact (Ehui *et al.,* 2009). One high quality cow could produce up to 32 embryos per year compared to the conventional method where the farmer has to wait for nine months for a calf that could be either male or female (Muchemi, 2011). The reproductive potential of a female newborn calf is enormous. There are an estimated 150,000 potential ova in the cow. Through natural breeding, only a fraction of the reproductive potential is realized and the average cow will have one calf per year. Under normal management programmes a cow produces an average of 8 to 10 calves in her lifetime. The reproductive potential of the female has largely been underutilized. Embryo transfer is a technique that can greatly increase the number of offspring that a genetically superior cow can produce (Glenn, 2004).

The first successful embryo transfer was performed in rabbits in 1890. The first successful transfer of sheep, pig and cattle embryos was reported in the early 1950s. Surgical transfer of the embryos into the uterus of the recipients was the most successful technique used in the early days. Birth of calves and pigs following nonsurgical transcervicals embryos was reported in the 1960s. However, it was not until the mid 1970s that transcervical embryo transfer replaced surgical embryo transfer as a routine procedure in cattle. The advent of practically feasible technique for the superovulation of the higher producing cows and embryo transfer to animals with the low genetic potential has opened up for further possibility for the herd improvement (Brockington *et al.,* 2000). The technique permits a move away from the present situation in a herd producing calves for replacement to one with the propagation of only a few of the highly producing cows. The cow to cow pathways of inheritance would thus contribute more to the overall genetic improvement of the herd (Cunningham, 2001). MOET has the capacity to reduce generation interval. It is also useful in progeny testing program due to reduction in generation interval (Harkurkar *et al.,* 2004).

Factors such as species, treatment protocols, breed, age, health, nutrition, season, ovarian status, gonadotrophin preparation and repeated superovulation have been shown to affect superovulation and the quantity and quality of embryos produced (Lamb, 2012; Mapletoft, 2012). Other factors include lactation status of donors and recipients and the time of embryo recovery after insemination. The site of embryo placement in the recipient uterus, embryo size, quality and stage of development influence implantation and overall embryo transfer success rate (Mapletoft, 2012). The success of MOET programmes has been shown to be influenced by the superovulatory responses and fertilization rates of the donors and the survival rates of transferred embryos (Bari *et al.,* 2003).

Over 80% of all milk is produced by small scale farmers in rural areas of Kenya. Most dairy cattle breeders do not have adequate high quality replacement heifers due to low reproductive capacity and inadequate number of high quality breeding stock. Most of these small scale farmers have no breeding objectives and may use artificial insemination or natural service to sustain milk production. The aim is for the cow to produce milk after calving and not the quality of the heifer produced. To sustain their demand, some of these farmers turn to large scale breeders for quality breeding stock. Attempts to bridge this gap with newer biotechnologies including Multiple Ovulation and Embryo Transfer (MOET), Invitro Embryo Production (IVEP) and gender selected semen has not been successful due to variable output and lack of suitable protocols. This has led to high cost of production of heifers thus discouraging the adoption of such high value technologies. These biotechnologies have a great impact on the dairy industry in Kenya especially the accessibility to high quality breeding stock with high productivity. The objective of the study was to determine the success rate of multiple ovulation and embryo transfer in Kenya and lessons learnt.

Materials and Methods

The study was done based on secondary data on multiple ovulation and embryo transfer from Agricultural Development Corporation (ADC) farm in Trans-Nzoia county in the north rift part of Kenya. Data of the number of donor cows super-ovulated, Follicular stimulating hormone (FSH) protocol used, response to superovulation, lactation status of the donors, the number of embryos harvested, the quality and stage of the embryos, number of embryos transferred, and position of placement of embryos, conceptions rates, and lactation status of recipients. This retrospective study examined data collected over a period of six years from 2007 to 2012 from the Agricultural Development Corporation (ADC) ADC being the custodian of the national cattle stud herds has been the leaders in adoption of new technologies and MOET in particular. Over 70% of the country's MOET programs have been done at ADC.

Data were stored in two different Databases (DB), namely: i) DB1 for donors contained data regarding super-ovulation and flushing (n=44), data regarding embryos (n=108), data regarding breed type (n=4) and the lactation stage in days; ii) DB2 data containing the transfers (n=115), the stages of the embryos transferred (n=4), the deposit site (n=3) and the stage of the recipient in days after estrus (n=2).

Super-Ovulation Procedure

On day 1 donor cows were given 2ml of prostaglandin (estroplan or prosolvin), day 2 and 3 heat observation and fertagyl 2.5ml administered on day 3. On day 8, estrus was induced using controlled intravaginal drug release (CIDR) device plus 2mls ciderol and 20ml kyropos. On day 12 superovulation was performed by administering LH/FSH folltropinat the rate of 2 injections at 12 hours interval with decreasing doses for three days. On day 15 an injection of prostaglandin (prosolvin) was administered alongside follitropin. On day 16, the CIDR devices were removed and the last treatment of follitropin administered. Twelve hours after the last treatment, double insemination using frozen semen was done 12 hours apart on observed standing heat.

Embryo Recovery

Embryo recovery was performed 7 days after estrus using standard nonsurgical method (Elsden *et al.*, 1976). To collect the embryos non-surgically, a small synthetic rubber catheter (silicone catheter) was inserted through the cervix and a flushing medium was flushed into and out of the uterus to harvest the embryos. Each uterine horn was filled and emptied 5 to 10 times using an embryo filter with a pore size of 60-70 microns.

Embryo Evaluation

The embryos collected by uterine flushing were classified according to the rules of the international Embryos Transfer Society (IETS) (Robertson & Nelson, 1998). The embryos were classified into: A-Morula, B-Early blastocyst, C-Blastocyst, D-Mature blastocyst and E-Hatched blastocysts. Only B, C and D were considered as transferable.

Recipient Synchronization

To maximize embryo survival in recipients, the condition of their reproductive tract should closely resemble those in the donor. This requires synchronization of estrus cycle between the donor and the recipients, optimally within one day of each other and done as follows: On day 1, 20mls of multivitamin injection was administered and on day 7 estrus was induced using CIDR device and 2ml ciderol. On day 12 folligon/chronogestinjections was administered at a dose of 2mls or 2.5 ml respectively and on day 15 CIDR device was removed and 2mls prosolvin administered. On day 16, 1ml ciderol injection was administered; heat observation was done for the next 2 days and on day 24 embryo transplant was performed.

Table 1. Super-ovulation and synchronization protocol

Day	Time	Donor program	Recipient program
1	AM	Inject 2ml PG+Mult V	Inject 20ml Mult V
7	AM		Insert CIDR+2ml ciderol
8	AM	InsertCIDR+2mlciderol+20mi mult V	
12	PM	Inject 4ml LH/FSH follitropin	Inject estrumate
13	AM	Inject 3ml follitropin	
	PM	Inject 3ml follitropin	
14	AM	Inject 2.5ml follitropin	Inject estrumate
	PM	Inject 2.5ml follitropin	
15	AM	Inject 2ml follitropin+2ml estrumate	Remove CIDR
16	AM	Remove CIDR 1.5ml follitropin	Observe heat
17	AM	Observe heat	
	PM	Inseminate	
18	AM	Inseminate	
	PM	Inseminate	
21	AM	Flush	Transfer

Embryo Transfer

Prior to ET, recipient cow was palpated for the presence of Corpus Luteum (CL) and determine which ovary reacted. An epidural injection of 5-7mls of Lidocaine2 was administered for smooth handling of the genitalia. An embryo was then loaded into ¼ml insemination straw and put on the transfer gun which was carefully passed through the cervix to the uterine horn. The embryo was then deposited in the uterine horn epsilateral to the side of the CL. All transfers were performed non-surgically.

Data Type

In DB1, information was collected on the following parameters; breed of the donor (Holstein Friesian, Guernsey, Jersey, Aryshire), body Condition Score, lactation stage in days and number of embryos flushed.In DB2, the effects of the following parameters on conception rate were investigated: Embryo stage, stage of the recipient after estrus and deposit site.

Data Analysis

The data were analyzed using correlations and regression analyses. Simple descriptive statistics was used. In each DB the variables have been tested looking for the significant relations. The regression model was used to study the effect of factors on the conception rate. The model was specified as:

$$Y_i = \beta_0 + \beta_1 X_i + \varepsilon$$

Where: Y_i was the number of embryos; X_i were the independent variable (X_1=BT1, X_2= BC, X_3=LAS) and ε=error term BT1-Breed Type; BC-Body Condition; LAS-Lactation Stage

Results

Though there were no particular trends, the super ovulations resulted in variable embryo production ranging from 0 to 11 transferable embryos as seen on Table 2.

Table 2. Number of transferable embryos per donor cow

Number of Embryo	Frequency	%PG
0	14	32.6
1	8	18.6
2	7	16.3
3	2	4.7
4	2	4.7
5	1	2.3
6	2	4.7
7	4	9.3
8	2	4.7
11	1	2.3
Total	**43**	**100.0**

Factors Influencing Super-Ovulation of Donors

The regression results indicated that the significant factors that influence the ovulation were the BT ($p \leq 0.05$) and LAS ($p \leq 0.01$) (Table 7). The Body Condition Score ranges from 1 to 5 where 1 is very emaciated and 5 is very obese. The Body Condition Score (BCS) has a significant effect on the number of embryos obtained. A higher number of embryos were obtained from animals at BCS 4 compared to those animals in BCS3. Lactation stage of the donor which ranges between 60 and 210 has significant effect on the number of viable embryos harvested (Flushed). The number of viable embryos decreased with the decrease in lactation stage (days). Animals that were ninety days and above post partum super ovulated better than those that were in the early stage of lactation especially sixty-five and below.

Table 3. Regression model on factors influencing number of embryos flushed (dependent variable = number of embryos)

	Unstandardized Coefficients		Standardized Coefficients	T	Sig.
	B	**Std. Error**	**Beta**		
Constant	-1.651	2.776		-0.595	0.555
BT1	-0.755	0.339	-0.271	-2.227	0.032
BC	0.541	0.707	0.093	0.765	0.449
LS	0.042	0.008	0.617	5.076	0.000
R-squ	43%				
F-value	9.754***				
N	43				

Key: ***; **; * significant at 1%, 5% and 10% respectively

In 2007 there was 25% Pregnancy Rate (PR) which increased to 34.38 and 36.84 in 2009 and 2011 respectively. However, as the pregnancy rate increased steady, the overall pregnancy rate is still low at 32.17% but within the range of 0-67 worldwide as indicated by Yang *et al.* (2010). This low success rates may be attributed to lack of adequate facilities available for the program.

Table 4. Percentage pregnancy over years

Year	N	% PG
2007	32	25.00
2009	64	34.38
2011	19	36.84
Over all	115	32.17

During transplant, the embryo was deposited in the uterine horn on the same side of the ovary with an active CL. The uterine horn was categorized into three deposit sites namely: the lower third located just after the uterus (L), the mid third (MI) and the upper third that connects to the fallopian tube (U). There were three deposit sites for the embryo transplant (L, MI and U). Results showed higher pregnancy rate was achieved when embryos were transplanted at the MI compared to ‘L’ and ‘U’ as indicated on Table 4.

Table 5. Percentage pregnancy rate in relation to deposit site

DS	N	%PG
L	26	26.92
MI	43	48.84
U	44	20.93

Lactation stage of the donor has significant effect on the number of viable embryos harvested (Flushed). The number of viable embryos decreased with the decrease in lactation stage (days). Animals that were ninety days and above post partum ovulated better than those that were in the early stage of lactation, especially sixty-five and below as shown in Figure 1.

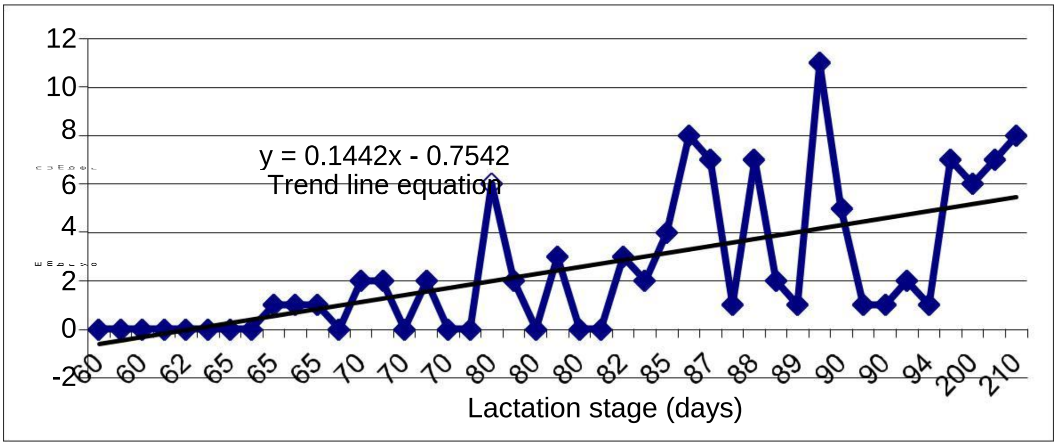

Figure 3. Number of embryos per donor cow over lactation period

Table 6. Percentage pregnancy by stage of recipient after estrus cycle

Stage of recipient after estrus	% recipients that conceived	
	No pregnancy (n=78)	**With pregnancy (n=37)**
7 days after estrus (n=39)	76.9%	23.1%
8 days after estrus (n=76)	63.2%	36.8%
All n(n=115)	67.8%	32.2%

Factors Influencing Pregnancy Rate (PR) in Recipients

The stage of the embryo has a significant effect on the PR. Factors regarding the transfer: The Deposit Site (DS) has a significant effect on the PR ($x^2=5.756$; $p<0.05$). The PR in the mid transfers is higher than in the lower transfers.

Table 7. Correlation analysis on association between variables that have effect on pregnancy rate

	Embryo **stage (es1)**	**Stage of recipient (Star)**	**Pregnancy (Pg)**
Embryo stage (es1)	1		
Stage of recipient (Star)	-0.1129	1	
Pregnancy (Pg)	-0.0444	0.1395	1
Deposit site (ds1)	0.0636	0.0106	-0.0997

Factors Influencing Pregnancy Rate of Recipients

A logic regression analysis was done on factors influencing pregnancy rate. Pregnancy was the response variable and the other three remaining (embryo stage, deposit site and stage of recipient after estrus) were explanatory variables. The deposit site significantly influenced the pregnancy rate with the mid uterine horn (ds-mi) yielding higher success rate at ($p \le 0.05$) than the upper (ds-u) and lower uterine horn (ds-l). The embryo stages and the stage of the recipient after estrus had no significant influence on PR compared to the deposit site as shown on Table 8 and 9.

Table 8. Logic regression analysis on factors influencing PG (dependent variable 1 = positive; 0 = negative)

Variable	Coef.	Std. Err.	z	P>z
Embryo blastocycyst (es_b)	0.506431	0.797393	0.64	0.525
Embryo stage early blastocycystes_eb	0.107933	0.664189	0.16	0.871
EmbryostageMorula (es_m)	0.985359	0.641127	1.54	0.124
Deposit site lower (ds_l)	0.239134	0.598414	0.4	0.689
Deposit site mid (ds_mi)	1.26206	0.502049	2.51	0.012
Stage of recipient after estrus (sr)	0.742708	0.492882	1.51	0.132
_cons	-7.53411	3.895576	-1.93	0.053
Number of obs	115			
LR chi2(6)	14.05**			
Prob > chi2	0.0291			
Log likelihood	-65.214998			
Pseudo R2	0.0972			

Table 9. Marginal effects of logic regression analysis on factors influencing PG

Variable	dy/dx	Std. Err.	z	P>z	X
Embryo blastocycyst (es_b)	0.1136928	0.18838	0.6	0.546	0.113043
Embryo stage early blastocycystes_eb	0.0228393	0.14152	0.16	0.872	0.330435
Embryostage Morula (es_m)	0.2140608	0.14061	1.52	0.128	0.373913
Deposit site lower (ds_l)	0.051493	0.13166	0.39	0.696	0.226087
Deposit site mid (ds_mi)	0.2739546**	0.10758	2.55	0.011	0.382609
Stage of recipient after estrus (sr)	0.156048	0.10291	1.52	0.129	7.66087

Discussion

A significantly higher number of viable embryos were obtained from Aryshire cows when compared to other breeds (Jersey, Guernsey and Friesian). Other Authors obtained different results from different breeds (Breuel *et al.*, 1991; Bo, 2006). It is still unknown whether this could depend from physiological status influenced from management and production levels.

PR is deeply influenced by the quality of the embryos utilized, (obviously the better is the embryo the higher is the PR). Generally the PR reduced as the growth of the embryo stage advanced. There was more PR reported at the morulae stage compared to the proceeding stages. These results somewhat coincide with those of Chebel *et al.* (2002) that early blastocyst obtained better PR when transplanted fresh than when frozen. This is also in agreement with results reported by Sirard *et al.* (2002). However, the number of unsuccessful pregnancy exceeds the number of successful pregnancy results in each Stage of growth. This was attributed to poor facilities available for the program. PR is also deeply influenced by the depth of the transfer. The PR in the mid third is higher than in the upper third and the lower third of the uterine horn. This is in agreement with the observation by Steel *et al.* (2004) that PR in deep transfers is higher than in the superficial transfers.

Conclusion and Recommendations

The success rate of ET was about 33% out of 115 recipients. Some of the key influencing factors on the success rate were Embryo stage and stage of the recipient after estrus. The objective of the study was to determine the success rate of multiple ovulation and embryo transfer in Kenya and lessons learnt. Some of the key influencing factors on the super-ovulation were Breed Type (BT) and Lactation Stage (LS).

Research may be done on why some breeds are more superior in ovulation than others. On-farm transfer of fresh embryos has practical difficulties and requires trained personnel and equipments for evaluation and packaging of embryos at the farm site. The application of MOET can improve reproductive efficiency in dairy cattle and harvested embryos can be frozen and conserved in a bank for future use. It is recommended that the donors used for super-ovulation be 90 days postpartum

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