Adoption of Embryo Transfer in Kenya and its improvement through use of Optimal FSH dosage during superovulation

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A thesis submitted in fulfillment of the requirements for Degree of Doctor of Philosophy in Theriogenology

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2019

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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DEDICATION

I dedicate this work to my wife, Rebecca who endured my absence from time to time and the long hours of study. Without her support, I could not have made such strides. I thank my children, Joy and Jean for their understanding and encouragement without forgetting my parents Job and Rosa. My parents have continued to play a key role in shaping my destiny from the beginning. To all my other family members and friends, it's a big thank you for your support and encouragement.

Ecclesiastes 3:1: To everything there is a season, and a time for every purpose under the heaven.

Thank you all.

ACKNOWLEDGEMENT

I thank God for the journey mercies through the research period. I express my gratitude to my supervisors: Prof. Victor Tsuma and Prof. Henry Mutembei of the University of Nairobi, College of Agriculture and Veterinary Sciences, Faculty of Veterinary Medicine, Department of Clinical Studies, for the guidance, encouragement and mentorship. Without them, the long walk to success and the ultimate prize of my PhD education would not have been possible. My appreciation also goes to the Dean, Faculty of Veterinary Medicine, and Chairman and staff of Department of Clinical Studies for their support and assistance. To Prof. Mutiga and Madam Rachael Gitau, thank you for your support. I also appreciate the Faculty of Veterinary Medicine and the University of Nairobi for granting me the opportunity to pursue my PhD education.

I acknowledge and thank the German Academic Exchange Service (DAAD) for sponsoring my PhD studies by taking care of tuition and my upkeep and part payment for the research. Thank you Mr. Bonface Mutua of DAAD office for your support. I acknowledge East Africa Agricultural Productivity Project (EAAPP) for providing part of the materials for research and in particular I wish to appreciate Dr. Douglas Indetie and Dr. Muchemi Kariuki for this support.

I am grateful to the University of Eldoret for their support in providing time and experimental animals used in the research. Same goes to East Africa Semen and Embryo Transfer Association (EASETA) and Agricultural Development Corporation (ADC) for availing the secondary data on embryo transfer and the farmers who responded to my questionnaire.

Anybody not here mentioned who in anyway contributed to my study is also appreciated. The almighty God bless you all.

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LIST OF ABBREVIATIONS

ADC: Agricultural Development Corporation

AI: Artificial Insemination

ARTS: Assisted Reproductive Techniques

ASAL: Arid and Semi-Arid Land

BCS: Body Condition Score

CIDR: Controlled Intravaginal Drug Release

CL: Corpora Lutea

EAAPP: East African Agricultural Productivity Project

EASETA: East Africa Semen and Embryo Transfer Association

EDFA: Eldoret Dairy Farmers Association

ET: Embryo transfer

FSH: Follicle Stimulating Hormone

GDP: Gross Domestic Product

GMO: Genetically Modified Organism

IETS: International Embryo Technology Society

IVEP: *In vitro* Embryo Production

KAGRC: Kenya Animal Genetic Resources Centre

KALRO: Kenya Agricultural and Livestock Research Organization

KNBS: Kenya National Bureau of Statistics

KNDMP: Kenya National Dairy Master Plan

Ksh: Kenya Shilling

LH: Luteinizing Hormone

mg: Milligrams

MHz: Megahertz

mL: Milliliters

MOET: Multiple Ovulations and Embryo transfer

UOE: University of Eldoret

UON: University of Nairobi

USD: US Dollar

ABSTRACT

Agriculture is a key sector in national development contributing 32% of the gross domestic product (GDP) in Kenya. The livestock sub sector accounts for 37% of the agricultural GDP equivalent to 12% of the national GDP with dairy cattle contributing 4% of the national GDP. Dairy farmers in Kenya are required to embrace assisted reproductive techniques like embryo transfer (ET) to meet an ever-rising demand for milk that is projected to rise to 12.76 billion litres annually by the year 2030. Although ET has been in this county for decades, its practice has not been optimal to meet the demand of the need to improve dairy cattle through production of high quality heifer stock. The major challenge the ET practitioners have been citing for its inefficiency have been low embryo output during flushing and the cost of superovulation due to dosage of Follicle Stimulating Hormone (FSH). This study sort to utilize ultrasonography to create an understanding of the follicular dynamics during superovulation in order to enhance adoption of ET through reduced dosage rate of FSH. The specific objectives of the study were to evaluate the follicular dynamics during super ovulation using different follicle stimulating hormone (FSH) dose rates, to determine the embryo yield (quantity and quality) for the different FSH superovulation dose rates, to assess any other general factors likely to affect adoption of embryo transfer technology in Kenya, and to analyze the success rate of currently used MOET protocol used on dairy cattle in Kenya in relation with regards to embryo yield. The research was carried out in dairy cattle kept at the University of Eldoret farm in Uasin Gishu County. Cows were restrained in a crush and ultrasonography was done using a portable ultrasound device equipped with a 5.0/7.5 MHz linear bi-frequential probe (frequency of 6.5 MHz was used as standard). These were done daily before superovulation in three consecutive estrous cycle repeats. The probe was secured in an examination sleeve to protect the diaphragm and also to hold the acoustic gel in place. The probe was then introduced into the rectum after fecal evacuation was done and moved back and forth over the pelvic area to scan the ovaries

from above. Follicles of different sizes and the CL were identified and their location on the ovaries was noted. The diameters of the three largest follicles and *corpus luteum* (CL) were then measured and their location sketched on a research book to ease their identification. These follicle traits were used to track and evaluate for follicular dynamics. Follicular populations were obtained by manual count of all visible follicles. Numbers and sizes of ovarian follicles were determined and follicles were considered small (2 to 5 mm) or medium (6 to 8 mm). The dominant follicle (DF) of a wave was defined as the follicle that measured at least 9 mm in diameter and exceeded the diameter of all other follicles in the wave. The estrous cycles were described as being in recruitment, selection or dominant phase based on numbers and sizes of follicles noticed in the ovary. Cows observed to be in either recruitment or selection phases of the estrous cycle were super ovulated and later inseminated twice at 12 hours apart and embryos flushed after seven days. Numbers and grades of the harvested embryos were assessed. A questionnaire was also administered to 293 farmers in Uasin Gishu and Trans Nzoia Counties to evaluate factors affecting adoption of MOET technology. Secondary data from ADC Namandala and Sasini farms retrieved and analyzed to success rate of the currently used MOET protocol. Cows were seen to be in the three phases of estrous cycle during superovulation hence the usefulness of ultrasonography during superovulation. One third of the donors failed to ovulate, another third produced 1 – 3 embryos while a third produced over three embryos. The number of embryos recovered after flushing was similar to those of the MOET protocol conducted in Sasini farm but more than those for ADC Namandala farm. The season, technique, super ovulation protocol used and animal factors were shown to influence embryo production and recovery. The low adoption of embryo transfer was associated with inadequate sensitization about the technology, unavailability of the technology in Kenya, high cost of embryo production, and few professionals trained to handle the process. A FSH dosage rate of 200mg produced similar results to that of 400mg. To reduce the cost of embryo production

associated with the use of 400 mg of FSH per donor, it is recommended that super ovulations may be carried out in Kenya using 200 mg of FSH per donor. Ultrasound technique should also be used to monitor dynamics of follicular activity in order not to waste FSH on animals in the dominant phase who will not respond. Once this is done adoption of ET in Kenya is likely to be enhanced.

CHAPTER 1

1.0. INTRODUCTION

1.1. Agriculture Sector in Kenya

Agriculture sector contributes 32% of the gross domestic product (GDP) (KNBS, 2018), with the livestock sub-sector accounting for 12% of GDP, which represents 37% of the agricultural GDP in Kenya (Kabubo-Mariara, 2009; Kios *et al*., 2012). Agriculture sector is considered the backbone of our economy and one of the critical pillars in the Big Four agenda being implemented in the country. The government of Kenya's Big Four agenda has emphasized on; 1) 100% food and nutrition security, 2) Universal health care, 3) affordable housing and 4) manufacturing. The government has therefore laid more emphasize on these four key issues on their development agenda for the period 2017 to 2022.

The agriculture sector therefore plays a key role in ensuring 100% food and nutrition security, provision of raw materials for manufacturing and improvement of human health due to reduction in malnutrition. With the right intervention; agriculture sector will contribute immensely to poverty reduction, wealth creation and wellbeing of the people involved directly and indirectly. Though crop production plays a bigger role compared to livestock, the livestock sub sector at 37% contribution to overall agricultural gross domestic product is significant. In the arid and semi-arid lands where crop production is impossible, livestock production is the major activity of the majority of the population. Livestock are therefore the main source of wealth in arid and semi-arid lands and therefore needs greater attention.

1.1.1. Livestock Subsector

The Livestock farmers in the rural areas derive a larger share of their income from their livestock. Livestock keeping is attractive to many households due to the ease in establishment

and management especially dairy farming and chicken production. The national average population living in extreme poverty in Kenya is estimated at 8.6% with the rural areas being higher at 11.2% (KNBS, 2018). These are people in dire need of food and may not afford a single meal a day. Most of these people reside in counties where livestock is the backbone of their economy (KNBS, 2018). Improvement of the production and productivity of their livestock therefore, will lead to transformation of their living and nutritive standards. To meet the current and future demands for livestock products and enhanced food security, it is critical to improve livestock production in developing countries (Mutembei *et al*., 2015).

1.1.2. Dairy Subsector

Dairy subsector is estimated to contribute over 4% of the gross domestic product in Kenya (KNDMP, 2010), through milk production and processing, sale of breeding stock, meat and hides from culled cows slaughtered, manure and social use including dowry and batter trade. Demand for milk in Kenya is projected to rise to 12.76 billion litres per year by the year 2030 from the current 5.3 billion litres (KNDMP, 2010; KNBS, 2018). The current deficit for milk stands at 475 million litres annually (KNBS, 2018). The deficit has led to occasional importation of raw milk from Uganda and also purchase of powder milk from other countries.

The high demand for milk from the year 2030 will therefore be met through increased quantity and quality of milk production per cow and improved farm productivity. If improvement of the quality of the dairy cow is not achieved, then the country may depend heavily on milk importation to be able to meet the demand of the rising human population. This will lead to food and nutrition insecurity and pressure on the Kenyan shilling.

Milk plays an important role in human nutrition especially provision of much needed cheaper proteins, minerals and other nutrients. This is key contribution to the Big Four agenda of the government of Kenya specifically; 100% food and nutrition security, manufacturing and health. It is also a source of income to many households and the people employed within the dairy farms. Milk producers provide raw material to the milk processing industry which supports many people directly or indirectly through employment, returns from sale of milk and milk products.

There are also many players involved in informal milk and dairy cattle trade. The sale of heifers provides extra income to a milk producer and it is crucial in the sustainability of the dairy industry. Over 80% of all milk in Kenya is produced by small scale farmers in rural areas (KNDMP, 2010). Small scale farmers have smaller lands hence keep a few dairy cows that urgently need genetic improvement if any meaningful improvement of milk production is to be achieved.

1.2. Assisted Reproductive Techniques

Most of the small scale farmers lack guidance on breeding objectives and use their herds purely for milk production without thinking of replacement. Most of the small scale dairy farmers turn to the only breeding option available whenever their cows come on heat namely; natural service or haphazard use of artificial insemination (Lawrence *et al*., 2015). Such they believe will lead to achievement of pregnancy hence assured of future milk production with minimal input on quality of the calf for future replacement.

The use of assisted reproductive technologies has supported many countries to achieve sustainable production of milk and replacement heifers. These biotechnologies can have a great

impact on the dairy industry in Kenya especially on the accessibility to quality breeding stock for enhanced productivity. Reproductive efficiency of the top producing cows through Multiple ovulation and embryo transfer may provide the solution to this demand which has driven the prices of replacement breeding stock way above what the ordinary small scale dairy cattle farmers in Kenya can afford.

1.2.1. Embryo Transfer

It has been demonstrated that biotechnology if transferred to the small scale dairy cattle farmers has the potential to improve the production of their livestock (Mutembei *et al*., 2015). Multiple ovulation and embryo transfer can greatly increase the number of offspring that a genetically superior cow can produce. The reproductive potential of a cow could be enormously enhanced considering the numerous viable ova they contain in their ovaries (Mutembei *et al*., 2015). Through natural mating or artificial insemination, only a fraction of the reproductive potential of the cow is realized and the average cow will at best have one calf per year. Thus, a cow only produces 8 to 10 calves during her lifetime (Kios *et al*., 2013).

MOET is a process that involves the super ovulation of donor cows using hormones to increase the number of ova ovulated, followed by insemination and flushing of the uterus to recover embryos. Super ovulation is the most expensive process in MOET. The cost of super ovulation of one donor is currently approximated to be equivalent to Ksh. 25,000 (USD 250) (Mapletoft, 2012). With the cost of freezing one embryo being estimated at Ksh. 5,000 (USD 50) (Mapletoft, 2012).

A minimum of three donor cows are always prepared in any MOET protocol in Kenya at a minimum direct cost of Ksh. 75,000 excluding the cost of recipient preparation, donor and

recipient maintenance, transport and professional fee. This is to ensure that a reasonable number of transferable embryos are collected to avoid a situation where recipients are ready but there is lack of embryos.

It has been shown that some 1/3 of donor cows fail to super ovulate, another 1/3 produce few embryos and only 1/3 produce a reasonable number of embryos (Galli *et al*., 2003; Viana and Carmago, 2007; Mutembei *et al*., 2015). It has also been shown that factors such as technician skills, species, breed, age, health, nutrition, season (Lerner *et al*., 1986; Mollo *et al*., 2007; Mapletoft, 2012), ovarian status, gonadotrophin preparation, treatment protocols and repeated super ovulation affect the quantity and quality of embryos produced in a MOET process (Arendonk and Bijma, 2003; Lamb, 2012).

1.3. Statement of the Problem

In Kenya, quality replacement heifers are inadequate and those available are usually expensive (Mutembei *et al*., 2015), hence unaffordable to the small scale dairy farmers. This inadequacy is partly due to low adoption of reproductive technologies, improper implementation of breeding plans and absence of quality breeding stock (Muraya *et al*., 2015; Mutembei *et al*., 2015). The demand for replacement heifers far outstrips the supply hence creating a never-ending struggle by the farmers.

To meet the demand for replacement heifers, small scale farmers turn to large scale dairy cattle breeders for quality breeding stock. This gap has led to a high demand for dairy breeding stock leading to extremely high prices in the range of Kenya shillings (Ksh.) 200,000 to 300,000 (USD 2,000 to 3,000) a situation that is not only unsustainable but also out of reach to most of

the small scale farmers. Despite the high price, quality replacement heifers are hardly available in the market.

In the year 2014, 2015 and 2016, Eldoret Dairy Farmers Association (EDFA) in Uasin Gishu county imported over 300 dairy cattle heifers from the Republic of South Africa. The move was to alleviate the acute shortage of quality dairy cattle being experienced in Kenya. The heifers from South Africa arrived at a cost of over Kenya shillings two hundred thousand (Ksh. 200,000) which was expensive to majority of the Kenyan farmers most of whom are smallscale in their operations (personal communication with Mr. Nicholas Kositany, Chairman, Eldoret Dairy Farmers Association in the year 2016).

The logistics of importation especially transportation overland is difficult and almost impossible for many farmers. Such a venture is non tenable due to risks of spreading diseases, effect of genotype x environment interactions, abortions and deaths from long distance travels. Approximately 30% of the in calf heifers aborted on the way from South Africa. The heifers took long to adapt to the Kenyan environment and their production was similar to the local dairy cattle. The large scale dairy cattle farmers could try this option but the small scale farmers have none or minimal opportunity due to high costs involved.

A conventional method of heifer production through artificial insemination and natural service has not been able to wholly satisfy farmer demands for quality dairy cattle. The use of a combination of the existing assisted reproductive techniques is therefore important if supply has to meet the demand. Attempts could be made to bridge this gap by incorporating the old technologies like artificial insemination (AI) with these newer biotechnologies like multiple ovulation and embryo transfer (MOET), *invitro* embryo production (IVEP) and gender

selection of semen (sexing of semen) (Muasa *et al*., 2015; Muraya *et al*., 2015; Mutembei *et al*., 2015).

1.4. Justification

In spite of several attempts to introduce embryo transfer (ET) in Kenya, uptake of the technology has been insignificant. Although embryo transfer has been practiced on a small scale for over 30 years in Kenya, adoption of this technology by most dairy cattle breeders has been slow. There are perceptions among Kenyan dairy cattle farmers that the embryo technology is expensive and the embryo output from the donor cows has been low to meet the costs of embryo transfer. Again, the Kenyan practitioners have produced variable results of embryos recovered. The low adoption may be partly attributed to: Cost of follicle stimulating hormone (FSH), Technique used for super ovulation, Low conception rates, Lack of information on MOET availability and Low embryo recovery rates

To maximize on material utilization and lowered cost of production, there is need to consistently produce an average of six embryos per donor hence lower costs of production compared to variable embryo output (Mapletoft, 2012). Variable embryo output and lack of suitable synchronization and super ovulation protocols in Kenya has led to MOET being viewed as expensive with negative impact on the adoption of this technology by farmers. The MOET protocol currently used in Kenya was either adopted from the United States of America (USA) or the Republic of South Africa (RSA). These protocols were developed in the countries of origin after research on super ovulation hormones on their own cows under their production systems (Lerner *et al*., 1986).

The protocol described by Seidel and Seidel (2005) recommends 20% dose increase for donors above 800 kg. The average body weight recommended for the 400 mg FSH dose is not more than 800 kg. MOET protocol adopted from the USA recommends a blanket 30% reduction for their heifers and is silent on low weight mature cattle as kept in most farms in Kenya. Most of Kenyan Friesian dairy cattle have approximately 30 to 50% lower body weight compared to those of USA. The average weight of Kenyan dairy cattle was between 350 to 650 kg with an average of 500 kg in this study while those of the USA stood at 700 kg (www.holsteinusa.com). Furthermore, the studies on Brazilian Zebu cows has shown that donor cows responded well to lower doses of FSH (Lamb 2012).

Their donor cows have different physiological needs and raised in different environments. Thus, the follicle stimulating hormone (FSH) protocols for their situations may not be the most appropriate for Kenyan donor cows. Researchers in Brazil have shown that the *Bos indicus* donor heifers on low levels of follicle stimulating hormone (FSH) of 200 mg and 160 mg of Folltropin®-V, manufactured by Bioniche Animal Health, Canada produced an average of 9.37 and 9.60 transferable embryos respectively (Lamb, 2012). The studies on adult Brazilian Zebu donor cows also showed that adult Zebu cows responded well to lower doses of FSH compared to *Bos taurus* (Lamb, 2012).

Regular use of MOET will lead to production of heifers with good genetic merit that will be made available to most small scale farmers by private large scale animal breeders, government farms and Universities. The heifers will be more affordable as compared to the current situation due to improved availability in the market. When the supply improves, the pressure of demand drops and prices become more reasonable. Small scale farmers will be the beneficiaries of the drop in price due to improved supply of quality heifers.

1.5. Overall objective

To understand ovarian follicular dynamics in order to determine optimal dose rates of follicle stimulating hormone (FSH) for enhanced adoption of embryo transfer technology in Kenya.

1.5.1. Specific objectives

1. To evaluate the ovarian follicular dynamics during super ovulation using different follicle stimulating hormone (FSH) dose rates
2. To determine the embryo yield (quantity and quality) for the different FSH superovulation dose rates
3. To assess other general factors likely to affect adoption of embryo transfer technology in Kenya
4. To analyze the success rate of currently used MOET protocol used on dairy cattle in Kenya in relation with regards to embryo yield

1.6. Research Assumptions

i. Understanding ovarian follicular dynamics during super ovulation would enhance embryo yield
ii. Different follicle stimulating hormone (FSH) dose rates during superovulation would likely produce different embryo yield (quantity and quality)
iii. Other general factors are likely to affect adoption of embryo transfer technology in Kenya
iv. The success rate of currently used MOET protocol used on dairy cattle in Kenya may not be optimal

CHAPTER 2

2.0. LITERATURE REVIEW

2.1.Multiple Ovulation and Embryo Transfer (MOET)

MOET is a process that involves the selection of donor animals based on a predetermined criterion, followed by super ovulation of donors using hormones to increase the number of ova ovulated. After super ovulation, the donors are insemination with high quality semen followed by flushing of the uterus before implantation / hatching of the fertilized ova to recover embryos (Mapletoft, 2012). Flushing is normally carried out on day seven (7) for cattle.

2.1.1. Historical Background

Multiple ovulation and embryo transfer has been practiced for many years in a number of countries throughout the world. The commercialization of MOET has been shown to have begun in early 1970s (Mapletoft, 2012). The history of MOET has been extensively documented and shows that embryo transfer was first performed in rabbits in 1890 by Walter Heape who successfully transferred Angora rabbit embryos into an inseminated Belgian doe. The doe produced a mixture of Belgian and Angora kittens. This was proof enough that the Angora embryos transferred into the uterus of the Belgian doe had successful implantation. This was followed by embryo transfers in sheep (1930s), pig (1940s), and cattle (1950s) (Mapletoft, 2006). The technique is now more frequently used as a breeding tool for rapid improvement of animal genetic material and for conservation of threatened species and breeds of animals.

The initial technique for recovering and transferring cattle embryos were surgical but later, embryos were recovered and transferred non-surgically (Mapletoft, 2012). The surgical recovery of embryos was involving, expensive and limiting due to the need for a specialist

Veterinarian to perform the surgery. The introduction of non-surgical technique therefore improved the utilization of the MOET worldwide.

Cryopreservation of embryos also began in 1980s, followed by the introduction of embryo splitting, in vitro embryo production, direct transfer of frozen embryos and sexing of embryos (Mapletoft, 2012). These techniques enhanced the uptake and utilization of the MOET technique in many countries.

The recognition of the importance of follicular wave dynamics and the synchronization of the follicular wave in livestock has also increased embryo production per unit of time. Super ovulation of donor cows is now more frequent than in the past, and this has led to production of more embryos per year per donor in countries with regular practice (Mapletoft, 2013).

The International Embryo Technology Society (IETS) was founded in 1974. This was necessitated by the need to regulate the use of the new technology and provide guidelines to the members. IETS plays a critical role in the dissemination of information on embryo production and transfer. This has led to the rapid growth of the embryo transfer industry in the world (IETS, 2016).

IETS has several committees that play important roles in the growth of embryo transfer technology. The Health and Safety Advisory Committee of IETS, is instrumental in dissemination of scientific information about bovine embryo transfer and its potential in disease control for international trade on embryos (Thibier, 2011; IETS, 2016).

Embryos are now the preferred mode of importing or exporting genetic material compared with live animals (Thibier, 2011). The fear of disease transmission has been a challenge to live animal imports and export, but the advent of embryo transfer provided the solution for continued genetic exchange among nations. With proper processing of embryos, disease transmission is totally eliminated hence is acceptable for trade in many countries. Livestock diseases remain a great barrier in trade on genetic material.

The importation of live animals' costs more in comparison to importation of frozen embryos. There is also a reduced risk of disease transmission (Thibier, 2011) and reduction of quarantine costs in the use of embryos. Importation of embryos allows for the selection of animals from a wider genetic base and the genes of the donor animals remain within the exporting country. The animals produced from embryos are adapted to the environment of the importing country where they are born. This is due to the influence of surrogate dams carrying the embryos unlike imported live animals. Adaptation is important in the tropical and subtropical environments where the likelihood of influence of genotype x environment interaction is high.

MOET permits utilization of the superior cows hence exploiting their reproductive and productive potential. The cow to cow pathways of inheritance would thus contribute more to the overall genetic improvement of the herd (Kios *et al*., 2013). The number of off springs from superior dams is substantially increased in comparison with natural reproduction. A cow will naturally produce one calf every year and a maximum of 8 – 10 calves in a lifetime. With MOET, more calves are therefore produced from a superior cow (Kios *et al*., 2013).

MOET has also been shown to reduce generation interval hence useful in progeny testing programs (Mapletoft, 2012), and production of replacement heifers (Mutembei *et al*., 2015).

The MOET programme has also been used to genetically test artificial insemination (AI) sires reducing the waiting time from five-and-a-half years when using traditional progeny testing schemes to three-and-a-half years (Kios *et al*., 2013).

Embryo transfer is now commonly used to produce artificial insemination (AI) sires from proven cows and bulls in developed countries and a few developing nations (Mapletoft, 2013). The cattle industry will benefit through the use of bulls produced through such a MOET programme for rapid genetic improvement through artificial insemination. Embryo transfer is a technique that remains underutilized in developing countries despite the potential to transform the livestock industry (Kios *et al*., 2013; Mutembei *et al*., 2015). A well-designed MOET programme will lead to increased selection intensity resulting in improved genetic gains.

The average number of embryos recovered per donor has not changed substantially. The basic procedure of super ovulation of donor cattle has remained largely the same with minimal improvement over the past years (Arendonk and Bijma, 2003). This is despite the commercial embryo transfer being available over the last 40 years (Hasler, 2014). This has also been the situation in Kenya and the average number of transferable embryos recovered has largely remained low despite embryo transfer having been available since 1982 (Kios *et al*., 2013).

Devising methods for the synchronization of follicular wave emergence has simplified achievement of super ovulation, resulting in increased embryo production per unit of time. Donor cows are being super ovulated more frequently now than in the past, and more embryos are being produced per year per donor with no change in the actual super ovulation protocol being used (Mapletoft, 2013; Hasler, 2014).

2.1.2. MOET in Kenya

Embryo transfer in Kenya began in 1982 with the use of imported embryos transferred into the local breeds of cattle (Kios *et al*., 2013). It wasn't until in the year 1998 that the super ovulation of local donor cows and harvesting of embryos began in the country (Kios *et al*., 2013). Since then, more super ovulations, harvesting and transfer and preservation of embryos have been conducted. In the year 2005, more frequent embryo transfer programs began to be conducted in Kenya (Kios *et al*., 2013). Most of the embryo transfer programs have been carried out in either government farms or large-scale farms and some research organizations (Figure 1).

Figure 1. The author flushing one of the donor cows at ADC Namandala farm in Trans Nzoia County in the year 2006 during a MOET progamme. Superovulation was based on 400 mg of FSH administered uniformly to all donors.

MOET programs conducted between 2005 and 2008 at Agricultural Development Corporation (ADC) farms had variable embryo yield but much below the world average of six (6) transferrable embryos per donor (Kios *et al*., 2013). A total of 104 donors were super ovulated between the year 2005 and 2008 at ADC farms resulting in the production of 159 embryos; an average of 1.5 embryos per donor. Of the recovered embryos, 138 were transferred resulting in 56 pregnancies; 41% pregnancy rate achieved (Kios *et al*., 2013) (Figure 2, 3, 4 and 5).

The pregnancy rate achieved was however below the world average of 60 – 70% for fresh embryos. The potential to improve the average embryo output in Kenya is enormous given the right protocols for synchronization and super ovulation.

Figure 2. Some of the MOET calves born at ADC Namandala Farm in 2007 to Boran cattle surrogate dams. Embryos flushed were few despite the high dose of FSH of 400 mg per donor being used for super ovulation.

Figure 3. MOET Friesian calf suckling a Charolais cross surrogate dam at ADC Namandala farm in 2007. High quality calves were born hence an important technique for dairy cattle improvement in Kenya.

Recent MOET programs conducted in 2012 and 2013 at ADC Namandala in Kitale, Kenya Agricultural and Livestock Research Organization (KALRO), Naivasha and Makongi farm in Uasin Gishu County had high variable embryo output. The Sahiwal cattle produced an average of one (1) transferable embryo though there were individual donors who produced many embryos at KALRO, Naivasha while the Ayrshire cattle at Makongi farm in Uasin Gishu County yielded zero (0) embryos (personal communication with Mr Douglas Indetie of EAAPP and KALRO during the year 2014 and Mr Tim Chesire, Director of Makongi farm also in the same year 2014). This prompted Makongi farm to adopt the *invitro* embryo production technique instead of the conventional MOET programme so as to enhance the number of embryos produced.

Figure 4. Ayrshire MOET Calf born at ADC Namandala farm to a Boran surrogate dam in 2007. Strong high quality calves were born through this hence the need to improve its efficiency for meaningful adoption to take place.

On the other hand, the average output at ADC Namandala had improved to three (3) transferable embryos per donor though variations in embryo yield still existed between MOET programs (personal communication with Dr Musee, Veterinary Surgeon at ADC, during the year 2014). The high variability and low embryo yield were a major loss to the dairy cattle breeders who invested thousands of Kenya shillings in the program with the hope of improving their herds.

Figure 5: Friesian calf born on 30.01.07 out of MOET program at ADC Namandala farm in Transzoia County. Reduction of cost of production and improved efficiency will increase the population of quality dairy cattle in Kenya hence contribute to enhanced food security.

Other assisted reproductive techniques like *invitro* embryo production (IVEP) have been carried out hitherto in experimental basis at the University of Nairobi (UON) and International Livestock Research Institute (ILRI) and more recently at Makongi farm in Uasin Gishu County. More resources are needed to ensure frequent use of these breeding techniques together used in embryo production to boost their utilization in Kenya. The current level of utilization is insufficient to enhance efficiency in embryo production.

2.1.3. Factors that Affect Embryo Yield

It has been shown that factors such as technician skills, species, breed, age, health, nutrition, season (Mollo *et al.*, 2007; Mapletoft, 2012), ovarian status, gonadotrophin preparation,

treatment protocols and repeated super ovulation affect the quantity and quality of embryos produced (Arendonk and Bijma, 2003; Lamb, 2012). Other factors include; lactation status of donors and recipients and the time of embryo recovery after insemination, susceptibility to stress and physiological peculiarities (Lamb, 2012; Mapletoft, 2012).

Technician skills improves with the number of embryo production and transfer carried out by the technicians. Those with more frequent embryo production programs have improved output due to extensive experience compared to those with less opportunities to practice (Mapletoft, 2012). Breeds of animals have variable response to hormonal treatment and hence embryo output. Some breeds respond well to lower dose rates of super ovulatory hormones in particular the *Boss indicus* compared to *Bos taurus* as has been shown through research (Lamb, 2012).

Health status of the donor cows will influence the response to super ovulation hormonal treatment. Health donors respond well compared to unhealthy ones. Donors on good plane of nutrition also respond well to hormonal therapy compared to animals with nutritional deficiency (Mapletoft, 2012). Also lactation status of the donor cows will influence embryo output. Lactating donors have poor responds compared to the non-lactating ones (Lamb, 2012).

The site of embryo placement in the recipient uterus, embryo size, quality and stage of development has been shown to influence implantation and overall embryo transfer success rate (Arendonk and Bijma, 2003; Mapletoft, 2012; Kios *et al*., 2013). Embryos placed on the upper and middle thirds of the uterus of recipients have higher success rate of implantation and survival compared to those placed on the lower one third (Kios *et al*., 2013).

It has also been shown that quality of embryo determines success rate of the transfer. Grade one and two embryos have higher survival rates compared with grade three embryos (Bo and Mapletoft, 2013). Stage of development also influences the success rate of embryo transfer. Embryo harvesting is carried out on day seven after insemination of the donor cows. If on day seven, harvested embryo is at stage three; Young morula, success rate when implanted on surrogate dam at day seven after standing heat is low (Bo and Mapletoft, 2013).

Most of the research on factors affecting embryo output has been done in temperate countries with different climatic conditions (seasons) with Kenya. Little is known on the effects of such factors on dairy cattle embryo output in Kenya.

The success of MOET programs has also 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). The current MOET synchronization and super ovulation treatments may be a contributory factor to the low and variable embryo output in Kenya. Though progress has been made in manipulating the bovine follicular development, it has been shown that the high variability in the ovarian follicular response to gonadotropin stimulation remains a major problem (Lamb, 2012) that warrants further research.

The body size of the donors should be considered during super ovulation. Older donor cows which have a higher weight have been shown to respond well to higher doses of follicle stimulating hormone (FSH) while the younger cows which are also lighter in weight have poor response to high doses of FSH (Lerner *et al*., 1986). Most Kenyan dairy cattle weigh much below those from United States of America, Canada and Europe and this may contribute to the high variability in embryo output being experienced in Kenya among other factors. This

research was designed to address this concern using four levels of FSH to determine the appropriate levels for use in our dairy cattle donors.

2.2. Ovarian Follicular Cycle and Synchronization

Increasing efficiency of synchronization has been shown to positively influence MOET programs (Baruselli *et al*., 2006). It is possible to control the specific phases of follicular development with the strategic use of hormonal therapy. The control of the estrous cycle improves the efficiency of assisted reproductive techniques. The estrous cycle has two to four waves of follicular development in cattle (Binelli *et al*., 2006; Viana and Carmago, 2007; Muraya *et al*., 2015) each with three phases of; recruitment, selection, dominance and either ovulation or atresia. A follicular wave is characterized by the synchronous growth of a cohort of follicles, one of which continues growing while the others regress at variable intervals. Dairy cattle have an average of 21-day estrous cycle.

2.2.1. Recruitment

Recruitment of a cohort of follicles is stimulated by a transient rise in follicle stimulating hormone (FSH). FSH is produced by the pituitary gland and its production is triggered by gonadotrophin releasing hormone (GnRH) produced by the hypothalamus. At the onset of each follicular wave, approximately 20 to 30 small (3 to 5 mm) viable antral follicles have been detected in cattle (Barros and Nogueira, 2001).

Follicle waves emerge on days 2 and 11, or days 2, 9 and 16 for animals with two or three follicle wave cycles, respectively (Sirois and Fortune, 1988; Muraya *et al*., 2015). During estrous cycle in cattle, dominant follicles reach a maximum diameter of approximately 10–

20 mm (Muraya *et al*., 2015). The average inter-estrous interval is 21 days with two-wave cycles being shorter than three-wave cycles (Townson *et al*., 2002; Sartori *et al*., 2004).

2.2.2. Selection, Deviation, Dominance, Ovulation and Atresia

Selection is the process by which a single follicle from the recruited cohort continues to grow, while the remaining follicles of the cohort undergo atresia. With the decline in circulating FSH concentrations, small follicles are unable to continue with growth and the selected follicle shifts its dependency from FSH to luteinizing hormone (LH) (Barros and Nogueira, 2001). The decline in FSH is driven by increasing concentrations of estradiol and inhibin produced by the recruited follicles. This has negative feedback on the hypothalamic-pituitary axis to selectively suppress FSH secretion.

With the selection and establishment of a dominant follicle, follicular recruitment is inhibited until dominance is lost through atresia or ovulation. Inhibition of follicular recruitment may be mediated by the low concentrations of FSH. Destruction or ovulation of a dominant follicle results in the rise of FSH and subsequent initiation of a new follicular wave (Muraya *et al*., 2015). In cattle, one dominant follicle ovulates to release an ovum during the estrous cycle.

2.2.3. Luteal phase

The luteal phase begins with the formation of corpus luteum which begins with ovulation and ends with luteolysis. Progesterone is secreted by the corpus luteum and is regulated by secretions of the anterior pituitary gland, uterus, ovary, and embryo. The regulation of progesterone secretion is controlled by a balance of luteotropic (Luteinizing hormone; LH) and luteolytic (Prostaglandin; $PGF2\alpha$) stimuli (Barros and Nogueira, 2001). Progesterone plays a

critical role in the regulation of the estrous cycle and determines estrous cycle length and the maintenance of pregnancy.

2.3. Superovulation of Cattle

Super ovulation is the process of ovarian hyper stimulation using hormones to produce more than the usual number of mature ova. Cattle normally produce one mature ovum during the normal ovulatory process. Super ovulation is measured by the number of mature ova released by the donor cow after hormonal treatment.

The process of embryo transfer involves several steps that are crucial for its success (Seidel and Seidel, 2005; Moore and Thatcher, 2006). The first step involves the selection of donor animals based on sound reproductive and productive performance. The selection process is well defined and heavily depends on the objectives to be achieved by the breeder (Seidel and Seidel, 2005). Most breeders select donors with improved milk production and good linear traits. Production traits are more important in most developing countries including Kenya.

Synchronization of the estrous cycle is important to allow for ease of super ovulation, insemination, flushing and embryo transfer where applicable (Seidel and Seidel, 2005). Synchronization brings the estrous cycle of the donor and recipient cattle to the same point. Synchronization in MOET programs is achieved through use of progesterone and prostaglandin hormonal treatment (Hasler, 2004). All MOET protocols in use in Kenya have a standard synchronization process based on the two hormones.

After selection, the estrous cycle of the donor cattle and recipients if the transfer is anticipated immediately after flushing are synchronized together. After synchronization, super ovulation

is then performed based on the use of follicle stimulating hormone (FSH) (Lerner *et al*., 1986; Hasler, 2004; Seidel and Seidel, 2005; Lamb, 2012). Naturally, FSH is released by the anterior pituitary gland in small doses. FSH stimulates the growth of follicles in the ovary from recruitment stage through to the dominance stage.

The emergency of the dominant follicle triggers a negative feedback process that leads to decreased FSH production by the pituitary gland (Moore and Thatcher, 2006; Muraya *et al*., 2015). Decreased FSH production has detrimental effect on growing follicles in cattle. The growing follicles are dependent on FSH and hence the cohort that had grown with the dominant follicle will regress with decreased FSH (Muraya *et al*., 2015). The dominant follicle will be ovulated and if fertilization of the ovulated follicle fails, then another cohort of follicles is recruited as a new wave begins. During pregnancy, the waves continue to be witnessed but ovulation doesn't occur due to presence of progesterone that protects the pregnancy.

The use of external commercial FSH therefore overrides the negative feedback effects by availing the much needed FSH to the growing cohort of follicles which could have otherwise regressed. Most of the growing cohort of follicles will therefore reach maturity and are all ovulated by the donors receiving the commercial FSH. Before the advent of ultrasonography in cattle, it was difficult to predict the exact stage / phase of the oestrus cycle. It's now easy to examine the ovaries and record the stage of the cycle.

The stage of the ovarian cycle influences responds to super ovulation in cattle. The best time to begin FSH treatment is at the follicle recruitment stage when all follicles in the cohort recruited respond well to the external FSH. When dominance develops, it has influence on the

growing cohort of follicles and if the external dose of FSH is insufficient, then there is failure to super ovulate hence poor response achieved by the practitioner.

Commercial FSH used in super ovulation has led to production of more ova hence high number of embryos recovered during a MOET process (Seidel and Seidel, 2005; Lamb, 2012). Current protocol used in Kenya incorporate 400 mg of FSH per donor cow administered over a period of four days. The FSH is administered at a reducing rate twice daily 12 hours apart: day one (160 mg), of which 80 mg is administered in the morning and evening respectively. On day two (120 mg) on a divided dose as on day one. On day three (80 mg) and day four (40 mg) divided dose as was on day one and two (Seidel and Seidel, 2005; Lamb, 2012). This is because the FSH has a short half-life.

After gonadotrophin treatment and administration of prostaglandin, the donors are inseminated based on observed heat two to three times and 12 hours apart using high quality semen (Seidel and Seidel, 2005). The insemination procedure improves the ratio of fertilized to non-fertilized ova recovered during flushing. Flushing of the uterus to recover embryos is carried out on day seven (7) after insemination (Seidel and Seidel, 2005). Flushing on day seven has a high recovery rate of transferable embryos (compact morula and blastocysts). Recovered embryos are then graded and either transferred as fresh embryos or frozen for future use and export (Seidel and Seidel, 2005).

If transfer is to be carried out immediately using fresh embryos, the recipient animals are prepared alongside the donor cows so as to be in perfect synchrony during the transfer (Seidel and Seidel, 2005). Developmental stage of embryo which is in perfect synchrony with the recipient uterine conditions has a higher rate of survival compared to those not in perfect

synchrony (Seidel and Seidel, 2005). Asynchrony of 24 hours early or later than the developmental stage of the embryo is tolerated. Asynchrony of more than 24 hours has poor conception rates.

If transfer is not anticipated, then the embryos are prepared for freezing for later use. Such frozen embryos will be viable for a long time provided they are kept at the right temperatures. The advent of frozen embryos has allowed increased cross border trade on animal genetics and allowed superior genes for use in many parts of the world without restrictions due to trade barriers (Mapletoft, 2012).

2.4. Ovarian Ultrasonography in Cattle

The advent of bovine ovarian ultrasound led to immense progress being made in the understanding of folliculogenesis and the development of corpora lutea (Durocher *et al*., 2005). Ultrasonography is the imaging of deep structures of the body by recording the echoes of pulses of ultrasonic waves directed into the tissues and reflected by tissue planes where there is a change in acoustic impedance. Linear-array, real-time, B-mode ultrasound scanners are used.

Bovine reproductive organs are commonly scanned per rectum using a linear-array transducer. The description of ultrasound images is based on an evaluation of the shape, contour, size, and position of the structure being studied, as well as its echogenicity, which depends on the amplitude of the echoes received. Anechoic structures do not produce echoes; instead, they transmit the waves on to more deeply situated tissues. An example of anechoic structure is follicular fluid, which appears black on the screen.

Ultrasonography is particularly important as the practitioner is able to monitor the structure of interest. Follicular development can be monitored to evaluate how follicles develop and in the case of super ovulation it's possible to precisely detect ovulatory follicles. This technique will not only inform the practitioner of the number and sizes of the follicles and corpora lutea but can also be used to predict accurately the expected number of embryos to be harvested in a super ovulation program. It allows the practitioner to decide on when to start super ovulation treatment to maximize on response hence embryo yield.

2.5. Grading of Embryos

Embryos are graded according to the recommendation of the International Embryo Technology Society (IETS, 2016). The embryos are graded based on morphological appearance of cells forming the embryo and the developmental stage. The developmental stages are namely: Hatched blastocyst is denoted as eight (8). Hatchet blastocysts have broken out of the zona pellucida. These are embryos that are in preparation for implantation into the uterine wall of the dam. Expanded blastocyst is denoted as seven (7); blastocyst is denoted as six (6); early blastocyst is denoted as five (5); compact morula is denoted as four (4); early morula is denoted as three (3); 2 to 16 cells embryos were denoted as two (2) and unfertilized ovum is denoted as one (1) (Bo and Mapletoft, 2013). Embryos used in transfer are those of developmental stages four (4) to seven (7) (Bo and Mapletoft, 2013).

The hatched blastocysts, developmental stage 8 are difficult to identify during conventional MOET programmes since they have lost the zona pellucida. Developmental stage 3 embryos (early/young morula) are immature with poor survival rate after transfer, therefore, are not normally used during the transfers. Developmental stages of the embryos are presented as Figures 6 - 13.

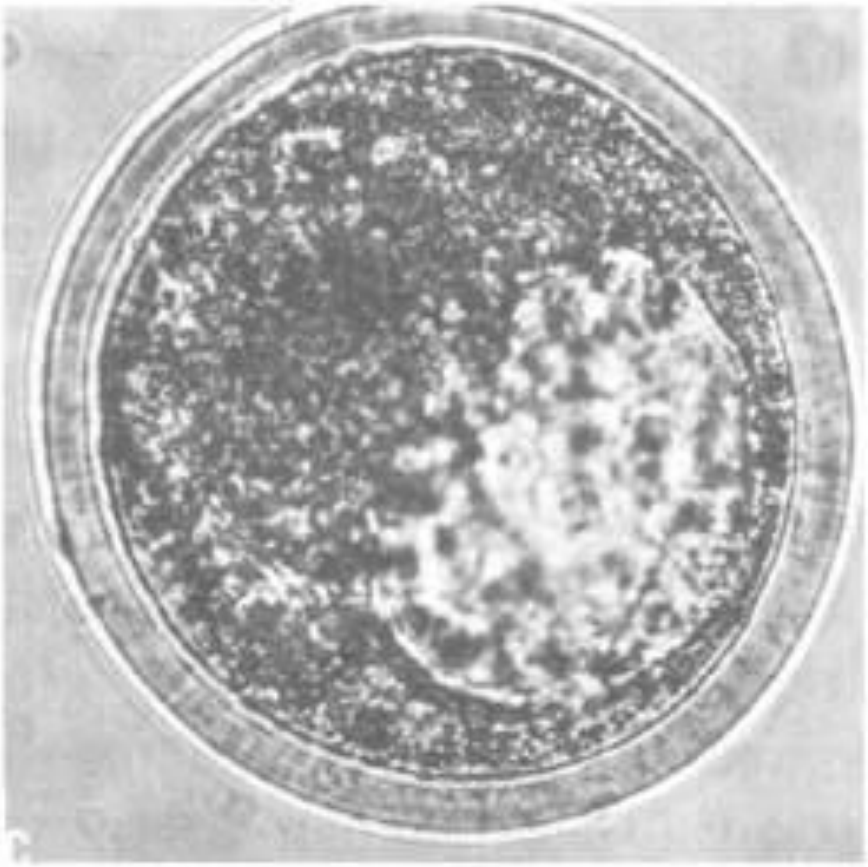

Figure 6. Embryo at an early Blastocysts stage denoted as developmental stage 5 and of quality grade 1. The cells forming the embryo were entire (no protrusion) (Seidel and Seidel, 2005)

Grading is also done based on the quality of the embryo as it appears under the microscope. The embryos are placed into four categories namely: Quality grade 1: embryos with 85% of cellular mass intact and viable and none or less than 15% extruded cells (Figures 6, 7, 8, 9 and 10). These embryos are graded as excellent or good (Bo and Mapletoft, 2013). Quality grade 2 embryos are those with 50% or more intact embryonic mass with extruded cells equal to or less than 50%. These embryos may also have intact embryonic mass but darker in color compared to normal embryos. These embryos are classified as fair (Figure 13) (Bo and Mapletoft, 2013).

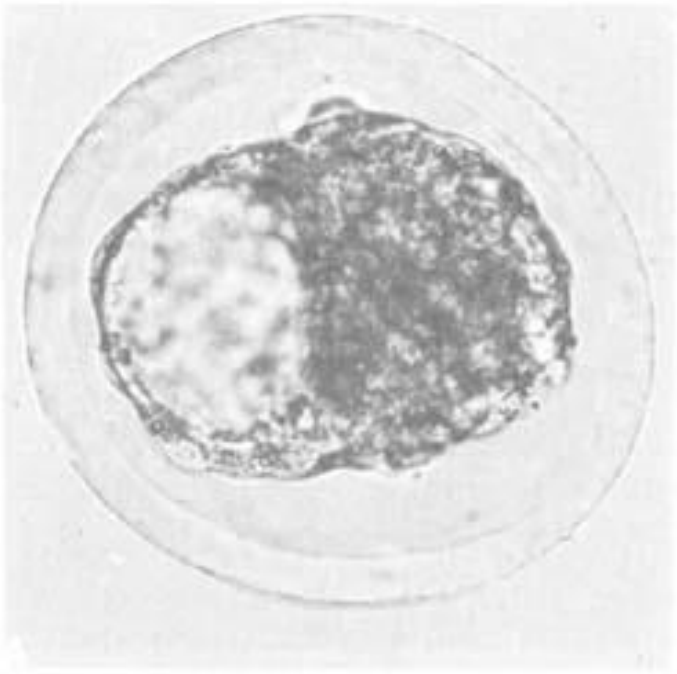

Figure 7. Embryo at a blastocyst stage denoted as developmental stage 6 and of quality grade 1 (Seidel and Seidel, 2005)

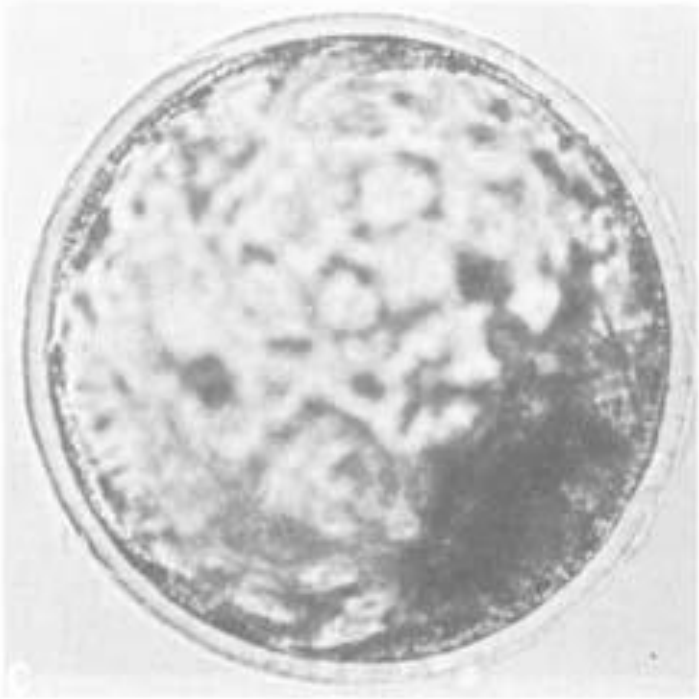

Figure 8. Embryo at expanded blastocyst stage denoted as developmental stage 7 and of quality grade 1 (Seidel and Seidel, 2005)

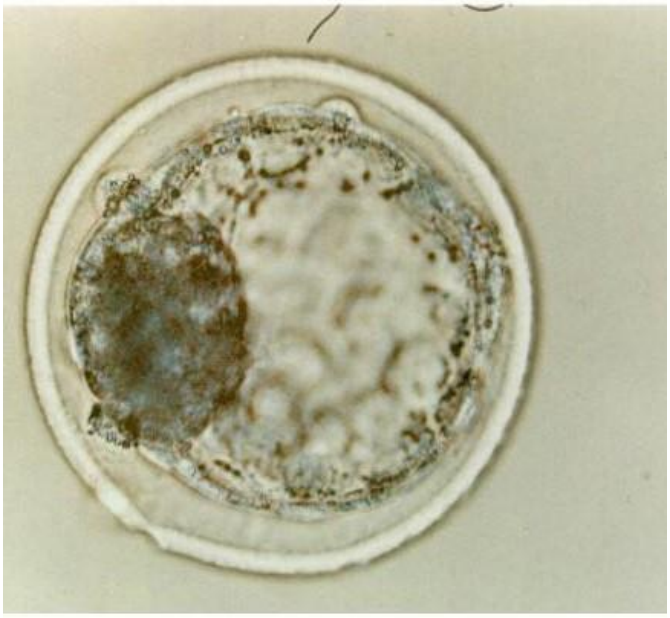

Figure 9. Embryo at expanded blastocyst stage denoted as developmental stage 7and quality grade 1(Bo and Mapletoft, 2013)

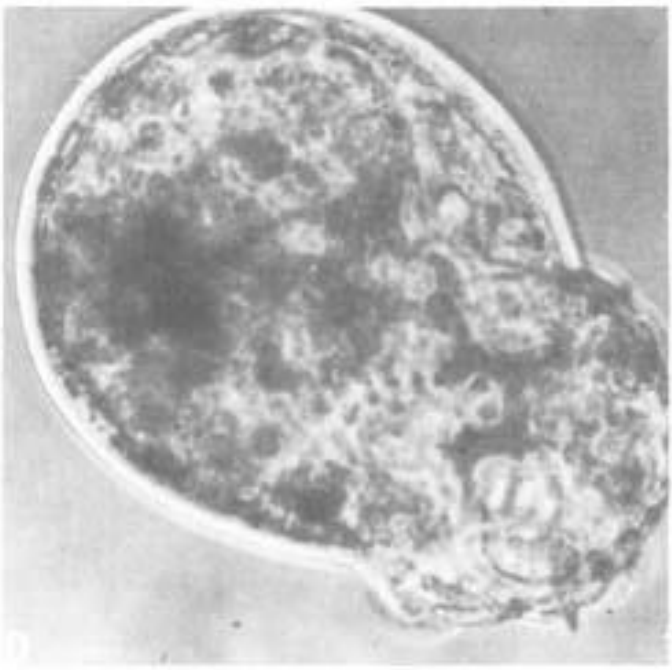

Figure 10: Embryo at hatching blastocyst stage denoted as developmental stage 8 and of quality grade 1 (Seidel and Seidel, 2005). The embryo is breaking out of zona pellucida in preparation for implantation.

Quality grade 3 embryos are those with major irregularities of embryo mass, color and density of individual cells. They are classified as poor with at least 25% of embryo cell mass intact (Bo and Mapletoft, 2013). Quality grade 4 embryos are those that have degenerated or dead. Also unfertilized ova are classified as quality grade 4 (Figure 11 and12) (Bo and Mapletoft, 2013). Quality grade 1 and 2 embryos have higher survival rate on transfer with grade 1 being superior. Quality grade 3 embryos have a lower post transfer survival rate with quality grade 4 embryos being rejected (Bo and Mapletoft, 2013).

During the packing of embryos into the straws, the developmental stage and quality grade are clearly indicated on the straw. The developmental stage is important in achieving synchrony with recipients which has been shown to influence post transfer pregnancy rate.

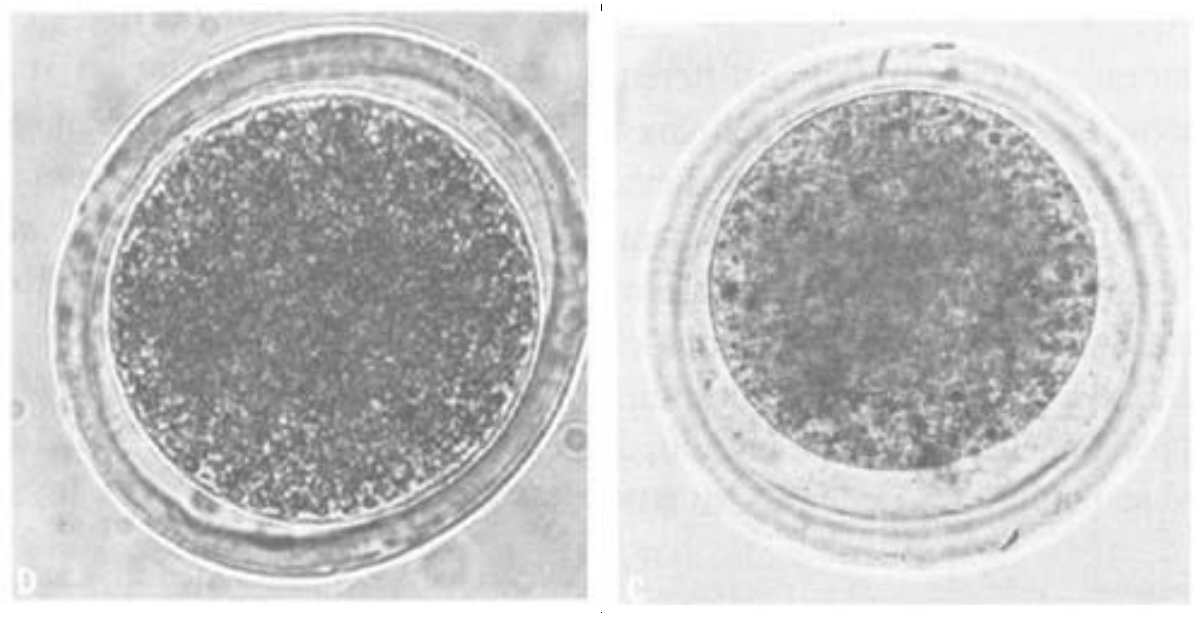

Figure 11. Two unfertilized Bovine ova with large zona pellucida. It is denoted as developmental stage 1, quality grade 4 (Seidel and Seidel, 2005)

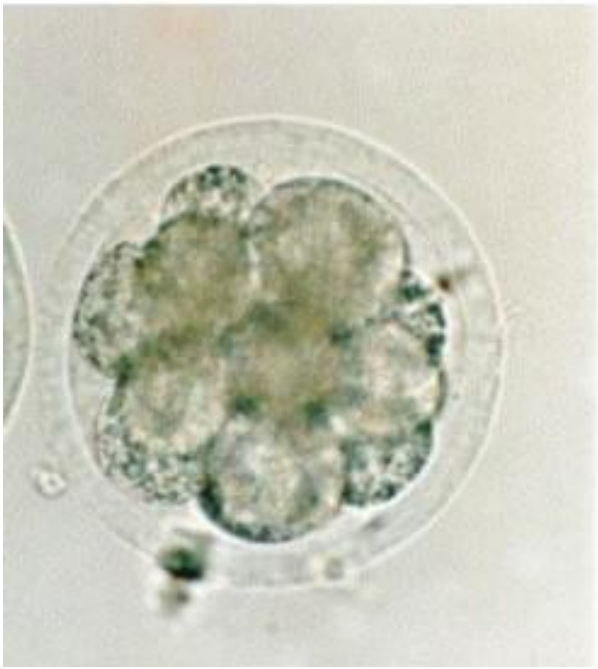

Figure 12. 16 cell embryo denoted as developmental stage 2 and of quality grade 4. The embryo exhibits arrested development since it was harvested on day 7 (Bo and Mapletoft, 2013)

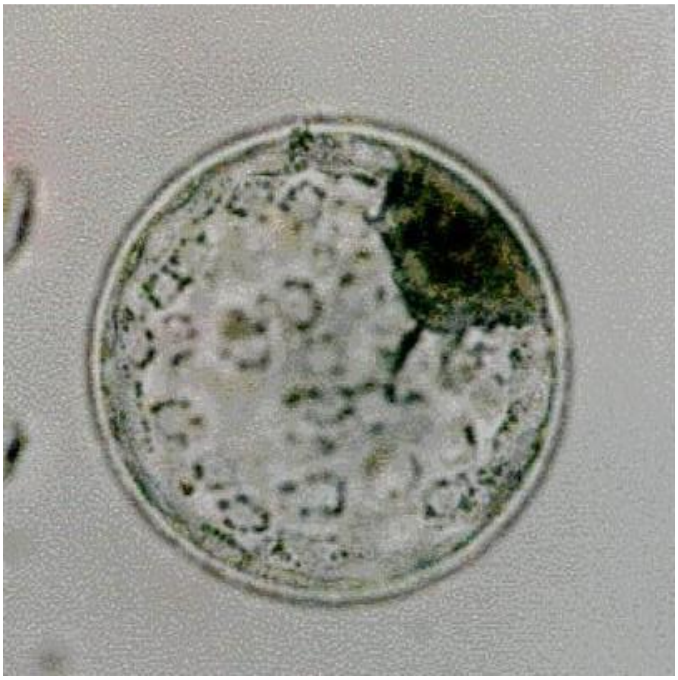

Figure 13. Embryo at expanded blastocyst stage denoted as developmental stage 7 and of quality grade 2. The embryo has a dark inner cell mass hence the reason for the classification as quality grade 2 (Bo and Mapletoft, 2013)

CHAPTER 3

3.0. MATERIALS AND METHODS

3.1. Follicular Dynamics, FSH Dose Rates and their Effect on Embryo Yield

3.1.1. Study Site

The study was carried out at the University of Eldoret (Chepkoilel) farm in Uasin Gishu County, North Rift, Kenya (Figure 14). The University is located approximately 9 km from Eldoret town along Eldoret – Ziwa road. The University is in a latitude of 0.5207° North and longitude of 35.2763°East.

Figure 14. The County of Uasin Gishu with its headquarters at Eldoret Town and the University of Eldoret in the neighborhood.

The University is situated at an altitude of 2154 metres above sea level. Average rainfall / precipitation is 1103 mm per year (Worldweatheronline, 2019). During the year under study (2015), the lowest precipitation of 29 mm was recorded in January and the highest of 172 mm and 196 mm recorded in July and August respectively. March and April had a precipitation of 57 mm and 150 mm respectively while May and June had a precipitation of 124 mm and 104 mm respectively (Worldweatheronline, 2019).

The temperatures of Eldoret (Uasin Gishu County) vary throughout the year with a high of 29°C recorded in March 2009 and a low of 9°C as recorded in June 2017 within the 10-year period of 2009 to 2019 (Worldweatheronline, 2019). During the experimental period in 2015, the temperatures were at a high of 26°C during the day in March to a low of 10°C at night in July. July had the lowest night temperatures over the ten year period and as also recorded during the 2015 experimental period (Worldweatheronline, 2019).

The University of Eldoret has 1000 acres of farm land used for production of both crops and livestock. The livestock section specializes in dairy cattle production and keeps mainly Friesian breed with a few Ayrshire breed of cattle. The total population of the dairy cattle at the University farm was 250 head with slightly over 120 adult cows at different stages of lactation and parity number.

The University of Eldoret was founded in 1946 as a large scale farmers training Centre by the white settler farmers in Kenya. It became a fully pledged University in 2013 after being awarded a charter by the President of the Republic of Kenya. The University is one of the institutions that produce breeding cows for dairy farmers although on small scale. The high

demand for breeding stock by dairy cattle farmers from the University farm and other large scale farms within Uasin Gishu County by far outstripped the supply.

3.1.2. Study Design

The study evaluated the effects four levels of experimental FSH on ovarian follicular dynamics and embryo yield of dairy cattle using ultrasonography based on four dependent variables as described: (1) Number of follicles (ovulatory and non-ovulatory) at the start of FSH treatment. These were follicles that were in the recruited cohort developing to maturity at the ovary (2) Number of ovulatory follicles at the end of FSH treatment. These were mature follicles just before ovulation. (3) Number of embryos/ova flushed from the experimental donor cows. These were the total transferrable embryos, degenerated embryos and ova flushed out from the uterus during the harvesting of embryos of the donor cows, (4) Quality of transferrable embryos harvested. These were the embryos that met the criteria for freezing or immediate transfer to recipients as previously described on the quality of embryos (Bo and Mapletoft, 2013).

Twelve (12) donor dairy cows on their second parity, aged and weighing approximately 40 months and 400 kg live weight, respectively were examined using ultrasound technique to evaluate the follicular response and test the embryo output (quantity and quality) based on the four levels of experimental FSH; (400 mg, 320 mg, 260 mg and 200 mg). The four FSH levels were informed by the relative weights of the Kenyan Friesian donor cows in comparison with the United States of America Holstein Friesians.

The donors had average body condition score (BCS) of three (3) on the 1 – 5 scale (where one (1) is emaciated and five (5) is extremely fat) at the beginning of the experiment and this was maintained throughout the experimental period. The scoring of the body condition was based

on a standard as described by Domecq *et al.* (1995). The controls were donors on 400 mg of FSH protocol, the standard procedure used currently in Kenya.

The twelve (12) experimental animals were randomly assigned to four (4) groups of three (3) donors each. A 4 x 4 cross over experimental design was used. Cross over experimental design has been shown to work well with repeated treatments comparing the response of different drugs or doses (Hedayat & Yang, 2005). The cross over design allowed the rotation of the four groups of donors on the four treatment levels randomly. The experimental donor cows crossed from one treatment to the other after a wash out period of two (2) months. The effects of the four (4) FSH (Folltropin®-V, manufactured by Bioniche Animal Health, Canada) treatment levels on ovarian follicular development and embryo yield over three different seasons (periods) were quantified and recorded.

Period 1, was March and April 2015, this is the period that marks the end of dry season and beginning of the long rains in Kenya. Its warmer with low to moderate rainfall at Eldoret in Uasin Gishu County. Period 2 (May and June) was colder than period 1 with moderate to high rainfall whereas period 3 (July and August) is the coldest season in Kenya with highest rainfall in the year.

3.1.2.1. Ultrasonography of Donor Cows

Donor cows were restrained in a crush and ultrasonography was done using a portable ultrasound device equipped with a 5.0/7.5 MHz linear bi-frequential probe (frequency of 6.5 MHz was used as standard). These were done daily before superovulation in three consecutive estrous cycle repeats. The probe was secured in an examination sleeve to protect the diaphragm and also to hold the acoustic gel in place. The probe was then introduced into the rectum after

fecal evacuation was done and moved back and forth over the pelvic area to scan the ovaries from above. Follicles of different sizes and the *corpus luteum* (CL) were identified and their location on the ovaries was noted.

The diameters of the three largest follicles and *corpus luteum* (CL) were then measured and their location sketched on a research book to ease their identification. These follicles traits were used to track and evaluate for follicular dynamics as previously described by Alvarez *et al*. (2000) and Muraya *et al.* (2015). Follicular populations were obtained by manual counting of all visible follicles. Numbers and sizes of ovarian follicles were determined and follicles were considered small (2 to 5 mm) or medium (6 to 8 mm). The dominant follicle (DF) of a wave was defined as the follicle that measured at least 9 mm in diameter and exceeded the diameter of all other follicles in the wave as defined previously (Ginther *et al.,* 1996).

The estrous cycles were described as being in recruitment, selection or dominant phase based on numbers and sizes of follicles noticed in the ovary as described previously by Muraya *et al*. (2015). Cows observed to be in either recruitment, selection or dominance phases of the estrous cycle were synchronized and super ovulated as described below and later inseminated twice at 12 hours apart and embryos flushed after seven days. Numbers and grades of the harvested embryos were assessed.

3.1.2.2. Synchronization and Superovulation

A17-day Synchronization and super ovulation protocol (Table 1) was uniformly administered during the four FSH treatment experiments. Synchronization was carried out using a combination of progesterone and prostaglandin to bring all the animals at the same phase of the estrous cycle during the super ovulation trial. It began with synchronization using a

controlled intravaginal drug release (CIDR) (EAZI-BREED™, CIDR® manufactured by Zoetis of United States of America) device impregnated with progesterone for slow release on day zero (0).

Table 1. FSH (Folltropin®-V) MOET Protocol for oestrus synchronization and super ovulation of donor cows at the University of Eldoret during the study.

DATE	DAY	TIME	DONOR PROGRAM	Remarks
	0	PM	Insert CIDR Inject 10 mL Multivitamin Inject 2 mL Cidirol	
	4		Ovarian ultrasonography	
	5	PM	Inject Folltropin (80 mg, 65 mg, 50 mg, 40 mg) (Table 2)	
	6	AM PM	Inject Folltropin (80 mg, 65 mg, 50 mg, 40 mg) (Table 2) Inject Folltropin (60 mg, 45 mg, 40 mg, 30 mg) (Table 2)	
	7	AM PM	Inject Folltropin (60 mg, 45 mg, 40 mg, 30 mg) (Table 2) Inject Folltropin (40 mg, 30 mg, 25 mg, 20 mg) + 5 mL Lutalyse (Table 2)	
	8	AM	Inject Folltropin (40 mg, 30 mg, 25 mg, 20 mg) + 5 mL Lutalyse (Table 2)	
		PM	Remove CIDR Inject Folltropin (20 mg, 20 mg, 15 mg, 10 mg) (Table 2)	
	9	AM	Inject Folltropin (20 mg, 20 mg, 15 mg, 10 mg) (Table 2)	
		PM	Ovarian ultrasonography Observe for Heat	
	10	AM	Inseminate	
		PM	Inseminate	
	16	AM/PM	Ovarian ultrasonography / Rectal palpation for corpora lutea	
	17	AM	Flushing and Embryo collection	

Table 2. Four FSH treatment levels for superovulation experiment on a reducing dose over a four-day period

	FSH LEVEL			
DAY	**Treatment 1**	**Treatment 2**	**Treatment 3**	**Treatment 4**
1	160 mg	130 mg	100 mg	80 mg
2	120 mg	90 mg	80 mg	60 mg
3	80 mg	60 mg	50 mg	40 mg
4	40 mg	40 mg	30 mg	20 mg
Total	**400 mg**	**320 mg**	**260 mg**	**200 mg**

The donors were also given a multivitamin injection to improve on appetite and estrogen; Cidirol® (Estradiol Benzoate) injection to improve on folliculogenesis. On the fourth day, ultrasonography was carried out on the ovaries using a 6.5 megahertz (MHz) dual frequency linear array probe as previously described (Figure 15).

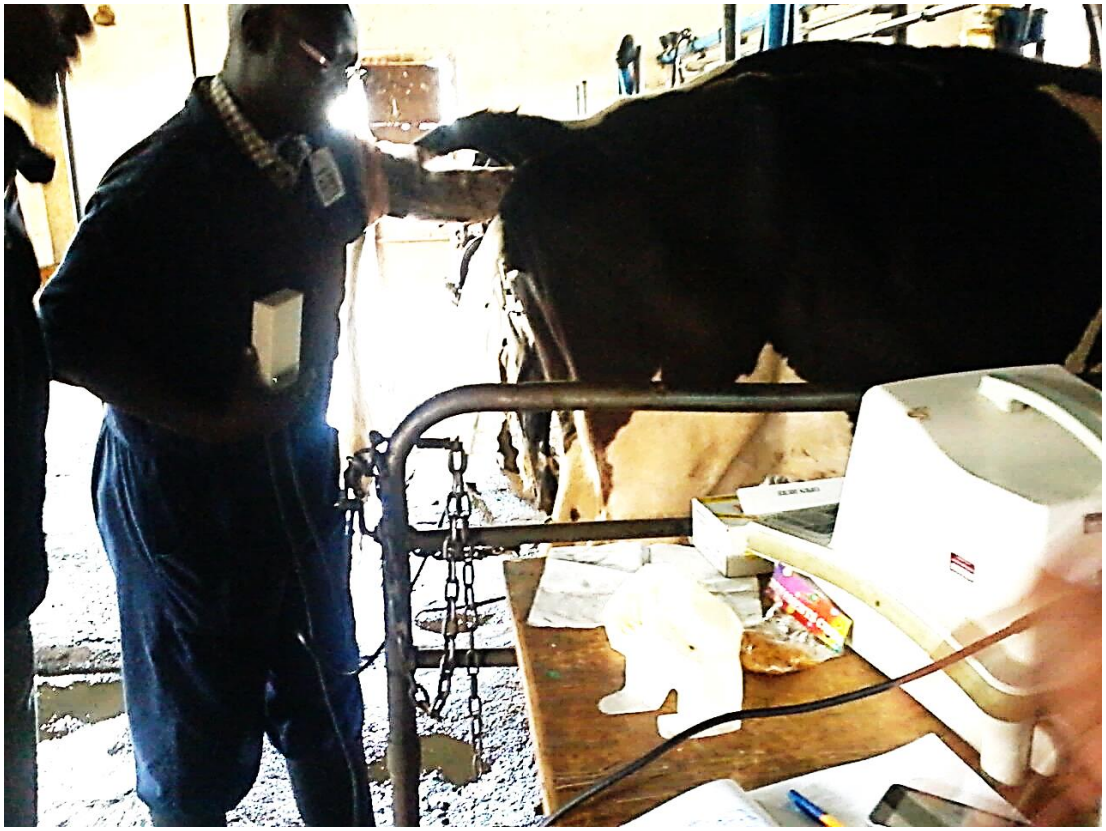

Figure 15. The author carrying out ultrasound evaluation of the follicles of one of the donor cows at University of Eldoret farm in 2015 during the FSH dose rate trials.

On the fifth day in the evening, the donors were treated with follicle stimulating hormone (FSH) based on the four experimental levels on a reducing dose for four days as shown on Table 2. FSH dose was divided into two equal portions and administered 12 hours apart. On day seven in the evening, the donors were administered with 5 mL *dinoprost tromethamine* injection, (Lutalyse®, Zoetis, United States of America) a prostaglandin (PGF2α) followed by a second administration of 5 mL on the morning of the following day.

Prostaglandin F2α injection removes *corpora lutea* in donors to allow the process of oestrus and ovulation to begin. CIDR was removed on day eight in the evening. On the last day of FSH treatment, the morning of day nine, the ovaries were scanned for any changes due to hormonal therapy using ultrasound transducer.

On the evening of day nine, the inseminator began to observe the donor cows for expression of oestrus (heat signs). Observation was carried out more frequently (every 2 – 3 hours). Donor cows standing to be mounted on by the other donors or females in the group indicated a donor on standing heat. The donors were inseminated twice after FSH treatment with high quality semen.

Rectal palpation combined with ultrasonography was used to predict the expected number of embryos based on the number of *corpora lutea* found on the ovaries on day 16. The expected output was compared with the actual number of embryos harvested. *Corpora lutea* are formed immediately after ovulation from the remaining structures of ovulating follicles. The presence of *corpora lutea* is a strong indicator of ovulation and can be used to estimate the number of embryos expected in a MOET program. This is also important for the practitioner especially when searching for the embryos as an indicator of the number of embryos expected.

Flushing the uterus of the donor cows for embryos and grading of embryos was carried out according to the International Embryo Technology Society (IETS) protocol on day 17. Flushing was carried out seven (7) days after insemination of the donors using three-way catheters introduced into the uterine horns (Figure 16). One uterine horn was flushed at a time.

Figure 16. The author flushing one of the Friesian donor cows at the University of Eldoret farm in the year 2015 during the FSH dose rate trials. The donors were administered with epidural anaesthesia before flushing to relax the pelvic region for ease of flushing.

Flushing media (Euroflush from IMV technologies in France) was introduced slowly to the uterine horn to flush out the embryos/ova which were then collected using an embryo (emcon) filter.

The donors were administered with epidural anaesthesia using lignocaine hydrochloride to reduce straining during flushing. After flushing, the donors were administered with 5mL of lutalyse® (prostaglandin injection) which destroys the corpora lutea. This is important to avoid implantation of embryos that may have been left behind in the uterus after flushing. Searching and grading of embryos was done using a special stereoscopic microscope designed for the purpose. Most embryos produced were frozen for later use and some were transfered fresh to available recipients.

3.1.3. Data Analysis

Two donor cows were replaced during the study due to hip dislocation in one cow and difficulty in cervical penetration for the other. The final data set had 35 observations. The number of ovarian follicles observed through ultrasonography before FSH treatment of the cows and four days thereafter were separately recorded. Ovarian follicles were classified as either ovulatory or non-ovulatory based on the size.

Ovulatory follicles were the large mature follicles at over 9 mm in diameter. Non ovulatory follicles were those below 9 mm in diameter as observed under the ultrasound transducer. The number of *corpora lutea* detected by ultrasonography and confirmed by rectal palpation together with embryos/ova and transferrable embryos flushed were also recorded.

Initial body weight of experimental donor cows was used to weight observations in the statistical analysis to take care of inadequacy of a uniform dose used on cows of varying body weight.

Response was taken on the same cow on different FSH dose rates and at different periods resulting in a repeated measures data structure. Response values for the same cow are correlated whereas values for different cows are assumed to be independent. Consequently, a mixed linear statistical model was postulated. A parameter estimation procedure that uses restricted maximum likelihood (REML) was used for variance and covariance estimation. SAS procedure for mixed linear models was deployed for analysis of the data.

3.1.4. Statistical Model

In the postulated linear mixed model (Duchateau *et al*., 1998), the number of follicles both ovulatory and non-ovulatory observed through ultrasonography, total embryos/ova flushed and transferrable embryos harvested constituted the dependent variables and the experimental factor FSH treatment was a fixed independent variable. Period of treatment is also a fixed variable whereas the variability among cows within period is random.

$Y_{ijk} = \mu + x_i + x_j + x_{ij} + e_{ijk}$

Were;

$i = 1,2,3 \;\; j = 1,2,3,4 \;\; k = 1,2, \ldots..18$

Y_{ijk}= Response of cow k that received hormone dose j in period i

μ is the overall mean effect

$x_i$is the fixed effect of period i

$x_j$is the fixed effect of hormone dose level j

x_{ij} is the effect of the interaction between period i and hormone dose level j

e_{ijk} is the random error of the measured response of cow k that received hormone dose j in period i.

Variance of a single observation was estimated by residual variance + covariance between two measurements within the same animal.

3.1.5. Research Hypothesis

The null hypothesis under investigation was that of no difference in response among all levels of hormone tested.

$$H_0: x_l - x_j = 0$$

$H_a: x_l - x_j \neq 0$ for $l \neq j = 1,2,3,4$

3.2. Factors Affecting Adoption of Embryo Transfer Technology in Kenya

3.2.1. Study Site

The study was carried out in Uasin Gishu and Trans Nzoia Counties situated along the Rift Valley in Kenya (Figure 17). The two Counties are among those designated as food baskets for the country. The two counties specialize in production of both crop and livestock especially dairy cattle farming. Livestock farming is a key enterprise in both counties and in an effort to boost production the county governments have begun a subsidized artificial insemination service to the farmers. This has been introduced to improve the quality of the livestock, thus improving their production. The two counties are key in implementation of intervention strategies in the Big Four agenda of the government to improve on milk production hence achievement of food and nutrition security.

The livestock farmers in the two counties produce the bulk of milk in the region and are ranked 3rd and 4th after Kiambu and Nyandarua Counties in Central Kenya. The two counties are known for the good quality dairy cattle owned by large scale farmers.

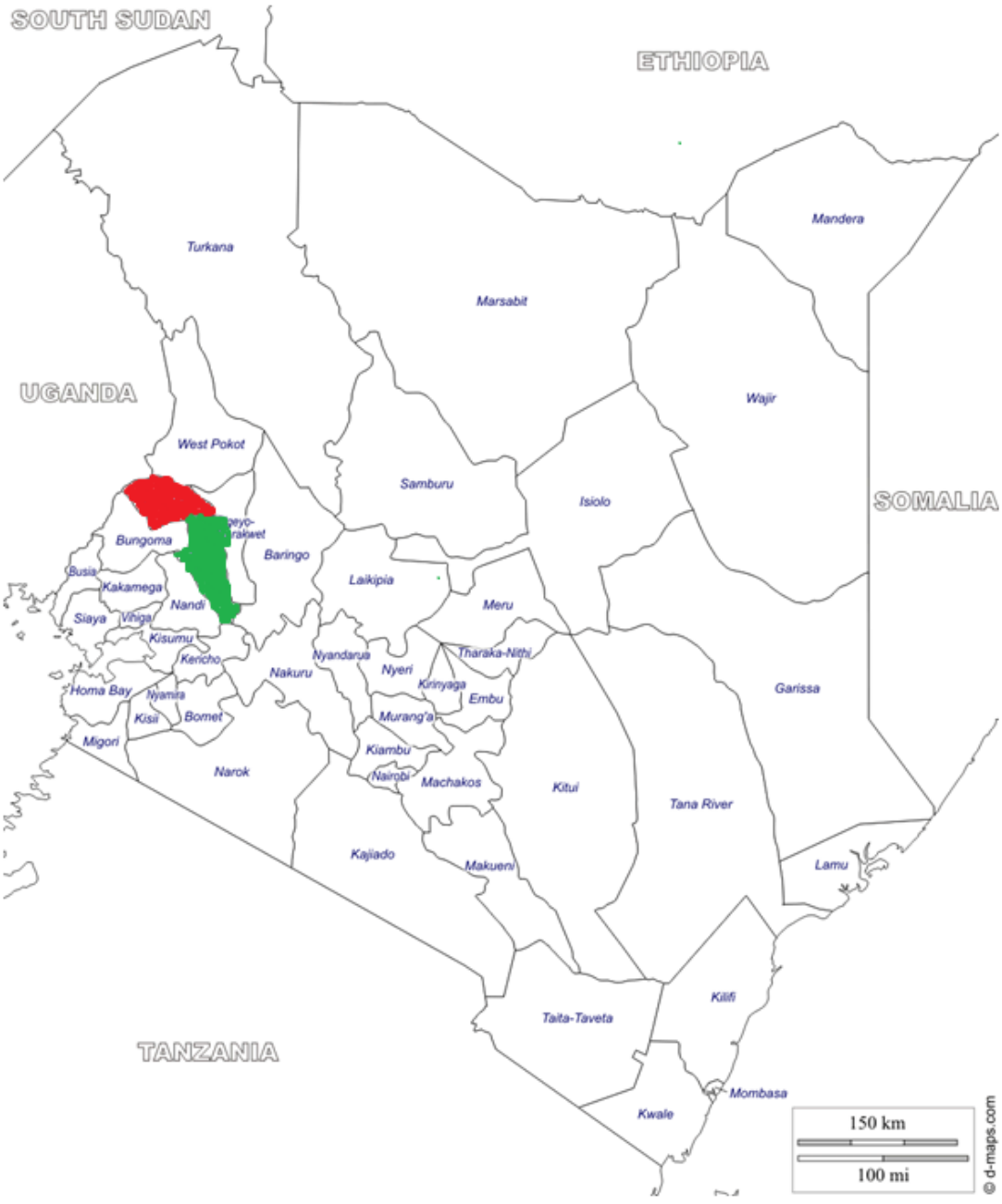

Figure 17. A map of Kenya showing Uasin Gishu (green) and Trans Nzoia (red) Counties were the research on adoption of MOET was carried out by the author.

Majority of the farmers were of small scale category while large scale farmers were only a few. Small scale farmers keep average quality dairy cows, mainly upgrades and produce the bulk of the milk. Dairy cattle farmers from other parts of the country and beyond purchase their replacement heifers from Uasin Gishu, Trans Nzoia and the surrounding Nandi County. This has led to higher prices hence has become unaffordable to the average dairy farmer. Notwithstanding the high prices, the in calf heifers are not readily available hence the would be buyers wait for long.

3.2.2. Study design, data handling and analysis

The aim of the study was to evaluate the factors that affected the adoption of MOET by dairy cattle farmers. A survey was carried out using a questionnaire administered to the farmers through individual face to face interview. The data was collected purposely from dairy cattle farmers already practicing artificial insemination technology for improvement of their livestock within Trans Nzoia and Uasin Gishu Counties through simple random method from a pool of data available at the County Veterinary Offices.

The choice of the farmers was purposive because these group of farmers who have practiced artificial insemination had a high likelihood of having used or known the existence of other assisted reproductive techniques in use in Kenya. The two counties were chosen because of closeness to the University of Eldoret, where the researcher was based.

The questionnaire was designed to capture the independent and dependent variables. It was first pretested with 20 respondents in Uasin Gishu County. Based on the data collected, the questionnaire was further refined (Appendix 1) and later administered to the 385 respondents.

Out of these, only 293 had practiced relevant artificial insemination technology and thus included in the analysis.

The sample size of 385 farmers (respondents) was derived using predetermined criteria of 95% confidence level, standard deviation of 0.5 and a margin of error of 5% and calculated as shown below:

$SS = Z^2 \times (p) \times (1 - p) / C^2$ (Creative research systems, 2015)

Where:

SS = the sample size

Z = Z value (1.96 for 95% confidence level),

p = percentage picking a choice, expressed as a decimal (0.5 used for the sample needed)

C = confidence interval expressed as a decimal

The questionnaire data was used to generate the list of factors affecting MOET as the independent variables against the dependent variable of the adoption of MOET by the farmer (the farmers were rated either as adopted or not adopted). SPSS software version 20 (https://www.ibm.com/analytics/us/en/spss/spss-statistics-version/) was used for the analysis of data.

The independent factors tested to have affected the adoption of MOET by the dairy cattle farmers included: (1) Availability of embryo transfer technology in the two counties under study, (2) Availability of embryo transfer experts to carry out the MOET process in the two counties, (3) Lack of awareness of the availability of the MOET technology in Kenya by the

farmers and (4) Whether the cost of the MOET process affected the adoption of the technology by the farmers.

The analytical comparison of associations for each of the independent variables at 99% confidence level ($P \leq 0.01$) were tested against the two levels of adoption (either adopted or not adopted) for the two counties based on Kendall's Tau correlations of coefficient matrix in SPSS software. It is a nonparametric measure of strength and direction of association that exists between two variables.

3.3. Evaluation of Success Rate of Current MOET Protocol in Kenya

3.3.1. Data Collection

The secondary data used during this study was purposely obtained from two repository centres; Agricultural Development Corporation (ADC) Namandala farm in Trans Nzoia County and Sasini Company Limited in Nyeri County. This was because the two centres had maintained records of regular MOET programs.

Information on past donor management practices and embryo production in dairy cattle was available from the two organizations. The two animal breeding organizations had fairly regular embryo transfer programs carried out over the period under study. Sasini Company Limited is a private organization owned by independent investors while ADC Namandala farm is wholly owned by the Government of Kenya.

3.3.2. Study Design

This was a quasi-experimental in that it aimed to evaluate data obtained by previous MOET protocol activities. Data of embryo yield (the number of recovered embryos and quantity) was

used to determine success rate of the protocol. This was carried out through retrospective analysis of secondary data of the MOET program in Kenya for the period 2013-2015. The data on the protocol used, type of donor management and number of embryos collected were used for the review.

The data was used to generate the list of the donor management practices as the independent variable against the dependent variable of the number of embryos recovered. The analytical comparison of associations for each of the independent variable (type of management) at 95% confidence level ($P \leq 0.05$) were tested against the dependent variable for the two study areas (Sasini and ADC farms). Significant difference in mean of harvested embryos was evaluated using the standard error of mean and tested using student t- test.

Sasini farm had deployed intensive system of animal production to manage the donor animals. The donor animals were confined with minimal movement. The aim was to ensure minimal variation in feeding, mineral supplementation and movement of the donor cows. Their use of total mixed ration (TMR) led to optimal feed intake and availability of all nutrients required by the donor cows.

ADC Namandala farm on the other hand practiced semi intensive management system of animal production to manage the donor cows. Such a system is prone to high variation in terms of quality feed and mineral intake since the cows were supplemented during milking only and intake in such cases may not be optimal. The donor cows at ADC Namandala farm were allowed to graze during the day, fed with supplements and minerals during milking and confined at night without feeding.

The FSH super ovulation protocol employed in the two firms were similar and each organization used 400 mg of FSH (Folltropin®-V, manufactured by Bioniche Animal Health, Canada) for superovulation and progesterone (EAZI-BREED™, CIDR® manufactured by Zoetis of United States of America) implant with prostaglandin injection for synchronization of the donors. The 17-day synchronization and superovulation protocol were deployed (Table 3). On day zero, the start of the program, CIDR® was implanted together with injection of 2 mL of oestrogen, Cidirol® (Estradiol Benzoate) and 10 mL of multivitamin given to the donors.

Table 3. FSH (Folltropin®-V) MOET Protocol for oestrus synchronization and super ovulation of donor cows during the Sasini and ADC Namandala embryo transfer programs in Nyeri and Trans Nzoia respectively.

DATE	DAY	TIME	DONOR PROGRAM	Remarks
	0	PM	Insert CIDR Inject 10mls Multivitamin Inject 2mls Cidirol	
	5	PM	Inject Folltropin (80 mg)	
	6	AM	Inject Folltropin (80 mg)	
		PM	Inject Folltropin (60 mg)	
	7	AM	Inject Folltropin (60 mg)	
		PM	Inject Folltropin (40 mg) + 5 mL Lutalyse	
	8	AM	Inject Folltropin (40 mg) + 5 mL Lutalyse	
		PM	Remove CIDR Inject Folltropin (20 mg)	
	9	AM	Inject Folltropin (20 mg)	
		PM	Observe for Heat	
	10	AM	Inseminate	
		PM	Inseminate	
	17	AM	Flushing and Embryo collection	

This was followed on day 5 with FSH (Folltropin®-V) treatment using 400 mg per donor for four days on a reducing balance divided dose twice as follows; 80 mg on the evening of day

five followed by 80 mg the following morning and 60 mg later in the evening of day six. On day seven, donors were given 60 mg of FSH in the morning and later 40 mg of FSH and 5mL of prostaglandin (Lutalyse® manufactured by Zoetis of United States of America) in the evening.

On day eight, the donors were given 40 mg of FSH and 5 mL of prostaglandin (Lutalyse®) in the morning and later 20 mg of FSH and the removal of CIDR® in the evening. On day nine, donors were given 20 mg of FSH in the morning which marked the end of super ovulation. Observation of the donors for heat signs began on the evening of day nine through day 10 with artificial insemination being carried from eight hours after observation of standing heat as shown on Table 3.

The column for dates was filled for individual donor groups. Different donor groups were treated at different dates during the study period. Dates are critical to ensure that each group of donors receive appropriate treatment on a timely manner and avoids mix up of the donors. The column is filled during research or commercial MOET programs.

Sasini farm synchronized and super ovulated 67 donor cows during the period under study while ADC Namandala farm synchronized and super ovulated 45 donors. Donor cows were selected on the two farms under study based on the same criteria as developed by the embryo transfer team. This included body condition score of three and above on the scale of 1 - 5, cycling donor cows, regular calving interval, above average milk production and good body structure and udder.

Super ovulation and embryo recovery in the two firms was carried out by a team from the East Africa Semen and Embryo Transfer Association (EASETA). The team comprised of members from the University of Nairobi, Clinical Studies Department of the Faculty of Veterinary Medicine, Director of Veterinary Services Staff, ADC staff and private embryo transfer practitioners. Kenya had only a few teams that could carry out super ovulation and embryo recovery due to the limited number of professionals trained on the procedure. MOET has not been fully commercialized in Kenya due to the limited number of trained personnel, unpredictable nature of super ovulation and embryo yields and lack of readily available and affordable FSH.

Though Kenya is classified under tropical climatic conditions, there are great weather variations throughout the year. January, February and first half of March exhibit hot and dry conditions. Second half of March to June is normally wet and warm whereas July to August and first half of September is extremely cold and wet. Last half of September to November is warm and wet while December is hot and dry. This weather pattern is exhibited mainly in the highlands where the two farms are located.

CHAPTER 4

4.0. RESULTS

4.1. Effects of Different FSH Dose Rates on the Number of Ovarian Follicles Stimulated

Restricted maximum likelihood (REML) estimates for the covariance of observations within the same cow and residual variance for the number of ovulatory and non-ovulatory follicles ≥3mm in diameter at the start of the treatment and ovulatory follicles at the end of the treatment (Figures 18, 19, 20, 21 and 22) as well as the residual variance and statistic -2 Log L were calculated. Model fit Statistic -2 Residual Log Likelihood indicated significant model fit for all the dependent variables.

The ovarian follicular growth of two donors (Figure 18 and 19) as recorded through ultrasonography during the study shows follicles that were in selection phase of one of the waves shortly before deviation. The follicles were of medium sized range in diameter.

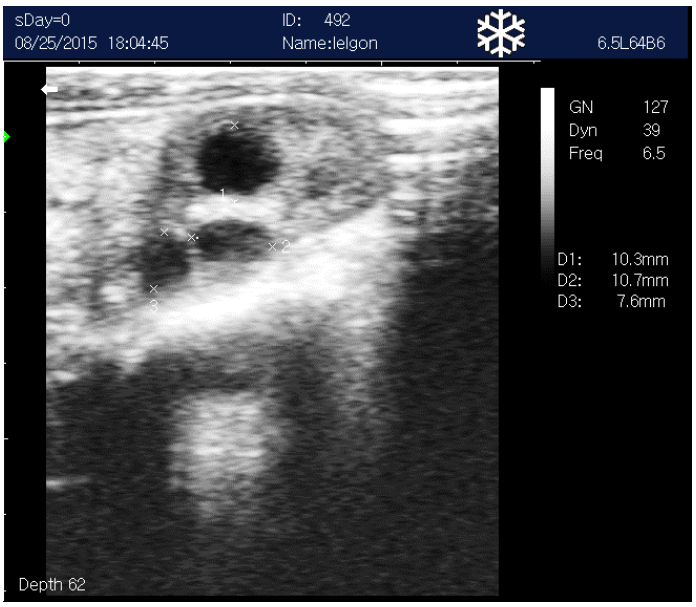

Figure 18. Ultrasound image of one of the donor cows recorded during the research. The follicles marked D1, D2 and D3 were at selection phase of development on day 4 before the start of the FSH superovulation treatment on donor cows as seen during this research.

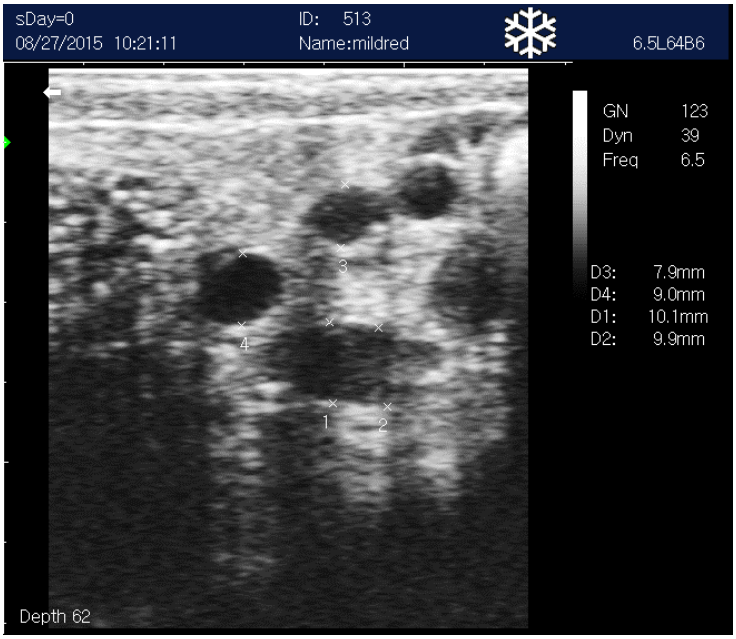

Figure 19. Example of ultrasonography images of four (D1-D4) super ovulated follicles (marked 1-4) whose borders are marked using X marks and sizes are indicated in mm as provided in right side; image is from one cow used in this research.

Ultrasonography image of ovarian follicles of a donor cow (Figure 20) shows a single follicle at dominance phase during one of the waves of the oestrus cycle as recorded through ovarian ultrasound technique. The image was recorded a day before the start of FSH superovulation treatment of donor cows during the research at the University of Eldoret (Figure 20). Most of the other follicles in the same cohort as the dominant follicle had regressed and were not visible as shown in the image (Figure 20).

Ultrasonographic image of the ovary of one of the donor cows (Figure 21) as recorded by ultrasonography during the study. Deviation of the large follicle was apparent and the process of follicular atresia had begun on the small follicles in the same cohort as the dominant follicle. The dominance had begun influencing negatively the growth of the smaller follicles (Figure 21).

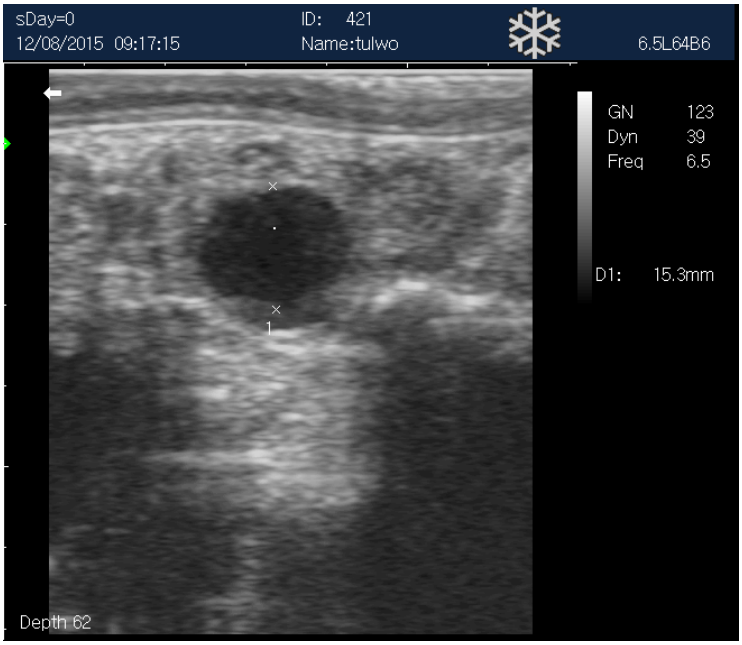

Figure 20. An ultrasonography image of a donor cow with a dominant follicle on day 4 at the beginning of FSH treatment as recorded through ovarian ultrasound technique during this study. The other follicles in the cohort had regressed with the onset of dominance.

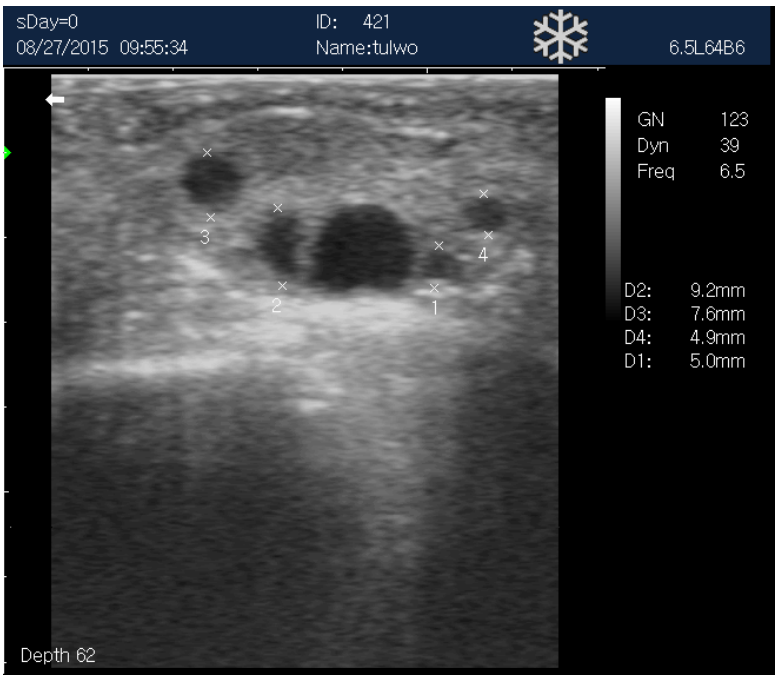

Figure 21. Ultrasonographic image of the ovary of a donor cow with a dominant follicle (unmarked) and other relatively smaller follicles as recorded at the beginning of FSH treatment during the study at the University of Eldoret.

Ultrasonographic image of one of the donor cows showing ovarian follicles at recruitment phase before selection and deviation begun as recorded through ultrasound technique (Figure 22). The cohort of follicles were smaller to medium in size.

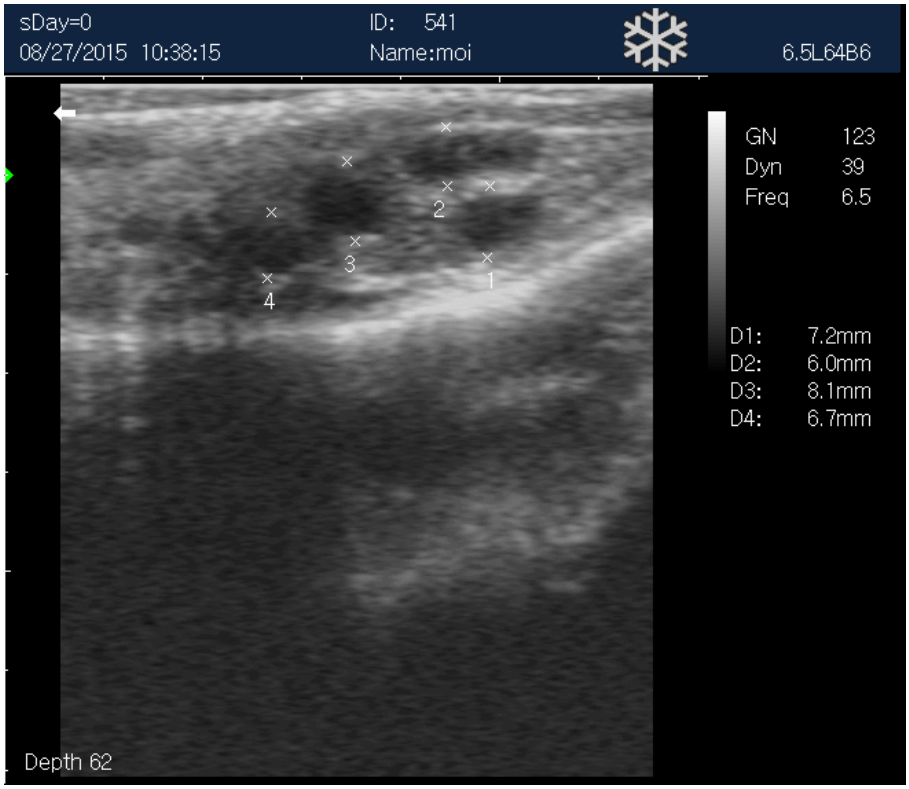

Figure 22. Ultrasonographic image of the ovary of a donor cow with follicles at recruitment stage at the beginning of FSH treatment as recorded through ultrasound technique during the study at the University of Eldoret.

Mean number of ovulatory follicles observed at the end of FSH treatment rose marginally with the level of FSH hormone dose but did not significantly differ (Table 4). Period three (3) mean was lower than that obtained for periods one (1) and two (2) but the difference was not statistically significant ($P > 0.05$) (Table 4).

The highest ovulatory follicle mean count was recorded for 400 mg dose in period two (2) due to an extreme observation of 22 ovulatory follicles from one cow. Mean follicle counts slightly improved for 400 mg dose from an equal response for all lower doses but not significantly. The mean ovulatory follicle counts for period three (3) was lowest (Table 4).

Table 4. Total follicular count (ovulatory and non-ovulatory ≥ 3mm) at the start of the FSH hormonal treatment and the total number of ovulatory (≥ 15mm in diameter) follicles at the end of FSH treatment through rectal ultrasound at three different periods (P1, P2 and P3) and four treatment levels of FSH experimental doses (400 mg, 320 mg, 260 mg and 200 mg) on donor dairy cattle at the University of Eldoret.

	At start of FSH treatment					**At end of FSH treatment**				
FSH Dose (mg)	**P1**	**P2**	**P3**	**Mean**	**SEM**	**P1**	**P2**	**P3**	**Mean**	**SEM**
200	10.7	10.7	7.7	**9.7**	**1.1**	5.7	8.7	4.0	**6.1**	**1.7**
260	12.3	11.0	8.0	**10.1**	**1.1**	10.0	5.7	3.0	**6.2**	**1.7**
320	12.7	11.5	8.3	**10.8**	**1.2**	8.3	6.0	5.3	**6.5**	**1.9**
400	10.0	13.7	10.3	**11.3**	**1.1**	6.0	10.0	7.3	**7.8**	**1.7**
Mean	**11.4**	**11.6**	**8.6**			**7.5**	**7.6**	**4.9**		
SEM	**0.9**	**1.0**	**0.9**			**1.5**	**1.6**	**1.5**		

Mean number of follicles observed at the start of the FSH treatment that progressed to ovulatory follicles ranged between 63% (9.7 to 6.1) for the lowest FSH dose and 69% (11.3 to 7.8) for the highest FSH dose (Table 4).

Period three (3) means were lower than those observed in periods one (1) and two (2) particularly for lower hormone doses (Table 4). The highest number of follicles at the start of the FSH treatment that progressed to ovulatory follicles (81%) was at period two (2) for 200 mg FSH hormone level (10.7 to 8.7) and period one (1) at 260 mg FSH level (12.3 to 10.0) (Table 4). The lowest number of follicles at the start of the FSH treatment that progressed to

ovulatory follicles (8.0 to 3.0) was at period three (3) for 260 mg FSH level at 38% followed by period three (3) for 200 mg FSH level (7.7 to 4.0) at 52% (Table 4).

Significance tests of fixed effects are shown in the analysis of variance (Table 5) for the two dependent variables under study. No significant hormone dose, period or interaction effect was detected ($P > 0.05$) but intercept model fit was significant ($P < 0.05$) for the two dependent variables. Least squares means for the fixed effects and interaction are presented below (Table 5).

Table 5. Analysis of variance (ANOVA) for fixed effects for two dependent variables (number of follicles (ovulatory and non-ovulatory) at the start of treatment and the number of ovulatory follicles at the end of the treatment)

Variable	Effect	Ndf	Ddf	F Value	Pr > F
Number of follicles at the start of FSH treatment	Dose	3	23	0.20	0.8975
	Period	2	23	1.01	0.3793
	Period*Dose	6	23	0.56	0.7539
Number of ovulatory follicles at the end of FSH treatment	Dose	3	23	0.47	0.7089
	Period	2	23	3.04	0.0675
	Period*Dose	6	23	0.57	0.7523

4.2. Effects of Different FSH Dose Rates on Embryos/Ova Yield and the Quality Grade

4.2.1. Embryos/Ova Yield and Quantity of Transferable Embryos

REML estimates for the covariance of observations within the same cow and residual variance for number of embryos/ova and transferable embryos flushed as well as the residual variance and statistic -2 Log L were calculated. Model fit Statistic -2 Residual Log Likelihood indicated

significant model fit for all the dependent variables (0.04953 for total embryos/ova and 0.04895 for transferable embryos) (Appendix 2).

Significance tests of fixed effects are shown in the analysis of variance (Table 6) for the two dependent variables under study. No significant hormone dose, period or interaction effect was detected ($P > 0.05$) but intercept model fit was significant ($P < 0.05$) for both dependent variables.

Table 6. Analysis of variance for flushed embryos/ova and transferable embryos

Variable	Effect	Ndf	Ddf	F Value	Pr > F
Embryos/Ova	Dose	3	23	0.24	0.8645
	Period	2	23	1.17	0.3279
	Period*Dose	6	23	0.49	0.8085
Transferable embryos	Dose	3	23	0.07	0.9764
	Period	2	23	0.62	0.5489
	Period*Dose	6	23	0.54	0.7737

Least squares means for the fixed effects and interaction are presented in Table 7. Mean number of embryos/ova flushed at FSH hormone dose 400 mg was marginally higher but did not significantly differ from the rest. Period three mean was lower than that obtained for periods one and two but the difference was not statistically significant (Table 7).

The highest mean number of embryos/ova were flushed at hormone dose 260 mg in period one (6.0) and dose 400 mg in period two (5.7) while the lowest mean yield (1.3) was observed for dose 260 mg in period three (Table 7).

Table 7. Mean number of structures and embryos resulting from the use of different FSH hormone dose levels during various periods on donor dairy cattle at the University of Eldoret farm.

	Structures					Embryos				
FSH Dose	**P1**	**P2**	**P3**	**Mean**	**SEM**	**P1**	**P2**	**P3**	**Mean**	**SEM**
200 mg (10 mL)	3.7	5.3	2.0	**3.7**	**1.1**	2.7	4.3	2.0	**3.0**	**0.9**
260 mg (13 mL)	6.0	3.7	1.3	**3.7**	**1.1**	4.3	2.7	1.3	**2.8**	**0.9**
320 mg (16 mL)	4.7	3.0	2.3	**3.3**	**1.2**	3.7	2.5	2.0	**2.7**	**0.9**
400 mg (20 mL)	3.7	5.7	4.7	**4.7**	**1.1**	2.3	3.7	3.7	**3.2**	**0.9**
Mean	**4.5**	**4.4**	**2.5**			**3.3**	**3.3**	**2.3**		
SEM	**1.0**	**1.1**	**1.0**			**0.7**	**0.8**	**0.7**		

Mean number of transferable embryos flushed was also not significantly different at all FSH hormone dose levels (Table 7) as was for the mean number of embryos/ova. Period three mean number of transferable embryos was lower than that obtained for periods one and two but the difference was not statistically significant (Table 7). The highest mean number of transferable embryos was flushed at FSH hormone dose 200 mg in period two (4.3) and dose 260 mg in period one (4.3) while the lowest mean yield of transferable embryos flushed was observed for hormone dose 260 mg in period three (1.3) (Table 7).

4.2.2. Quality of Flushed Embryos based on Different FSH Dose Rates

REML estimates for the covariance of observations within the same cow and residual variance for number of flushed embryos in various quality grades as well as the residual variance and statistic -2 Log L were calculated. Model fit Statistic -2 Residual Log Likelihood indicated significant model fit for all the dependent variables. Significance tests of fixed effects are shown in the analysis of variance table 8 for the three dependent variables. No significant hormone dose, period or interaction effect was detected ($P > 0.05$) but intercept was significant ($P < 0.05$) only for grade 1 embryos (Table 8).

Table 8. Analysis of variance for the quality of flushed embryos from experimental donor cows at the University of Eldoret farm based of different dose levels of FSH treatment.

Variable	Effect	Ndf	Ddf	F Value	Pr > F
G1	Dose	3	23	0.16	0.9207
	Period	2	23	0.73	0.4906
	Period*Dose	6	23	0.35	0.8997
G2	Dose	3	23	0.25	0.8625
	Period	2	23	0.75	0.4855
	Period*Dose	6	23	1.44	0.2439
UF	Dose	3	23	0.90	0.4584
	Period	2	23	2.07	0.1485
	Period*Dose	6	23	0.29	0.9370

G1 = grade 1 embryo, G2 = Grade 2 embryo and UF = unfertilized ova

Least squares means for the fixed effects and interaction for quality of embryos flushed are presented in Table 9. Mean number of grade one embryos flushed did not significantly differ with hormone dose or period of study ($P > 0.05$).

Table 9. Mean number of grade one and grade two embryos and unfertilized ova resulting from the use of different FSH hormone dose levels at different periods on donor cows at the University of Eldoret farm.

	Grade 1					**Grade 2**					**Unfertilized**				
Dose (mg)	**P1**	**P2**	**P3**	**Mean**	**SEM**	**P1**	**P2**	**P3**	**Mean**	**SEM**	**P1**	**P2**	**P3**	**Mean**	**SEM**
200	2.3	3.3	1.7	2.4	**0.7**	0.4	0.9	0.3	0.5	**0.2**	1.0	1.0	0.0	0.7	**0.4**
260	3.0	2.7	1.0	2.2	**0.7**	1.3	0.0	0.3	0.5	**0.2**	1.7	1.0	0.0	0.9	**0.4**
320	2.7	2.0	1.3	2.0	**0.7**	1.1	0.6	0.6	0.8	**0.2**	1.0	0.5	0.3	0.6	**0.4**
400	2.0	3.0	3.0	2.7	**0.7**	0.4	0.6	0.7	0.6	**0.2**	1.3	2.0	1.0	1.4	**0.4**
Mean	**2.5**	**2.8**	**1.8**			**0.8**	**0.5**	**0.5**			**1.3**	**1.1**	**0.3**		
SEM	**0.6**	**0.6**	**0.6**			**0.2**	**0.2**	**0.2**			**0.3**	**0.4**	**0.3**		

Fewer mean number of grade two embryos was flushed and no significant difference was observed across hormone levels or period of study (Table 9). No significant difference was observed in mean number of unfertilized ova flushed across hormone levels or period of study ($P > 0.05$) (Table 9). However, most of the zero responses by donor dairy cows at the University of Eldoret were recorded during study period 3 hence the lower average (Table 9).

4.3. Adoption of Embryo Transfer Technology

The study established that more than 50% of respondents in Uasin Gishu County practice extensive dairy cattle rearing system compared to only 10% of those in Trans Nzoia County. Two thirds (2/3) of the respondents practice semi-intensive system of production in Trans Nzoia compared to one third (1/3) in Uasin Gishu. Only 8% of respondents in Uasin Gishu and 12% in Trans Nzoia County use intensive system of livestock production (Appendix 3).

Almost one-third (1/3) of the respondents in Trans Nzoia County keep a mixed herd of Friesians and Ayrshires compared to 24% of those in Uasin Gishu. 25% of farmers in Uasin Gishu keep only Friesians. Sole Ayrshire cattle herds were reared by 21% of respondents in Uasin Gishu and 24% in Trans Nzoia. 21% of farmers in Trans Nzoia keep cross breed cattle compared to 28% in Uasin Gishu. Most respondents, 93% in Uasin Gishu and 74% in Trans Nzoia are smallholder dairy cattle farmers keeping less than 20 dairy cattle (Appendix 3).

In Uasin Gishu County, only 9% of the respondents have registered their animals with Kenya studbook compared to 23% in Trans Nzoia County mainly due to lack of awareness (62% in Uasin Gishu) and unavailability of the service (49% in Trans Nzoia). More than 70% of respondents in both counties sell heifers at price range of Ksh50,000 to 100,000. These are dairy cross heifers as kept by the farmers and ranged from 6 months old to in calf heifers. The

majority of the respondents; 54% in Uasin Gishu and 62% in Trans Nzoia use conventional artificial insemination (AI) while 40% in Uasin Gishu and 9% in Trans Nzoia used both conventional and sexed semen (Appendix 3).

Out of 385 respondents, only 293 provided the relevant data that was used for this study. A total of 143 farmers from Uasin Gishu and 150 from Trans Nzoia Counties were successfully interviewed to ascertain their perception on multiple ovulation and embryo transfer (MOET) and factors that may hinder the adoption of the technique in Kenya. The results are presented in Table 10, Figure 20 and Appendix 3.

Table 10. Proportions p (%) of respondents in Uasin Gishu and Trans Nzoia Counties that attributed none-use of MOET to various independent factors: Cost of MOET, lack of experts, Lack of awareness or unavailability of the technology

Factor	**Uasin Gishu**		**Trans Nzoia**	
	P (%)	SE(p)	P (%)	SE(p)
Cost of MOET	6.3	2.0	12.7	2.7
Lack of experts	28.0	3.8	31.3	3.8
Unawareness	58.7	4.1	46.7	4.1
Unavailability	7.0	2.1	9.3	2.4
Total	100.0		100.0	

The non-use of MOET by the respondents was mainly attributed to lack of awareness of the existence of the technique as shown by 58.7% (84 respondents) in Uasin Gishu County and 46.7% (70 respondents) in Trans Nzoia County) (Table 10, Figure 20). Lack of experts to carry out the procedure was also cited as the reason for the poor adoption of MOET by 28% and 31.3% of the respondents in Uasin Gishu and Trans Nzoia Counties respectively (Table 10).

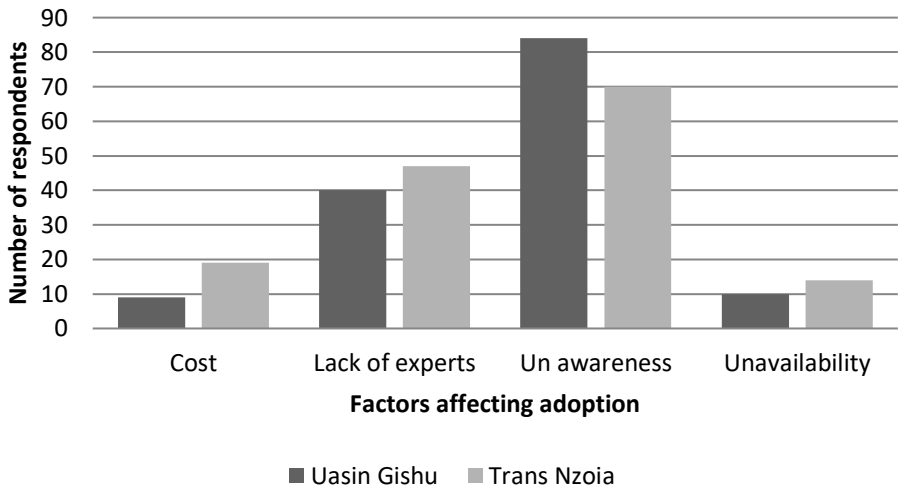

Figure 23. Factors affecting adoption of MOET in Uasin Gishu and Trans Nzoia Counties; cost of technology, lack of experts, lack of awareness and unavailability of the technology.

Only a few respondents cited the cost as a reason for the slow adoption of the technique (Table 10). This could have been due to the fact that they have not used MOET hence were not aware of the cost implications. However, majority of the respondents would adopt MOET if it was made available (72% in Uasin Gishu and 79% in Trans Nzoia Counties respectively). More than 60% of respondents in both counties bred their own replacement heifers and only 40% outsourced the heifers from other breeders.

The results of Kendall's Tau correlation analysis among the factors that influence adoption and respondents' willingness to adopt embryo transfer if availed is given in Table 11. Values below the diagonal pertain to Uasin Gishu and those above diagonal to Trans Nzoia Counties. The test was carried out at 99% confidence level and showed the correlations were significant as shown on Table 11.

Table 11. Kendall's tau Correlation matrix for factors affecting adoption of MOET by dairy cattle farmers in Uasin gishu and Transzoia Counties in Kenya.

	Lack of awareness	**Not available**	**Lack of Experts**	**Costly**	**Adoption If available**
Lack of awareness		-.356**	-.632**	-.300**	-.084
Not available	-.309**		-.257**	-.122	.194**
Lack of experts	-.744**	-.162**		-.217**	.345**
Costly	-.327**	-.071	-.171**		-.629**
Adoption if available	-.237**	.162**	.388**	-.379**	

** Correlation is significant at the 0.01 level (2-tailed).

4.4. Current Situation on Embryo Transfer in Kenya

The mean number of embryos flushed per donor during different periods of the year at Sasini farm in Nyeri County and ADC Namandala farm in Transzoia County, the two organizations that were chosen for the study, are shown below (Table 12 and Appendix 4). There were variations in the number of embryos produced among the donors, between the two farms and also between the different periods (Table 12).

Table 12. Mean number of embryos per donor harvested at Sasini farm and ADC Namandala farm at different periods between April 2013 and August 2015.

Sasini				**ADC**			
Period	**No. of donors**	**Average Embryos**	**SE**	**Period**	**No. of donors**	**Average Embryos**	**SE**
Oct 2014	11	2.1	1.6	Apr 2013	12	1.9	0.8
Dec 2014	13	5.8	2.3	Sept 2013	15	1.5	0.5
Feb 2015	17	5.9	1.3	Oct 2014	18	2.3	1.0
May 2015	15	6.7	1.7				
Aug 2015	11	1.8	0.4				
Mean		**4.5**	**1.6**	**Mean**		**1.9**	**0.8**

Harvesting of embryos was carried out by the same team of technical personnel on both farms. As previously described, the two farms practiced different donor management programs. Sasini

farm donor cows were on total mixed rations and fully confined whereas donor cows at ADC Namandala farm were on semi intensive system with grazing during the day and supplementation at milking.

The results of embryo production carried out at Sasini farm shows that one third (1/3) of the donors produced zero embryos despite the animals being inseminated after expressing heat signs. Another one third (1/3) produced most of the embryos harvested while the final one third (1/3) produced one or two embryos.

CHAPTER 5

5.0. DISCUSSION

5.1. Number of Follicles and Embryos/Ova

The research was carried out to monitor the ovarian follicular changes occurring during the process of donor cow super ovulation and sought to understand the dynamics of the use of different levels of FSH on ovarian follicular changes and embryo yield. The research showed that the lower dose level of FSH of 200 mg produced similar results compared to the higher dose levels of 400 mg.

All donors responded well to gonadotrophin and at the end of the treatment, all the donors had increased number of ovulatory follicles. Donor cows at recruitment and selection phase of the follicular wave of the ovarian cycle had more ovulatory follicles compared to the ones that were at the dominant phase as recorded through ultrasound technique. Ultrasonography played an important role in monitoring and evaluation of the ovarian follicular changes and subsequently the formation of *corpora lutea*.

5.1.1. Number of Follicles

Despite the large numbers of ovulatory follicles, ranging from 7.7 to 13.7, as observed through ultrasound technique, not all follicles ovulated. The proportion of ovulatory follicles that ovulated as indicated by the number of *corpora lutea* in the ovaries observed through ultrasound technique and manual rectal palpation ranged between 54% for 260 mg of FSH treatment to 62% for the 400 mg of FSH treatment. 38% to 46% of ovulatory follicles as seen through ultrasonography failed to ovulate.

Donor cows whose phases were at recruitment or selection during the ovarian follicular wave as recorded through ultrasonography had the largest number of ovulatory follicles at the end of FSH treatment across the four dose rates. Those donors that were at the dominance phase of the ovarian follicular wave had fewer ovulatory follicles. This was as a result of the dominant follicle exerting negative feedback influence on production of FSH and the fact that a number of the follicles in the cohort had begun to undergo follicular atresia and hence could not be rescued by the administration of the external FSH at that point in time.

Use of ultrasonography therefore plays a critical role and FSH treatment of donor cows at dominance phase may be postponed for a short period to allow loss of dominance for better ovarian follicular response to be achieved and hence improved number of embryos harvested.

It has been shown that despite the advances in super ovulation, there has been less appreciable increase in the number of harvested embryos per donor during a MOET program (Arendonk and Bijma, 2003; Lamb, 2012; Hasler, 2014). Research needs to be carried out to ascertain the cause of the non-ovulation of some of the ovulatory follicles and ways for improvement of the ratio of ovulated follicles. The findings will be of great value in the improvement of the number of embryos per donor.

One third of the donors failed to ovulate, though they showed heat signs on expected dates and time; ovarian ultrasonography and manual rectal palpation returned zero number of *corpora lutea* but ovulatory follicles were still present in the ovaries of those donors. One third of the donors ovulated with few *corpora lutea* of between one and three being recorded and these donors still had some ovulatory follicles, while the other one third responded to the FSH

treatment well with more than three *corpora lutea* recorded. Mapletoft (2012) had observed such a trend in several donor cow super ovulations.

The reasons for such variation in ovulation are not known but may be due partly to animal factors or hormone drug formulation especially the quantity of luteinizing hormone. Luteinizing hormone plays a critical role in ovulation process hence low levels or lack of it will result in poor ovulation hence low embryo recovery rates. The FSH treatment used (Folltropin®-V, manufactured by Bioniche Animal Health, Canada) has LH in low dose in the drug formulation. This is the FSH formulation being used by most MOET practitioners worldwide and is a popular brand in Kenya. It should also be noted that, though two thirds of the donors ovulated the number of *corpora lutea* formed in the ovary were highly variable amongst the donors.

These failures by some donors to ovulate is a challenge in super ovulation because they could not be predicted by the use of ultrasonography technique for scanning the ovaries. The failure to ovulate despite the growth of these follicles to ovulatory stage and subsequent turning on of heat signs should be investigated. Further research is needed to ascertain the cause and mitigation strategies to further optimize embryo recovery especially the quantities of luteinizing hormone in the drug formulation used in super ovulation.

5.1.2. Embryos/Ova Yield

The expected embryo yield based on ultrasound scan of ovaries and subsequent count through rectal palpation of the corpora lutea formed showed an average of 6.1 to 7.8 embryos. This was however not realized in actual mean number of embryos/ova flushed (3.3 to 4.7). Only 50 – 60% of the expected embryos/ova were flushed. Lack of regular practice in embryo flushing

and searching skills may have let to lower embryos/ova recovered. Regular practice is needed for consistent embryo production but this has been hindered by lack of affordable FSH hormone retailing at USD 150 per donor. Improvement of technical skills is needed if we are expected in Kenya to meet international standards. Most embryos/ova (over 40%) could have been left within the uterus hence leading to perceived high production costs. Technician skills have been shown to be among the reasons for the low embryo recovery / yield (Arendonk and Bijma, 2003; Hasler, 2004; Lamb, 2012; Mapletoft. 2012).

The average number of embryos flushed was 2.9 per donor which is lower than those harvested in the United States of America; 6 (Hasler, 2014) and Brazil; 4.1 - 7.3 (Peixoto *et al*., 2006) but higher than those of Ethiopia; 2.07 (Tadesse, 2016). This could be attributed to lack of experienced personnel in embryo flushing or animal factors. There has been little incentive among the private Veterinary professionals in Kenya to take up and disseminate this technology.

Most Veterinarians view embryo transfer as complicated and less rewarding due to variable embryo yield. The inputs especially the hormone used for superovulation is expensive (USD 150 per donor) and not readily available hence complicating the situation. Lack of regular post graduate training program on MOET in our Institutions of higher learning or other organizations involved in animal breeding for those interested in this technique further compounds the problem.

Most of the embryos flushed were of grade one quality across the different levels of FSH and seasons. Different FSH levels had no influence on the quality of embryos produced. More

unfertilized ova observed were at the higher level of FSH. There was no documented evidence on research to ascertain the influence of different FSH dose levels on quality of embryos.

5.1.3. Effect of Season

Period three (July / August) had the lowest proportion of follicles that ovulated at 50% compared to period two (April / May) which had the highest proportion at 63%. Season has been shown to influence embryo yield (Arendonk and Bijma, 2003; Lamb 2012; Mapletoft, 2012). The donor cows should be kept warm through proper housing facility during colder periods if optimum embryo production is to be realized. Donor cows in the current research were kept in the open field conditions as is always the case in most farms in Kenya without temperature regulators or heaters.

There were differences in ovulatory follicles recorded at different periods with the lowest being period three which was the period of July - August. This is the coldest and wettest period of the year in Kenya and affected the number of ovulatory follicles. This may be attributed mainly to cold stress affecting the animals since they were not housed and were prone to changes in environmental conditions.

Such periods should be avoided or donor animals kept warm for optimum production of embryos. Results from superovulation of donor cows at Sasini and ADC Namandala farms in Kenya also showed similar trends. Those donor cows super ovulated between the period of June through August at both farms had low response and hence poor embryo recovery as was also observed in the current study. This is the first time that seasonal influence has been documented in Kenya and may be of significant consideration in future embryo production

programs. The cold stress may affect the response to gonadotrophin hormone hence the observed poor response. Synchronization was otherwise effective.

An average of three embryos per donor was recovered during this research, although there was a potential of more than six embryos as assessed by the number of *corpora lutea* detected by the ultrasound images and manual rectal palpation. The number of embryos recovered was similar to those of Sasini farm but more than those harvested at ADC Namandala farm; both are located in Kenya.

One third of the donors failed to ovulate, another third produced 1 – 3 embryos while a third produced over three embryos. The season, technique, super ovulation protocol and animal factors were shown to influence embryo production and recovery. The low adoption of embryo transfer was associated with inadequate sensitization on embryo transfer, technology not readily available in Kenya, high cost of embryo production and few professionals trained to handle the MOET process.

5.2. Adoption of MOET

The study evaluated factors that contribute to the low utilization of MOET in Uasin Gishu and Trans Nzoia Counties in Kenya. MOET programme, though an important tool in animal breeding for livestock improvement is hardly used in the country. Most dairy cattle farmers interviewed were not aware of the existence of embryo transfer technique in Kenya. This was attributed to the fact that most of the farmers interviewed were small scale practicing either extensive or semi intensive form of dairy farming. There was also no documented literature to evaluate factors that may influence adoption of MOET in Kenya. None of the respondents in this study had used MOET to improve their livestock.

It has been shown that farm size, age of the farmer and type of farming system has influence in the adoption of new technologies (Howley *et al*., 2012; Gillespie *et al*., 2014). Large scale dairy farmers are most likely to embrace newer technologies compared to small scale farmers. Large scale farmers have the desire to maximize production hence their profits compared to the small scale farmers (Howley *et al*., 2012; Gillespie *et al*., 2014). In the current study, it has been clearly demonstrated that farmers in extensive and semi intensive production systems with small farm sizes have not adopted embryo transfer technology for improvement of their dairy cattle despite its existence for over the last 30 years (Kios *et al*., 2013).

There are only a few large-scale dairy cattle breeders who have attempted the use of MOET with variable success (Kios *et al*., 2013). Dairy cattle farming in Kenya are in the hands of smallholder farmers who keep one to less than twenty dairy cows (KNDMP, 2010). Only a few of the dairy cattle farmers are classified as large scale. Smallholder dairy cattle farmers will hardly search for newer technologies for improvement of their dairy herds in the market place. They depend solely on government, cooperative societies and nongovernmental organizations for extension services (Howley *et al*., 2012; Gillespie *et al*., 2014).

Small scale farmers comprise 90% of the total number of dairy cattle owners and contribute approximately 80% of the total milk production in Kenya (KNDMP, 2010). The subsector has failed to attract the youth since it's considered dirty and laborious. This has contributed to low adoption of technologies such as embryo transfer since most of the aging farmers may not have access to information on the newer techniques in dairy cattle production. Khanal and Gillespie (2013) have shown that younger farmers in specialized farms have higher chances of adopting newer technologies because of the long planning horizons. Small scale farming is considered

the backbone of the dairy industry in Kenya (KNDMP, 2010) unlike in developed countries where the production is in the hands of a few large scale farmers.

Small scale farmers keep dairy cattle characterized by low production of milk per cow per day. Due to the low production, most of the farmers may be categorized as subsistence milk producers. Any technique that will lead to improvement of their herds has the potential of being adopted if it is affordable as clearly shown in this study. This will lead to efficient milk production hence improved food security and wealth creation.

Most donor funded programs have been directed to small scale dairy cattle farmers in Kenya but little progress has been registered especially on daily average production of milk per cow due to low adoption of technologies as shown in this study. Such programs include but not limited to Agriculture Sector Development Project, East Africa Agricultural Productivity Project, Smallholder Dairy Cattle Commercialization Project and Kenya Agricultural Productivity Project among many that have been developed to improve production. The slow progress has been attributed to lack of awareness of existence of these technologies and availability of technicians to perform the procedures as indicated by the results of the survey during this study.

Lack of awareness as shown in this study, may have contributed to the slow adoption of embryo transfer technology in Kenya therefore leading to the high demand for breeding heifers with resultant stiff rise in prices. The prevailing prices of dairy in-calf heifers from many breeders range from Ksh. 100,000 to 300,000 as shown in the study. The low priced heifers were mainly cross breeds but the pure breed dairy in calf heifer prices were higher. These heifers are unaffordable and inaccessible to many small-scale dairy cattle farmers. These group of farmers

are likely to embrace the embryo transfer technology provided adequate sensitization is done and the issue of affordability is mitigated on. There is urgent need to build and improve the existing embryo transfer capacity and infrastructure for meaningful adoption to be realized.

In this study, there was positive correlations between adoption and unavailability of the technology or lack of expertise. This means that as embryo transfer is made available, many farmers will be willing to adopt the technology. When experts are availed, many farmers will adopt the technology. The cost of embryo transfer was however not presented to the farmers hence most of them may not be aware of the cost implication of such a technology. This shows that farmers are willing to adopt the technology to rapidly upgrade their dairy herds if made available and affordable.

5.3. Current Embryo Transfer Technology Situation

The study was carried out to evaluate the super ovulation protocol in current use and donor management practice in relation to embryos harvested. The results from analysis of past embryo recovery shows large variation in embryo output across the two farms, seasons and among the donors. The recovery rate of embryos was low at ADC Namandala farm compared to that of Sasini farm as clearly shown by the results of this study.

The difference in the two means of embryos harvested in the two farms under study was significant ($p \leq 0.05$). This may be attributed to one or more of the many factors which have been shown to affect embryo output including; donor management type, nutritional status of the donor cows, body condition score at the start of the program, season and technical competence of persons carrying out the embryo recovery (Mollo *et al*., 2007; Arendonk and Bijma, 2003; Lamb, 2012; Mapletoft, 2012).

The embryo production at ADC Namandala and Sasini farms were carried out using the same super ovulation protocol explained and team of experts. The variation observed could therefore be mainly due to donor cow management program and nutrition within the two farms before and during the embryo production. Donor cows at Sasini farm were heavier with better diet through the total mixed ration fed compared to those of ADC Namandala farm (Personal communication with Dr. Maurice Cherogony, E.O. LGS 2015). Mollo *et al*. (2007) and Mapletoft (2012) have shown that nutrition and donor management has influence on the quantity of embryos harvested.

Donor cows under zero grazing (intensive) management system of production at Sasini farm showed better embryo production as compared to those on semi intensive management system as practiced in ADC Namandala farm. Less movement of the donor cows and improved feeding regime plays an important role in a MOET program as shown clearly in the results of this study. Donor preparation therefore remains critical to the success of the super ovulation program.

The season of the year has influence on embryos harvested in Kenya as shown from the results of this study. There was low embryo production between the month of July through to October as observed from the results of Sasini farm, ADC Namandala farm and at the University of Eldoret farm. The low embryo output could be attributed to the heavy rains and colder weather condition compared to the other months of the year in Kenya.

Such weather condition may lead to stress of the donors who are mainly kept outdoors. Therefore, the effects of such environmental changes led to the low embryo output as clearly demonstrated in the study. Peixoto *et al*. (2006), has also shown that different months of the

year had effect on embryo production in Brazil Zebu cattle. Other ET practitioners have also demonstrated the effects of season on embryo production in different parts of the world (Mapletoft, 2012).

Most practitioners in Kenya may not be aware of the seasonal influence on embryo production due to lack of publication of the findings of most embryo production programs carried out in the country. Little has been documented on embryo recovery rate in Kenya and conventional embryo recovery and transfer has been carried out sparsely. MOET practitioners should therefore be encouraged to analyze their results and publish for the purpose of sharing experiences to improve on the technique.

The East Africa Semen and Embryo Transfer Association (EASETA) was formed to help coordinate MOET activities in Kenya and later the Eastern Africa region. Due to lack of funding, the association has not been able to regulate, publish embryo production and transfer results nor help coordinate the conduct of regular embryo transfer programs.

Though there is high demand for superior breeding stock in Kenya, it has not translated into more frequent embryo transfer programs. The low utilization of MOET has been contributed by the lack of a critical number of trained personnel to carry out the activity and also the high embryo output variability as seen in the two farms under study. Embryo transfer has the potential to influence future breeding programs in Kenya (Mutembei *et al.*, 2015). With the rising demand for replacement heifers in Kenya, there is need to make use of the embryo production and transfer technique available (Kios *et al.*, 2013). It's only with regular use of the technique that will lead to reduction in variability of embryo output.

The lower dose level of FSH will lead to a reduction of the cost of embryo production by approximately one quarter without compromising the quantity and quality of the harvested embryos in Kenya. The high cost of embryo production remains a key factor in the low levels of adoption and utilization of this novel technology for the improvement of dairy cattle herds. The cost of super ovulation is USD 250 (Ksh. 25,000) (Mapletoft, 2012), of which FSH costs Ksh. 15, 000 (USD 150) hence there is a reduction of USD 75 (Ksh. 7,500) per donor if super ovulation is carried out using 200 mg of FSH instead of the current 400 mg per donor.

Embryo production and transfer is perceived as expensive, hence the low adoption rate. This has led to infrequent, rarely used technique that is seldom utilized by the animal breeders in Kenya. Though it's a technique that is frequently used in developed countries and a few developing countries, it's hardly a tool of choice in most Sub Sahara Africa. Embryo output during this research had high variability with only one third of the donors producing most of the embryos and another one third yielding zero embryos. Lower FSH doses rate of 200 mg per donor cow had similar response to the higher dosage rate of 400 mg per donor cow currently in use in Kenya.

Embryo transfer therefore remains the most viable option provided that the correct super ovulation protocol is employed together with relevant training and experience. The use of inappropriate super ovulation protocol together with poor techniques and cold season may lead to poor response and low embryo recovery rates. The practitioners need to perfect the embryo harvesting techniques and sensitization of dairy cattle breeders to adopt and utilize the technique for faster dairy cattle improvement.

CHAPTER 6

6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

Based on the current research; observed follicular response, number of corpora lutea formed and actual quantity and quality of embryo output, the experimental model using 200 mg of follicle stimulating hormone (FSH) emerged the most appropriate for use in Kenya. Therefore, a FSH dose rate of 200 mg per donor is recommended. The higher dose rate of 400 mg per donor had the disadvantage of increased cost of embryo production. The use of 200 mg of FSH per donor cow during super ovulation may encourage more embryo transfer practitioners in Kenya to mount regular superovulation programs.

Optimization of embryo output will reduce variability and increase the utilization of the embryo transfer technique. Identification of donor cows that consistently produce high numbers of embryos both in quantity and quality is key for optimum embryo output. This is possible if recording, data analysis and sharing is encouraged amongst practitioners and other consumers.

Breeders as well as commercial milk producers should be encouraged to invest on regular embryo transfer. This will complement the artificial insemination services and reduce the need to purchase live animals from other countries. The regular use of embryo transfer will ease the prevailing high demand for breeding stock leading to improved affordability and accessibility of high quality replacement heifers.

The prohibitive cost of replacement heifers produced by the few large scale dairy cattle breeders continues to hinder envisaged milk production in the subsector. This has led some farmers to attempt importation of livestock from South Africa and Netherlands. The importation has its own challenges which include: high cost of purchase and transportation,

disease threat, effects of genotype x environment interaction on milk production. The use of overland alternative route to reduce cost of transportation has been tried with detrimental effect especially losses due to abortion and failure by the animals to adapt quickly to the new environment.

Most farmers interviewed had no idea of the existence of the embryo transfer technology. There is opportunity for the Universities in Kenya and other animal breeding organizations including; University of Nairobi and Kenya Animal Genetic Resources Centre (KAGRC) to provide leadership in training and dissemination of the embryo transfer technology. With devolution, the county governments were tasked with dissemination of information to farmers since agriculture is a fully devolved function. Unfortunately, most of the officers in service involved with dissemination of embryo production and transfer may not be fully equipped with knowledge and skills for the purpose hence the need for Universities and organizations involved in animal breeding to be in the lead to provide the service.

The high percentage of farmers willing to adopt the embryo transfer technology for rapid improvement of the livestock provides the much needed impetus for the utilization of embryo transfer technique. Despite embryo transfer technology being with us for the past 40 years, there has been low utilization in most developing countries compared to the developed ones. For Kenya to be food secure, there is need to empower the farmers to use modern technologies for food production. The study showed clearly that; sensitization of the farmers on the available assisted reproductive techniques and their potential is the first step on the road to achievement of food security as envisioned in the Big Four agenda, Vision 2030 and sustainable development goals (SDGs).

The multiple ovulation and embryo transfer technique has become more crucial especially in the production of sires for use in artificial insemination. It ensures that every cow contracted for sire production produces a male calf instead of the 50:50 ratios when using conventional methods. This has been necessitated by the shrinkage of the number of breeders for contract mating scheme for sire production due to sub division of the farm lands and diversification to other enterprises perceived to be more lucrative by the farmers. Many top sires in developed countries have been produced through MOET and it's a possibility in Kenya and other developing countries. Through this research, the lowered cost of embryo production will encourage bull stations to adopt the technology to produce high quality sires that will be used by the Kenyan farmers for improvement of their livestock.

The combination of regular embryo transfer program together with artificial insemination using conventional and gender selected semen may provide the much-needed impetus to improve the productivity of the dairy cattle in the hands of small holder dairy cattle farmers in Kenya. Through this research, a FSH super ovulation protocol was described that may address the high variability in the embryo output, reduce the cost of embryo production and increase the rate of adoption of the technology.

The cost of super ovulation is approximately Kenya shillings (KSh.) 25,000 per donor cow. FSH accounts for 60% of this cost at Ksh. 15,000 for 400 mg (20 mL) dose rate being used currently in the super ovulation programs in Kenya. This is the single most limiting factor that contributes to the high cost of conventional embryo production. The high embryo yield variability and poor recovery rates compound the already high cost of production. This has discouraged most would be embryo transfer practitioners from adopting this technology to ensure regular embryo production.

6.2. Recommendations

1. Super ovulation of donor dairy cows in Kenya responded well to the protocol based on 200 mg of follicle stimulating hormone (FSH). It's therefore the most appropriate dose rate for cattle kept in the tropics due to their small to medium sized bodies and physiological needs. This dose rate has also optimal returns due to lowered cost of production of embryos. This will translate to lower cost of embryo transfer technology and improved uptake by dairy cattle breeders. Dairy cattle farmers will benefit from lowered cost and availability of in calf heifers in the market.

2. Use of ultrasound technology is critical for production of embryos in conventional MOET program. This is important in monitoring the effectiveness of synchronization and super ovulation and moreso appropriate time for introduction of FSH hormonal treatment for cattle. Donor cattle that respond poorly to super ovulation as shown by the few ovulatory follicles recorded through ultrasonography may not be flushed to reduce on costs unless the donor has unique genotype that must be harnessed for future use. Donor cattle that repeatedly fail to ovulate despite responding well to super ovulation as shown by ultra sound scanning should not be considered in future embryo production programs to reduce on variability of embryo yield, cost of production and the overall cost of embryo transfer.

3. Super ovulation of donor cows should be carried out during the warm seasons / period unless there are contingency plans to keep the donor cattle warm during the cold seasons. This will avoid the low embryo yields due to stress associated with low temperatures as demonstrated in this study. The donor cows use most of the energy from the feed to generate enough heat to keep warm at the expense of reproduction.

4. Training and nurturing several teams of embryo transfer practitioners in the country is crucial to be able to carry out regular MOET programs. This will lead to increased efficiency and reduction in variability of embryo yield resulting from poor technique. Due to the high cost of training of embryo transfer experts since it heavily relise on expensive practicals it has been difficult for most institutions to have regular course.

5. Data on work carried out by embryo transfer personnel in most developing countries and Kenya in particular should be documented, analyzed and published to help on the development of intervention strategies to improve on the embryo transfer technique. The public documentation of such analysed MOET data is critical for future decisions on improvement of this technique. Most countries with regular embryo transfer as part of their livestock improvement strategy have published their work hence it is easy to solve or develop intervention strategy on any challenges encountered. This is possible and needs support by organizations involved in livestock genetic improvement especially the Kenya Livestock Breeders Organization who are beneficiaries of a functional embryo transfer technology.

6. There is need to ascertain the cause of failure by some donor cows to ovulate despite the observed ovarian follicular growth as recorded through ultrasonography technique. It is suspected that low levels of luteinizing hormone (LH) may be the main cause of ovulation failure and hence the need to introduce LH immediately after FSH treatment. Further research is needed in this area to provide intervention strategies to optimize embryo production in future.

7. Creation of awareness amongst dairy cattle breeders in Kenya on the availability of embryo transfer in the country as an alternative reproductive technology for improvement of their breeding stock should be carried out. The adoption of the embryo transfer technology will lead to self-sustenance in provision of the much needed high quality replacement stock. This will alleviate the high cost of in calf heifers currently being witnessed in the country hence a direct benefit and relieve to the dairy cattle farmers in the country.

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8.0. APPENDICES

8.1. Appendix 1. Questionnaire for data collection on multiple ovulation and embryo transfer (MOET) in Kenya

Breeder's details

1. Name of the farmer / farm....……
2. Physical address……..County.....……
3. Telephone No…….……..
4. Type of dairy enterprise: (a) extensive (b) intensive (c) semi intensive
5. No. of animals……Breed.....……
6. Have you registered your animals? (a) yes (b) no
7. If no, why? (a) expensive (b) not available (c) not aware of its existence (d) no reason
8. Do you sale animals to other farmers? (a) yes (b) no
9. If yes, at what price (Ksh.)? (a) <50,000 (b) 50,000 – 100,000 (c) 100,000 – 150,000 (d) 150,000 – 200,000 (e) >200,000

Multiple Ovulation and Embryo Transfer (MOET) and other Breeding Techniques

10. Do you use: (a) artificial insemination (A.I) (b) sexed semen (c)embryo transfer (d) all
11. Have you heard of MOET? (a) yes (b) no
12. If yes, have you used it to improve your dairy cattle? (a) yes (b) no
13. If yes, what is your experience with MOET? (a) excellent (b) good (c) fair (d) poor
14. How often do you use MOET? (a) frequent (b) rarely (c) occasionally (d) very rare
15. What is the major obstacle to the use of MOET? (a) cost (b) lack of experts (c) lack of information about MOET (d) unavailability
16. How long have you used MOET? (a) > 15 yrs (b) 10 – 15 yrs (c) 5 – 10 yrs (d) 0 – 5 yrs

17. What influenced your decision to use MOET? (a) need to improve stock (b) demand for heifers (c) hobby (d) others (specify)

18. If MOET is made available, would you use it? (a) yes (b) no (c) not sure

19. If no, why? (a) cost (b) religious reasons (c) cultural believes (d) no reason

20. How do you view MOET? (a) GMO (b) breeding technique (c) foreign (d) no views

21. Do you know any farmer who uses MOET? (a) yes (b) no

22. What do you thing needs to be done for farmers to adopt MOET?: .....

.....

.....

23. Where do you get heifers from?.....

24. Are the Veterinarians available to carry out embryo transfer: .....

.....

8.2. Appendix 2. Total embryos / ova

Appendix 2.1. FSH Experiment Embryos Total 1

20:40 Thursday, February 16, 2016

The Mixed Procedure

Model Information

Data Set	SASUSER.D2
Dependent Variable	ET
Weight Variable	WT
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Satterthwaite

Class Level Information

Class	Levels	Values
COW	18	Anniversary 493 Certificate 431 Cherop 411 Dikir Dona 540 Esnuz Expert 553 Eznus Holiday 471 Larry 473 Lelgon 492 Manu U 447 Mildred Moi Mureno 531 Sotik 440 Tulwo 421 Winner 389
PD	3	1 2 3
DS	4	10 13 16 20

Dimensions

Covariance Parameters	2
Columns in X	20
Columns in Z	35
Subjects	1
Max Obs Per Subject	35

Number of Observations

Number of Observations Read	35
Number of Observations Used	35
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	154.43045996	
1	2	153.59259459	0.00000010
2	1	153.59258681	0.00000007

The Mixed Procedure

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
3	1	153.59258163	0.00000005
4	1	153.59257817	0.00000003
5	1	153.59257587	0.00000002
6	1	153.59257434	0.00000001
7	1	153.59257331	0.00000001

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate	Alpha	Lower	Upper
COW(PD)	26.6956	0.05	16.1256	52.5306
Residual	0.03191	0.05	.	.

Fit Statistics

-2 Res Log Likelihood	153.6
AIC (smaller is better)	157.6
AICC (smaller is better)	158.2
BIC (smaller is better)	160.7

Solution for Fixed Effects

Effect	PD	DS	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha
Intercept			7.3333	2.9830	23	2.46	0.0219	0.05
DS		10	-3.3333	4.2187	23	-0.79	0.4375	0.05
DS		13	-4.3333	4.2187	23	-1.03	0.3150	0.05
DS		16	-2.0000	4.2187	23	-0.47	0.6399	0.05

Solution for Fixed Effects

Effect	PD	DS	Lower	Upper
Intercept			1.1624	13.5042
DS		10	-12.0603	5.3936

DS		13	-13.0603	4.3936
DS		16	-10.7270	6.7270

The Mixed Procedure

Solution for Fixed Effects

Effect	PD	DS	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha
DS		20	0	.	.	.	.	.
PD	1		-1.3333	4.2187	23	-0.32	0.7548	0.05
PD	2		2.6667	4.2187	23	0.63	0.5335	0.05
PD	3		0	.	.	.	.	.
PD*DS	1	10	3.0000	5.9661	23	0.50	0.6199	0.05
PD*DS	1	13	8.3333	5.9661	23	1.40	0.1758	0.05
PD*DS	1	16	4.3333	5.9661	23	0.73	0.4750	0.05
PD*DS	1	20	0	.	.	.	.	.
PD*DS	2	10	2.0000	5.9661	23	0.34	0.7405	0.05
PD*DS	2	13	-9.62E-7	5.9661	23	-0.00	1.0000	0.05
PD*DS	2	16	-2.0000	6.3280	23	-0.32	0.7548	0.05
PD*DS	2	20	0	.	.	.	.	.
PD*DS	3	10	0	.	.	.	.	.
PD*DS	3	13	0	.	.	.	.	.
PD*DS	3	16	0	.	.	.	.	.
PD*DS	3	20	0	.	.	.	.	.

Solution for Fixed Effects

Effect	PD	DS	Lower	Upper
DS		20	.	.
PD	1		-10.0603	7.3936
PD	2		-6.0603	11.3936
PD	3		.	.
PD*DS	1	10	-9.3418	15.3418

PD*DS	1	13	-4.0085	20.6751
PD*DS	1	16	-8.0085	16.6751
PD*DS	1	20	.	.
PD*DS	2	10	-10.3418	14.3418
PD*DS	2	13	-12.3418	12.3418
PD*DS	2	16	-15.0904	11.0904
PD*DS	2	20	.	.
PD*DS	3	10	.	.
PD*DS	3	13	.	.
PD*DS	3	16	.	.
PD*DS	3	20	.	.

The Mixed Procedure

Solution for Random Effects

Effect	COW	PD	Estimate	Std Err Pred	DF	t Value	Pr > |t|	Alpha
COW(PD)	Anniversary 493	1	0.3333	2.9830	23	0.11	0.9120	0.05
COW(PD)	Certificate 431	1	5.0000	2.9830	23	1.68	0.1073	0.05
COW(PD)	Cherop 411	1	4.3333	2.9830	23	1.45	0.1598	0.05
COW(PD)	Dona 540	1	3.0000	2.9830	23	1.01	0.3250	0.05
COW(PD)	Expert 553	1	-4.6667	2.9830	23	-1.56	0.1314	0.05
COW(PD)	Holiday 471	1	6.6667	2.9830	23	2.23	0.0354	0.05
COW(PD)	Larry 473	1	-4.0000	2.9830	23	-1.34	0.1930	0.05
COW(PD)	Lelgon 492	1	3.0000	2.9830	23	1.01	0.3250	0.05
COW(PD)	Manu U 447	1	-6.0000	2.9830	23	-2.01	0.0561	0.05
COW(PD)	Mureno 531	1	1.6667	2.9830	23	0.56	0.5818	0.05
COW(PD)	Sotik 440	1	-1.0000	2.9830	23	-0.34	0.7405	0.05
COW(PD)	Winner 389	1	-8.3333	2.9830	23	-2.79	0.0103	0.05
COW(PD)	Cherop 411	2	2.3333	2.9830	23	0.78	0.4421	0.05
COW(PD)	Dona 540	2	2.3333	2.9830	23	0.78	0.4421	0.05
COW(PD)	Eznus	2	-7.0000	2.9830	23	-2.35	0.0279	0.05

COW(PD)	Larry 473	2	1.0000	3.6535	23	0.27
0.7867	0.05					
COW(PD)	Lelgon 492	2	1.3333	2.9830	23	0.45
0.6591	0.05					
COW(PD)	Mildred	2	-3.6667	2.9830	23	-1.23
0.2314	0.05					
COW(PD)	Moi	2	-5.6667	2.9830	23	-1.90
0.0701	0.05					
COW(PD)	Mureno 531	2	-1.0000	2.9830	23	-0.34
0.7405	0.05					
COW(PD)	Sotik 440	2	8.0000	2.9830	23	2.68
0.0133	0.05					
COW(PD)	Tulwo 421	2	-1.0000	3.6535	23	-0.27
0.7867	0.05					
COW(PD)	Winner 389	2	3.3333	2.9830	23	1.12
0.2754	0.05					
COW(PD)	Cherop 411	3	5.6667	2.9830	23	1.90
0.0701	0.05					
COW(PD)	Dikir	3	-2.0000	2.9831	23	-0.67
0.5092	0.05					
COW(PD)	Dona 540	3	2.6667	2.9830	23	0.89
0.3806	0.05					
COW(PD)	Esnuz	3	-3.0000	2.9830	23	-1.01
0.3250	0.05					
COW(PD)	Larry 473	3	-3.0000	2.9830	23	-1.01
0.3250	0.05					
COW(PD)	Lelgon 492	3	0.6667	2.9830	23	0.22
0.8251	0.05					
COW(PD)	Mildred	3	-7.3333	2.9830	23	-2.46
0.0219	0.05					
COW(PD)	Moi	3	-3.3333	2.9830	23	-1.12
0.2754	0.05					
COW(PD)	Mureno 531	3	3.0000	2.9830	23	1.01
0.3250	0.05					
COW(PD)	Sotik 440	3	6.0000	2.9830	23	2.01
0.0561	0.05					
COW(PD)	Tulwo 421	3	-1.0000	2.9830	23	-0.34
0.7405	0.05					
COW(PD)	Winner 389	3	1.6667	2.9830	23	0.56
0.5818	0.05					

Solution for Random Effects

Effect	COW	PD	Lower	Upper
COW(PD)	Anniversary 493	1	-5.8376	6.5043
COW(PD)	Certificate 431	1	-1.1709	11.1709
COW(PD)	Cherop 411	1	-1.8376	10.5042
COW(PD)	Dona 540	1	-3.1709	9.1709

The Mixed Procedure

Solution for Random Effects

Effect	COW	PD	Lower	Upper
COW(PD)	Expert 553	1	-10.8376	1.5043
COW(PD)	Holiday 471	1	0.4957	12.8376
COW(PD)	Larry 473	1	-10.1709	2.1709
COW(PD)	Lelgon 492	1	-3.1709	9.1709
COW(PD)	Manu U 447	1	-12.1709	0.1709
COW(PD)	Mureno 531	1	-4.5043	7.8376
COW(PD)	Sotik 440	1	-7.1709	5.1709
COW(PD)	Winner 389	1	-14.5042	-2.1624
COW(PD)	Cherop 411	2	-3.8376	8.5042
COW(PD)	Dona 540	2	-3.8376	8.5043
COW(PD)	Eznus	2	-13.1709	-0.8291
COW(PD)	Larry 473	2	-6.5578	8.5578
COW(PD)	Lelgon 492	2	-4.8376	7.5042
COW(PD)	Mildred	2	-9.8376	2.5043
COW(PD)	Moi	2	-11.8376	0.5043
COW(PD)	Mureno 531	2	-7.1709	5.1709
COW(PD)	Sotik 440	2	1.8291	14.1709
COW(PD)	Tulwo 421	2	-8.5578	6.5578
COW(PD)	Winner 389	2	-2.8376	9.5042
COW(PD)	Cherop 411	3	-0.5043	11.8376
COW(PD)	Dikir	3	-8.1709	4.1710
COW(PD)	Dona 540	3	-3.5043	8.8376
COW(PD)	Esnuz	3	-9.1709	3.1709
COW(PD)	Larry 473	3	-9.1709	3.1709
COW(PD)	Lelgon 492	3	-5.5043	6.8376
COW(PD)	Mildred	3	-13.5042	-1.1624
COW(PD)	Moi	3	-9.5042	2.8376
COW(PD)	Mureno 531	3	-3.1709	9.1709
COW(PD)	Sotik 440	3	-0.1709	12.1709
COW(PD)	Tulwo 421	3	-7.1709	5.1709
COW(PD)	Winner 389	3	-4.5043	7.8376

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
DS	3	23	0.20	0.8975
PD	2	23	1.01	0.3793
PD*DS	6	23	0.56	0.7539

The Mixed Procedure

Least Squares Means

Standard

Effect	PD	DS	Estimate	Error	DF	t Value	Pr > |t|	Alpha
DS		10	6.1111	1.7223	23	3.55	0.0017	0.05
DS		13	6.2222	1.7223	23	3.61	0.0015	0.05
DS		16	6.5556	1.8603	23	3.52	0.0018	0.05
DS		20	7.7778	1.7223	23	4.52	0.0002	0.05
PD	1		7.5000	1.4915	23	5.03	<.0001	0.05
PD	2		7.5833	1.5820	23	4.79	<.0001	0.05
PD	3		4.9167	1.4915	23	3.30	0.0032	0.05
PD*DS	1	10	5.6667	2.9830	23	1.90	0.0701	0.05
PD*DS	1	13	10.0000	2.9830	23	3.35	0.0028	0.05
PD*DS	1	16	8.3333	2.9830	23	2.79	0.0103	0.05
PD*DS	1	20	6.0000	2.9830	23	2.01	0.0561	0.05
PD*DS	2	10	8.6667	2.9830	23	2.91	0.0080	0.05
PD*DS	2	13	5.6667	2.9830	23	1.90	0.0701	0.05
PD*DS	2	16	6.0000	3.6535	23	1.64	0.1141	0.05
PD*DS	2	20	10.0000	2.9830	23	3.35	0.0028	0.05
PD*DS	3	10	4.0000	2.9830	23	1.34	0.1930	0.05
PD*DS	3	13	3.0000	2.9830	23	1.01	0.3250	0.05
PD*DS	3	16	5.3333	2.9830	23	1.79	0.0870	0.05
PD*DS	3	20	7.3333	2.9830	23	2.46	0.0219	0.05

Least Squares Means

Effect	PD	DS	Lower	Upper
DS		10	2.5483	9.6739
DS		13	2.6595	9.7850
DS		16	2.7073	10.4038
DS		20	4.2150	11.3405
PD	1		4.4146	10.5854
PD	2		4.3107	10.8559
PD	3		1.8312	8.0021
PD*DS	1	10	-0.5042	11.8376
PD*DS	1	13	3.8291	16.1709
PD*DS	1	16	2.1624	14.5042
PD*DS	1	20	-0.1709	12.1709
PD*DS	2	10	2.4958	14.8376
PD*DS	2	13	-0.5042	11.8376

PD*DS	2	16	-1.5578	13.5578
PD*DS	2	20	3.8291	16.1709
PD*DS	3	10	-2.1709	10.1709
PD*DS	3	13	-3.1709	9.1709
PD*DS	3	16	-0.8376	11.5042
PD*DS	3	20	1.1624	13.5042

Obs	COW	WT	PD	DS	FD0R	FD0L	FD0T
1	Anniversary 493	453	1	10	3	0	3
2	Certificate 431	510	1	13	2	0	2
3	Cherop 411	487	1	10	1	1	2
4	Cherop 411	487	2	13	4	1	5
5	Cherop 411	487	3	20	3	0	3
6	Dikir	318	3	13	1	3	4
7	Dona 540	373	1	20	5	3	8
8	Dona 540	373	2	10	3	3	6
9	Dona 540	373	3	16	1	2	3
10	Esnuz	500	3	10	3	4	7
11	Expert 553	368	1	10	0	0	0
12	Eznus	500	2	20	6	5	11
13	Holiday 471	492	1	16	2	1	3

Obs	FD4R	FD4L	FD4T	ER	EL	ET	PRT
1	6	4	10	4	2	6	0.6
2	7	7	14	8	7	15	1
3	6	7	13	5	5	10	0.77
4	8	5	13	5	3	8	0.62
5	6	6	12	7	6	13	1

```
  6            3            3            6            0            1            1
0.17
  7            5            5           10            5            4            9
0.9
  8            6            5           11            6            5           11
1
  9            5            5           10            6            2            8
0.8
 10            3            2            5            1            0            1
0.2
 11            4            5            9            0            1            1
0.11
 12            4            4            8            1            2            3
0.38
 13            9            7           16            8            7           15
0.94

                                        Predicted Std Err
Obs          PRR          PRL F17 F18 F19    Mean     Pred     DF   Alpha   Lower
Upper  Residual

  1         0.67          0.5              5.6667  2.98304 23.0000  0.05 -0.50423
11.8376  0.33333
  2            1            1             10.0000  2.98304 23.0000  0.05  3.82910
16.1709  5.00000
  3         0.83         0.71              5.6667  2.98304 23.0000  0.05 -0.50423
11.8376  4.33333
  4         0.63          0.6              5.6667  2.98304 23.0000  0.05 -0.50423
11.8376  2.33333
  5            1            1              7.3333  2.98304 23.0000  0.05  1.16244
13.5042  5.66667
  6            0         0.33              3.0000  2.98304 23.0000  0.05 -3.17090
9.1709 -2.00000
  7            1          0.8              6.0000  2.98304 23.0000  0.05 -0.17090
12.1709  3.00000
  8            1            1              8.6667  2.98304 23.0000  0.05  2.49577
14.8376  2.33333
  9            1          0.4              5.3333  2.98304 23.0000  0.05 -0.83756
11.5042  2.66667
 10         0.33            0              4.0000  2.98304 23.0000  0.05 -2.17090
10.1709 -3.00000
 11            0          0.2              5.6667  2.98304 23.0000  0.05 -0.50423
11.8376 -4.66667
 12         0.25          0.5             10.0000  2.98304 23.0000  0.05  3.82910
16.1709 -7.00000
 13         0.89            1              8.3333  2.98304 23.0000  0.05  2.16244
14.5042  6.66667


                FSH EXPERIMENT EMBRYOS TOTAL                                  2
                                        Predictions           20:40 Thursday,
February 16, 2016


Obs COW                      WT           PD           DS         FD0R
FD0L         FD0T

 14 Larry 473               667            1           13            1
0            1
```

```
 15 Larry 473                667            2           16            0
5            5
 16 Larry 473                667            3           10            2
2            4
 17 Lelgon 492               490            1           20            0
0            0
 18 Lelgon 492               490            2           10            1
5            6
 19 Lelgon 492               490            3           16            2
2            4
 20 Manu U 447               500            1           20            1
3            4
 21 Mildred                  471            2           10            4
1            5
 22 Mildred                  471            3           20            1
2            3
 23 Moi                      497            2           13            4
4            8
 24 Moi                      497            3           16            3
0            3
 25 Mureno 531               364            1           16            0
0            0
 26 Mureno 531               364            2           20            1
0            1
```

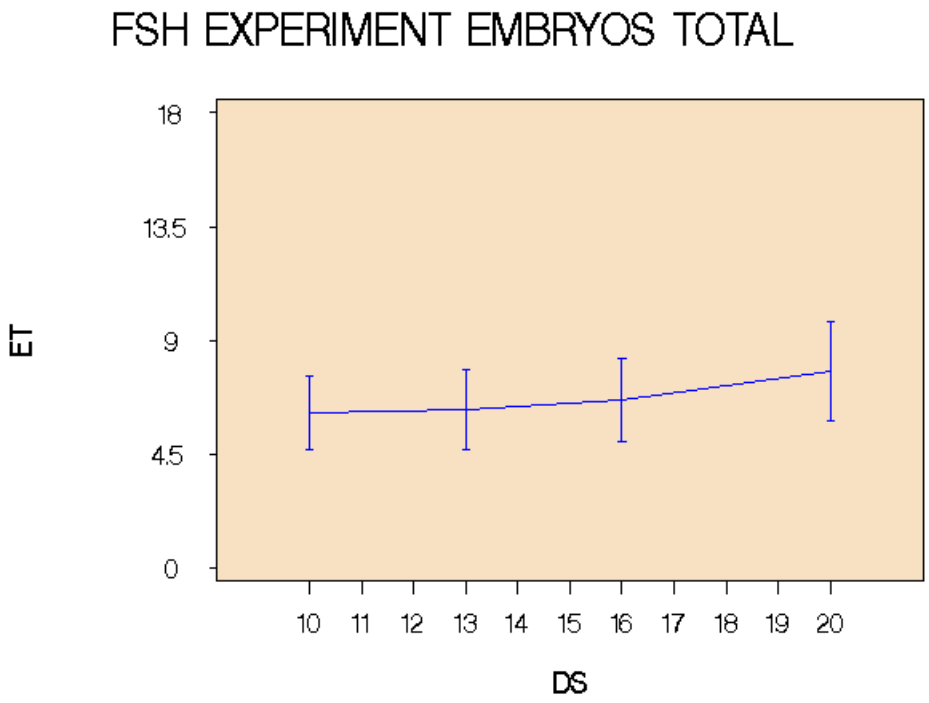
Obs	FD4R	FD4L	FD4T	ER	EL	ET	PRT
14	5	5	10	5	1	6	0.6
15	5	5	10	3	4	7	0.7
16	2	3	5	1	0	1	0.2
17	4	7	11	4	5	9	0.82
18	5	3	8	5	5	10	1
19	5	3	8	4	2	6	0.75
20	5	4	9	0	0	0	0
21	5	8	13	2	3	5	0.38
22	5	6	11	0	0	0	0
23	5	4	9	0	0	0	0
24	4	3	7	1	1	2	0.29
25	4	6	10	5	5	10	1
26	6	5	11	4	5	9	0.82

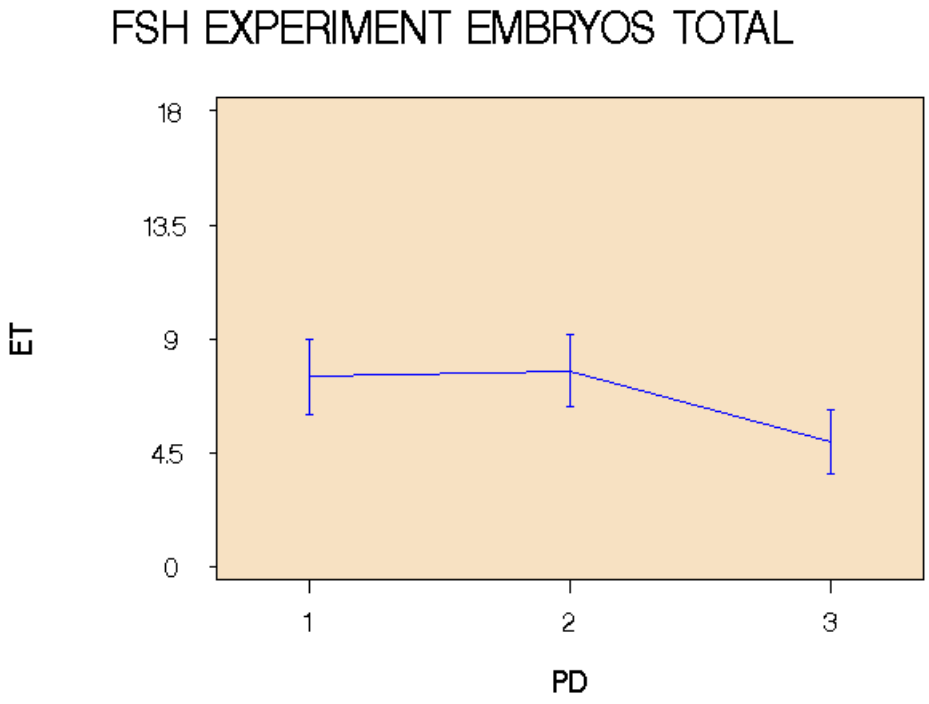
Obs	PRR	PRL	F17	F18	F19	Predicted Mean	Std Err Pred	DF	Alpha	Lower	Upper	Residual

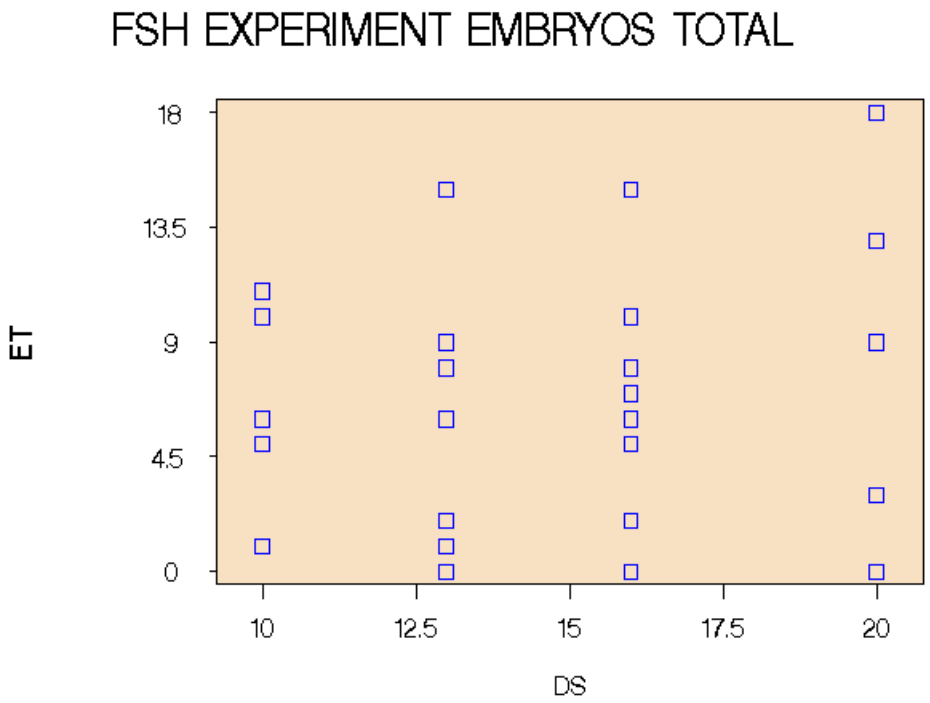
```
 14            1          0.2             10.0000  2.98304 23.0000  0.05  3.82910
16.1709 -4.00000
 15          0.6          0.8              6.0000  3.65347 23.0000  0.05 -1.55777
13.5578  1.00000
 16          0.5            0              4.0000  2.98304 23.0000  0.05 -2.17090
10.1709 -3.00000
 17            1         0.71              6.0000  2.98304 23.0000  0.05 -0.17090
12.1709  3.00000
 18            1            1              8.6667  2.98304 23.0000  0.05  2.49577
14.8376  1.33333
 19          0.8         0.67              5.3333  2.98304 23.0000  0.05 -0.83756
11.5042  0.66667
 20            0            0              6.0000  2.98304 23.0000  0.05 -0.17090
12.1709 -6.00000
 21          0.4         0.38              8.6667  2.98304 23.0000  0.05  2.49577
14.8376 -3.66667
 22            0            0              7.3333  2.98304 23.0000  0.05  1.16244
13.5042 -7.33333
 23            0            0              5.6667  2.98304 23.0000  0.05 -0.50423
11.8376 -5.66667
 24         0.25         0.33              5.3333  2.98304 23.0000  0.05 -0.83756
11.5042 -3.33333
 25            1         0.83              8.3333  2.98304 23.0000  0.05  2.16244
14.5042  1.66667
 26         0.67            1             10.0000  2.98304 23.0000  0.05  3.82910
16.1709 -1.00000


 FSH EXPERIMENT EMBRYOS TOTAL                                  3
                                      Predictions           20:40 Thursday,
February 16, 2016


Obs COW                    WT          PD          DS         FD0R
FD0L         FD0T

 27 Mureno 531             364          3          13           1
5            6
 28 Sotik 440              524          1          13           1
1            2
 29 Sotik 440              524          2          20           4
5            9
 30 Sotik 440              524          3          10           2
1            3
 31 Tulwo 421              532          2          16           2
5            7
 32 Tulwo 421              532          3          13           1
3            4
 33 Winner 389             433          1          16           2
2            4
 34 Winner 389             433          2          13           4
1            5
 35 Winner 389             433          3          20           0
0            0


Obs         FD4R         FD4L         FD4T           ER           EL           ET
PRT
```

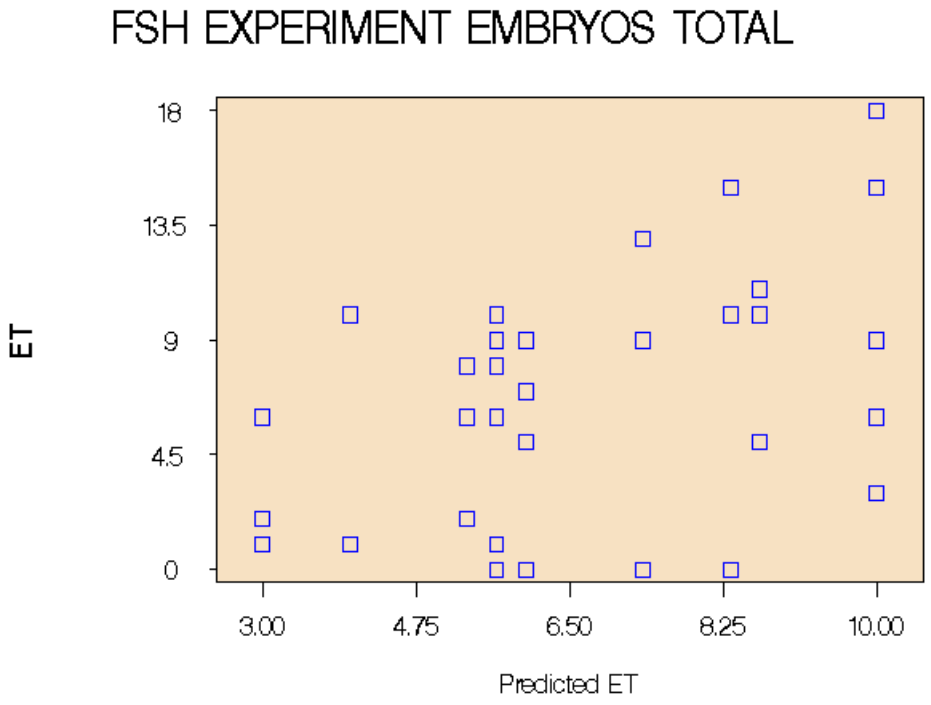
27	4	4	8	4	2	6
0.75						
28	8	5	13	4	5	9
0.69						
29	12	10	22	10	8	18
0.82						
30	8	5	13	7	3	10
0.77						
31	9	4	13	2	3	5
0.38						
32	5	5	10	1	1	2
0.2						
33	7	5	12	0	0	0
0						
34	5	3	8	6	3	9
1						
35	4	4	8	5	4	9
1						

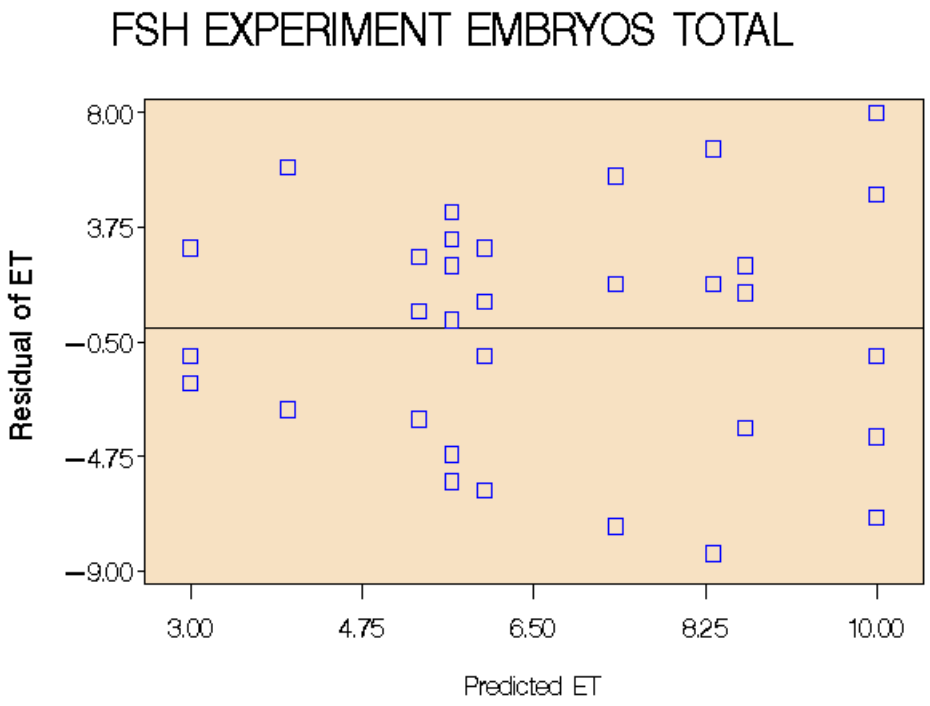
Obs	PRR	PRL	F17	F18	F19	Predicted Mean	Std Err Pred	DF	Alpha	Lower	Upper	Residual
27	1	0.5				3.0000	2.98304	23.0000	0.05	-3.17090	9.1709	3.00000
28	0.5	1				10.0000	2.98304	23.0000	0.05	3.82910	16.1709	-1.00000
29	0.83	0.8				10.0000	2.98304	23.0000	0.05	3.82910	16.1709	8.00000
30	0.88	0.6				4.0000	2.98304	23.0000	0.05	-2.17090	10.1709	6.00000
31	0.22	0.75				6.0000	3.65347	23.0000	0.05	-1.55777	13.5578	-1.00000
32	0.2	0.2				3.0000	2.98304	23.0000	0.05	-3.17090	9.1709	-1.00000
33	0	0				8.3333	2.98304	23.0000	0.05	2.16244	14.5042	-8.33333
34	1	1				5.6667	2.98304	23.0000	0.05	-0.50423	11.8376	3.33333
35	1	1				7.3333	2.98304	23.0000	0.05	1.16244	13.5042	1.66667

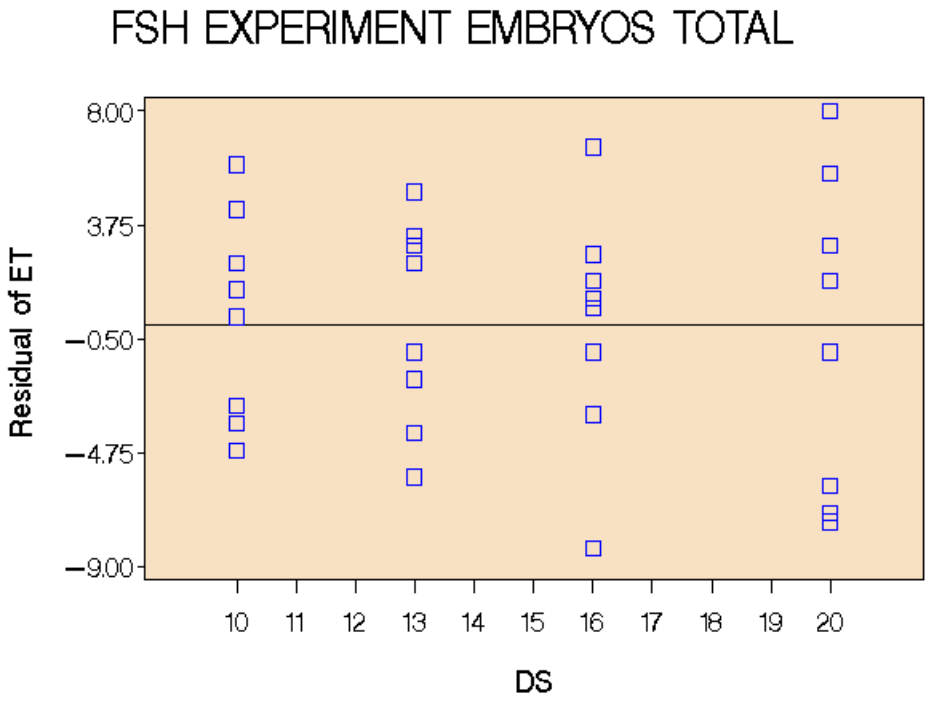

FSH EXPERIMENT EMBRYOS TOTAL
ET
18
13.5
9
4.5
0
10 11 12 13 14 15 16 17 18 19 20
DS

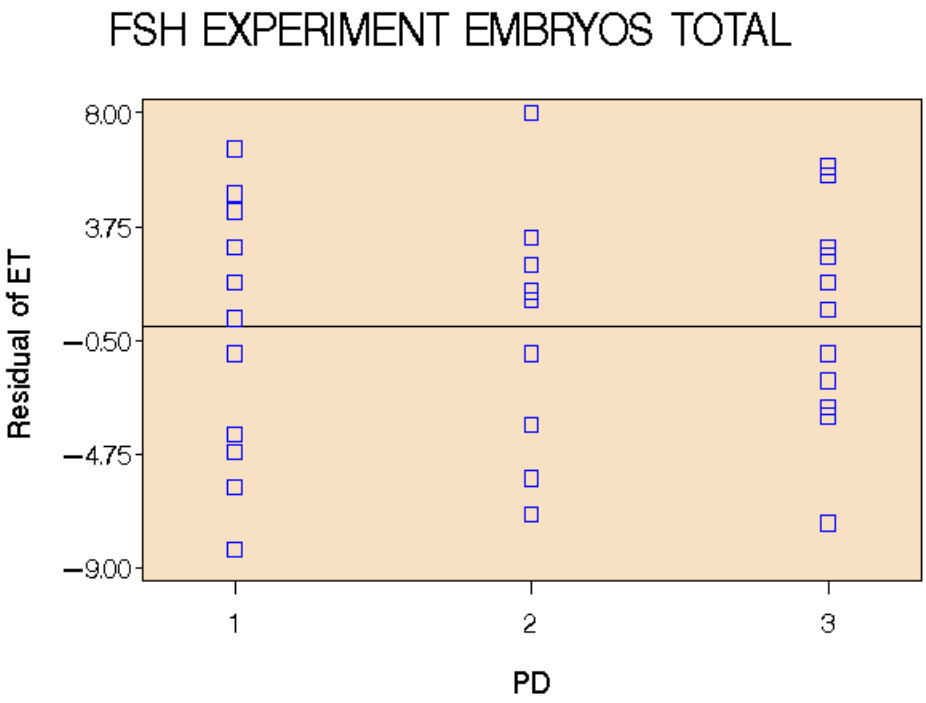

FSH EXPERIMENT EMBRYOS TOTAL
ET
18
13.5
9
4.5
0
1 2 3
PD


FSH EXPERIMENT EMBRYOS TOTAL
ET
18
13.5
9
4.5
0
10
12.5
15
17.5
20
DS


FSH EXPERIMENT EMBRYOS TOTAL
ET
18
13.5
9
4.5
0
3.00
4.75
6.50
8.25
10.00
Predicted ET


FSH EXPERIMENT EMBRYOS TOTAL
Residual of ET
8.00
3.75
−0.50
−4.75
−9.00
3.00
4.75
6.50
8.25
10.00
Predicted ET


FSH EXPERIMENT EMBRYOS TOTAL
Residual of ET
8.00
3.75
−0.50
−4.75
−9.00
10
11
12
13
14
15
16
17
18
19
20
DS


FSH EXPERIMENT EMBRYOS TOTAL
Residual of ET
8.00
3.75
-0.50
-4.75
-9.00
1
2
3
PD

8.3. Appendix 3. Adoption of MOET

```
GET DATA /TYPE=XLSX
  /FILE='D:\DATA FILES\IMPRINT\DATA\Kios data\RESPONDENTS MOET.xlsx'
  /SHEET=name 'TNZ responses'
  /CELLRANGE=full
  /READNAMES=on
  /ASSUMEDSTRWIDTH=32767.
EXECUTE.
DATASET NAME DataSet1 WINDOW=FRONT.
```

Appendix 3.1. Frequencies Variables in TransNzoia County = Adoption Lack of awareness, Technology not available, Lack of experts, Costly, Adoption if available

```
  /ORDER=ANALYSIS.
```

Frequencies

Notes

Output Created		17-SEP-2017 21:35:32
Comments		
Input	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	150
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data.
Syntax		FREQUENCIES VARIABLES=Adoption Lackofawareness Technologynotavailable Lackofexperts Costly Adoptionifavailable /ORDER=ANALYSIS.
Resources	Processor Time	00:00:00.02
	Elapsed Time	00:00:00.01

[DataSet1]

Statistics

		Adoption	Lack of awareness	Technology not available	Lack of experts	Costly
N	Valid	150	150	150	150	150
	Missing	0	0	0	0	0

Statistics

		Adoption if available
N	Valid	150
	Missing	0

Frequency Table

Adoption

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	150	100.0	100.0	100.0

Lack of awareness

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	80	53.3	53.3	53.3
	1	70	46.7	46.7	100.0
	Total	150	100.0	100.0	

Technology not available

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	131	87.3	87.3	87.3
	1	19	12.7	12.7	100.0
	Total	150	100.0	100.0	

Lack of experts

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	103	68.7	68.7	68.7
	1	47	31.3	31.3	100.0
	Total	150	100.0	100.0	

Costly

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	136	90.7	90.7	90.7
	1	14	9.3	9.3	100.0
	Total	150	100.0	100.0	

Adoption if available

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	31	20.7	20.7	20.7
	1	119	79.3	79.3	100.0
	Total	150	100.0	100.0	

Appendix 3.2. Frequencies Variables in UasinGishu County = Adoption, Lack of awareness, Technology not available, Lack of experts, Costly, Adoption if available
/ORDER=ANALYSIS.

Frequencies

Notes

Output Created		17-SEP-2017 17:26:11
Comments		
Input	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	143
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics are based on all cases with valid data.
Syntax		FREQUENCIES VARIABLES=Adoption Lackofawareness Technologynotavailable Lackofexperts Costly Adoptionifavailable /ORDER=ANALYSIS.
Resources	Processor Time	00:00:00.02

Elapsed Time	00:00:00.02

[DataSet1]

Statistics

		Adoption	Lack of awareness	Technology not available	Lack of experts	Costly
N	Valid	143	143	143	143	143
	Missing	0	0	0	0	0

Statistics

		Adoption if available
N	Valid	143
	Missing	0

Frequency Table

Adoption

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	143	100.0	100.0	100.0

Lack of awareness

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	59	41.3	41.3	41.3
	1	84	58.7	58.7	100.0
	Total	143	100.0	100.0	

Technology not available

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	134	93.7	93.7	93.7
	1	9	6.3	6.3	100.0
	Total	143	100.0	100.0	

Lack of experts

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	103	72.0	72.0	72.0
	1	40	28.0	28.0	100.0
	Total	143	100.0	100.0	

Costly

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	133	93.0	93.0	93.0
	1	10	7.0	7.0	100.0
	Total	143	100.0	100.0	

Adoption if available

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	0	40	28.0	28.0	28.0
	1	103	72.0	72.0	100.0
	Total	143	100.0	100.0	

```
SAVE OUTFILE='D:\DATA FILES\IMPRINT\DATA\Kios data\OBJ1UGDATA.sav'
  /COMPRESSED.
FREQUENCIES VARIABLES=Adoption, Lack of awareness, Technology not available,
Lack of experts, Costly, Adoption if available.
  /ORDER=ANALYSIS.
```

Appendix 3.3. Correlations

```
  /VARIABLES=Adoption Lackofawareness Technologynotavailable Lackofexperts Costly
Adoptionifavailable
  /PRINT=TWOTAIL NOSIG
  /MISSING=PAIRWISE.
```

Correlations

Notes

Output Created		18-SEP-2017 14:09:09
Comments		
Input	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	143
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
Syntax		CORRELATIONS /VARIABLES=Adoption Lackofawareness Technologynotavailable Lackofexperts Costly Adoptionifavailable /PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE.
Resources	Processor Time	00:00:00.03
	Elapsed Time	00:00:00.03

[DataSet1]

Correlations

		Adoption	Lack of awareness	Technology not available
Adoption	Pearson Correlation	.[a]	.[a]	.[a]
	Sig. (2-tailed)		.	.
	N	143	143	143
Lack of awareness	Pearson Correlation	.[a]	1	-.309**
	Sig. (2-tailed)	.		.000
	N	143	143	143
Technology not available	Pearson Correlation	.[a]	-.309**	1
	Sig. (2-tailed)	.	.000	
	N	143	143	143
Lack of experts	Pearson Correlation	.[a]	-.744**	-.162
	Sig. (2-tailed)	.	.000	.054
	N	143	143	143
Costly	Pearson Correlation	.[a]	-.327**	-.071
	Sig. (2-tailed)	.	.000	.399
	N	143	143	143
Adoption if available	Pearson Correlation	.[a]	-.237**	.162
	Sig. (2-tailed)	.	.004	.054
	N	143	143	143

Correlations

		Lack of experts	Costly	Adoption if available
Adoption	Pearson Correlation	.[a]	.[a]	.[a]
	Sig. (2-tailed)	.	.	.
	N	143	143	143
Lack of awareness	Pearson Correlation	-.744[a]	-.327	-.237**
	Sig. (2-tailed)	.000	.000	.004
	N	143	143	143
Technology not available	Pearson Correlation	-.162[a]	-.071**	.162
	Sig. (2-tailed)	.054	.399	.054
	N	143	143	143
Lack of experts	Pearson Correlation	1[a]	-.171**	.388
	Sig. (2-tailed)		.041	.000
	N	143	143	143
Costly	Pearson Correlation	-.171[a]	1**	-.379
	Sig. (2-tailed)	.041		.000
	N	143	143	143
Adoption if available	Pearson Correlation	.388[a]	-.379**	1
	Sig. (2-tailed)	.000	.000	
	N	143	143	143

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

```
NONPAR CORR
  /VARIABLES=Adoption Lack of awareness Technology not available Lack of experts
Costly Adoption if available
  /PRINT=BOTH TWOTAIL NOSIG
  /MISSING=PAIRWISE.
```

Nonparametric Correlations

Notes

Output Created		18-SEP-2017 14:09:10
Comments		
Input	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	143
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
Syntax		NONPAR CORR /VARIABLES=Adoption Lackofawareness Technologynotavailable Lackofexperts Costly Adoptionifavailable /PRINT=BOTH TWOTAIL NOSIG /MISSING=PAIRWISE.
Resources	Processor Time	00:00:00.02
	Elapsed Time	00:00:00.02
	Number of Cases Allowed	92521 cases[a]

a. Based on availability of workspace memory

[DataSet1]

Correlations

			Adoption	Lack of awareness
Kendall's tau_b	Adoption	Correlation Coefficient	.	.
		Sig. (2-tailed)	.	.
		N	143	143
	Lack of awareness	Correlation Coefficient	.	1.000
		Sig. (2-tailed)	.	.
		N	143	143
	Technology not available	Correlation Coefficient	.	-.309**
		Sig. (2-tailed)	.	.000
		N	143	143
	Lack of experts	Correlation Coefficient	.	-.744**
		Sig. (2-tailed)	.	.000
		N	143	143
	Costly	Correlation Coefficient	.	-.327**
		Sig. (2-tailed)	.	.000
		N	143	143
	Adoption if available	Correlation Coefficient	.	-.237**
		Sig. (2-tailed)	.	.005
		N	143	143
Spearman's rho	Adoption	Correlation Coefficient	.	.
		Sig. (2-tailed)	.	.
		N	143	143
	Lack of awareness	Correlation Coefficient	.	1.000
		Sig. (2-tailed)	.	.
		N	143	143
	Technology not available	Correlation Coefficient	.	-.309**
		Sig. (2-tailed)	.	.000
		N	143	143
	Lack of experts	Correlation Coefficient	.	-.744**
		Sig. (2-tailed)	.	.000
		N	143	143
	Costly	Correlation Coefficient	.	-.327**
		Sig. (2-tailed)	.	.000

Correlations

			Technology not available	Lack of experts
Kendall's tau_b	Adoption	Correlation Coefficient	.	.
		Sig. (2-tailed)	.	.
		N	143	143
	Lack of awareness	Correlation Coefficient	-.309	-.744
		Sig. (2-tailed)	.000	.000
		N	143	143
	Technology not available	Correlation Coefficient	1.000	-.162**
		Sig. (2-tailed)	.	.054
		N	143	143
	Lack of experts	Correlation Coefficient	-.162	1.000**
		Sig. (2-tailed)	.054	.
		N	143	143
	Costly	Correlation Coefficient	-.071	-.171**
		Sig. (2-tailed)	.397	.042
		N	143	143
	Adoption if available	Correlation Coefficient	.162	.388**
		Sig. (2-tailed)	.054	.000
		N	143	143
Spearman's rho	Adoption	Correlation Coefficient	.	.
		Sig. (2-tailed)	.	.
		N	143	143
	Lack of awareness	Correlation Coefficient	-.309	-.744
		Sig. (2-tailed)	.000	.000
		N	143	143
	Technology not available	Correlation Coefficient	1.000	-.162**
		Sig. (2-tailed)	.	.054
		N	143	143
	Lack of experts	Correlation Coefficient	-.162	1.000**
		Sig. (2-tailed)	.054	.
		N	143	143
	Costly	Correlation Coefficient	-.071	-.171**
		Sig. (2-tailed)	.399	.041

Correlations

			Costly	Adoption if available
Kendall's tau_b	Adoption	Correlation Coefficient	.	.
		Sig. (2-tailed)	.	.
		N	143	143
	Lack of awareness	Correlation Coefficient	-.327	-.237
		Sig. (2-tailed)	.000	.005
		N	143	143
	Technology not available	Correlation Coefficient	-.071	.162**
		Sig. (2-tailed)	.397	.054
		N	143	143
	Lack of experts	Correlation Coefficient	-.171	.388**
		Sig. (2-tailed)	.042	.000
		N	143	143
	Costly	Correlation Coefficient	1.000	-.379**
		Sig. (2-tailed)	.	.000
		N	143	143
	Adoption if available	Correlation Coefficient	-.379	1.000**
		Sig. (2-tailed)	.000	.
		N	143	143
Spearman's rho	Adoption	Correlation Coefficient	.	.
		Sig. (2-tailed)	.	.
		N	143	143
	Lack of awareness	Correlation Coefficient	-.327	-.237
		Sig. (2-tailed)	.000	.004
		N	143	143
	Technology not available	Correlation Coefficient	-.071	.162**
		Sig. (2-tailed)	.399	.054
		N	143	143
	Lack of experts	Correlation Coefficient	-.171	.388**
		Sig. (2-tailed)	.041	.000
		N	143	143
	Costly	Correlation Coefficient	1.000	-.379**
		Sig. (2-tailed)	.	.000

Correlations

			Adoption	Lack of awareness
Spearman's rho	Costly	N	143	143
		Correlation Coefficient	.	-.237
	Adoption if available	Sig. (2-tailed)	.	.004
		N	143	143

Correlations

			Technology not available	Lack of experts
Spearman's rho	Costly	N	143	143
		Correlation Coefficient	.162	.388
	Adoption if available	Sig. (2-tailed)	.054	.000
		N	143	143

Correlations

			Costly	Adoption if available
Spearman's rho	Costly	N	143	143
		Correlation Coefficient	-.379	1.000
	Adoption if available	Sig. (2-tailed)	.000	.
		N	143	143

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

```
CORRELATIONS
  /VARIABLES=Adoption Lackofawareness Technologynotavailable Lackofexperts Costly
Adoptionifavailable
  /PRINT=TWOTAIL NOSIG
  /MISSING=PAIRWISE.
```

Correlations

Notes

Output Created		17-SEP-2017 21:40:25
Comments		
Input	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	150
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
Syntax		CORRELATIONS /VARIABLES=Adoption Lackofawareness Technologynotavailable Lackofexperts Costly Adoptionifavailable /PRINT=TWOTAIL NOSIG /MISSING=PAIRWISE.
Resources	Processor Time	00:00:00.03
	Elapsed Time	00:00:00.09

[DataSet1]

Correlations

		Adoption	Lack of awareness	Technology not available
Adoption	Pearson Correlation	.[a]	.[a]	.[a]
	Sig. (2-tailed)		.	.
	N	150	150	150
Lack of awareness	Pearson Correlation	.[a]	1	-.356**
	Sig. (2-tailed)	.		.000
	N	150	150	150
Technology not available	Pearson Correlation	.[a]	-.356**	1
	Sig. (2-tailed)	.	.000	
	N	150	150	150
Lack of experts	Pearson Correlation	.[a]	-.632**	-.257**
	Sig. (2-tailed)	.	.000	.001
	N	150	150	150
Costly	Pearson Correlation	.[a]	-.300**	-.122
	Sig. (2-tailed)	.	.000	.136
	N	150	150	150
Adoption if available	Pearson Correlation	.[a]	-.084	.194*
	Sig. (2-tailed)	.	.309	.017
	N	150	150	150

Correlations

		Lack of experts	Costly	Adoption if available
Adoption	Pearson Correlation	.[a]	.[a]	.[a]
	Sig. (2-tailed)	.	.	.
	N	150	150	150
Lack of awareness	Pearson Correlation	-.632[a]	-.300	-.084**
	Sig. (2-tailed)	.000	.000	.309
	N	150	150	150
Technology not available	Pearson Correlation	-.257[a]	-.122**	.194
	Sig. (2-tailed)	.001	.136	.017
	N	150	150	150
Lack of experts	Pearson Correlation	1[a]	-.217**	.345**
	Sig. (2-tailed)		.008	.000
	N	150	150	150
Costly	Pearson Correlation	-.217[a]	1**	-.629
	Sig. (2-tailed)	.008		.000
	N	150	150	150
Adoption if available	Pearson Correlation	.345[a]	-.629	1*
	Sig. (2-tailed)	.000	.000	

N	150	150	150

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

```
NONPAR CORR
  /VARIABLES=Adoption Lackofawareness Technologynotavailable Lackofexperts Costly
Adoptionifavailable
  /PRINT=BOTH TWOTAIL NOSIG
  /MISSING=PAIRWISE.
```

Nonparametric Correlations

Notes

Output Created		17-SEP-2017 21:40:25
Comments		
Input	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	150
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each pair of variables are based on all the cases with valid data for that pair.
Syntax		NONPAR CORR /VARIABLES=Adoption Lackofawareness Technologynotavailable Lackofexperts Costly Adoptionifavailable /PRINT=BOTH TWOTAIL NOSIG /MISSING=PAIRWISE.
Resources	Processor Time	00:00:00.02
	Elapsed Time	00:00:00.03
	Number of Cases Allowed	92521 cases[a]

a. Based on availability of workspace memory

[DataSet1]

Correlations

			Adoption	Lack of awareness
Kendall's tau_b	Adoption	Correlation Coefficient	.	.
		Sig. (2-tailed)	.	.
		N	150	150
	Lack of awareness	Correlation Coefficient	.	1.000
		Sig. (2-tailed)	.	.
		N	150	150
	Technology not available	Correlation Coefficient	.	-.356**
		Sig. (2-tailed)	.	.000
		N	150	150
	Lack of experts	Correlation Coefficient	.	-.632**
		Sig. (2-tailed)	.	.000
		N	150	150
	Costly	Correlation Coefficient	.	-.300**
		Sig. (2-tailed)	.	.000
		N	150	150
	Adoption if available	Correlation Coefficient	.	-.084
		Sig. (2-tailed)	.	.307
		N	150	150
Spearman's rho	Adoption	Correlation Coefficient	.	.
		Sig. (2-tailed)	.	.
		N	150	150
	Lack of awareness	Correlation Coefficient	.	1.000
		Sig. (2-tailed)	.	.
		N	150	150
	Technology not available	Correlation Coefficient	.	-.356**
		Sig. (2-tailed)	.	.000
		N	150	150
	Lack of experts	Correlation Coefficient	.	-.632**
		Sig. (2-tailed)	.	.000
		N	150	150
	Costly	Correlation Coefficient	.	-.300**
		Sig. (2-tailed)	.	.000

Correlations

			Technology not available	Lack of experts
Kendall's tau_b	Adoption	Correlation Coefficient	.	.
		Sig. (2-tailed)	.	.
		N	150	150
	Lack of awareness	Correlation Coefficient	-.356	-.632
		Sig. (2-tailed)	.000	.000
		N	150	150
	Technology not available	Correlation Coefficient	1.000	-.257**
		Sig. (2-tailed)	.	.002
		N	150	150
	Lack of experts	Correlation Coefficient	-.257	1.000**
		Sig. (2-tailed)	.002	.
		N	150	150
	Costly	Correlation Coefficient	-.122	-.217**
		Sig. (2-tailed)	.136	.008
		N	150	150
	Adoption if available	Correlation Coefficient	.194	.345
		Sig. (2-tailed)	.018	.000
		N	150	150
Spearman's rho	Adoption	Correlation Coefficient	.	.
		Sig. (2-tailed)	.	.
		N	150	150
	Lack of awareness	Correlation Coefficient	-.356	-.632
		Sig. (2-tailed)	.000	.000
		N	150	150
	Technology not available	Correlation Coefficient	1.000	-.257**
		Sig. (2-tailed)	.	.001
		N	150	150
	Lack of experts	Correlation Coefficient	-.257	1.000**
		Sig. (2-tailed)	.001	.
		N	150	150
	Costly	Correlation Coefficient	-.122	-.217**
		Sig. (2-tailed)	.136	.008

Correlations

			Costly	Adoption if available
Kendall's tau_b	Adoption	Correlation Coefficient	.	.
		Sig. (2-tailed)	.	.
		N	150	150
	Lack of awareness	Correlation Coefficient	-.300	-.084
		Sig. (2-tailed)	.000	.307
		N	150	150
	Technology not available	Correlation Coefficient	-.122	.194**
		Sig. (2-tailed)	.136	.018
		N	150	150
	Lack of experts	Correlation Coefficient	-.217	.345**
		Sig. (2-tailed)	.008	.000
		N	150	150
	Costly	Correlation Coefficient	1.000	-.629**
		Sig. (2-tailed)	.	.000
		N	150	150
	Adoption if available	Correlation Coefficient	-.629	1.000
		Sig. (2-tailed)	.000	.
		N	150	150
Spearman's rho	Adoption	Correlation Coefficient	.	.
		Sig. (2-tailed)	.	.
		N	150	150
	Lack of awareness	Correlation Coefficient	-.300	-.084
		Sig. (2-tailed)	.000	.309
		N	150	150
	Technology not available	Correlation Coefficient	-.122	.194**
		Sig. (2-tailed)	.136	.017
		N	150	150
	Lack of experts	Correlation Coefficient	-.217	.345**
		Sig. (2-tailed)	.008	.000
		N	150	150
	Costly	Correlation Coefficient	1.000	-.629**
		Sig. (2-tailed)	.	.000

Correlations

			Adoption	Lack of awareness
Spearman's rho	Costly	N	150	150
		Correlation Coefficient	.	-.084
	Adoption if available	Sig. (2-tailed)	.	.309
		N	150	150

Correlations

			Technology not available	Lack of experts
Spearman's rho	Costly	N	150	150
		Correlation Coefficient	.194	.345
	Adoption if available	Sig. (2-tailed)	.017	.000
		N	150	150

Correlations

			Costly	Adoption if available
Spearman's rho	Costly	N	150	150
		Correlation Coefficient	-.629	1.000
	Adoption if available	Sig. (2-tailed)	.000	.
		N	150	150

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

8.4. Appendix 4. ADC Namandala and Sasini farms Embryo Analysis

```
GET DATA /TYPE=XLSX
  /FILE='D:\DATA FILES\IMPRINT\DATA\Kios data\ADC MOET 2013.2014.xlsx'
  /SHEET=name 'Sheet5'
  /CELLRANGE=full
  /READNAMES=on
  /ASSUMEDSTRWIDTH=32767.
EXECUTE.
DATASET NAME DataSet1 WINDOW=FRONT.
T-TEST GROUPS=SITE('ADC' 'SASINI')
  /MISSING=ANALYSIS
  /VARIABLES=EMBRYOS
  /CRITERIA=CI(.95).
```

T-Test

Notes

Output Created		18-SEP-2017 12:09:04
Comments		
Input	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	122
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=SITE('ADC' 'SASINI') /MISSING=ANALYSIS /VARIABLES=EMBRYOS /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00.02
	Elapsed Time	00:00:00.02

[DataSet1]

Group Statistics

	SITE	N	Mean	Std. Deviation	Std. Error Mean
EMBRYOS	ADC	45	1.93	3.158	.471
	SASINI	77	4.13	5.533	.631

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
EMBRYOS	Equal variances assumed	11.335	.001	-2.438	120
	Equal variances not assumed			-2.791	119.960

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference
					Lower
EMBRYOS	Equal variances assumed	.016	-2.197	.901	-3.980
	Equal variances not assumed	.006	-2.197	.787	-3.755

Independent Samples Test

		t-test for Equality of Means
		95% Confidence Interval of the Difference
		Upper
EMBRYOS	Equal variances assumed	-.413
	Equal variances not assumed	-.639