BLOOD PROGESTERONE DETERMINATION BY LATERAL FLOW IMMUNOASSAY FOR ASSESSMENT OF REPRODUCTIVE STATUS OF DAIRY CATTLE IN KENYA.

A thesis submitted in partial fulfillment of the requirements for the degree of Masters in Veterinary Theriogenology at the Department of Clinical Studies, Faculty of Veterinary Medicine, University of Nairobi.

By: Dr.Wilkister Nakami Nabulindo (BVM, UoN)

2016

DECLARATION

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

Dr. Wilkister Nakami Nabulindo (BVM) Department of Clinical Studies, University of Nairobi.

Signature …….. Date ……

J56/75896/2014

SUPERVISORS:

This thesis has been submitted for examination with our approval as the University supervisors:

Prof. V.T. Tsuma (BVM, Dip. Anim. Repro., FRVCS, PhD), Department of Clinical Studies, University of Nairobi.

Signature …….. Date ……

Prof. H.M. Mutembei (BVM, MSc, Diplom in Reprod., PhD), Department of Clinical Studies, University of Nairobi.

Signature…….. Date ……

Dr. J.K. Muthee (BVM, MSc, PhD), Department of Clinical Studies, University of Nairobi.

Signature…….. Date ……

DEDICATION

To family: mum, dad, siblings and Maurice Karani Murungi who have always been an inspiration.

To my friends: Grace Kahinga, Antony Kahinga, Yvonne, Lydia and Patrick.

ACKNOWLEDGEMENT

I thank the heavenly God for giving me the wisdom and strength to carry out this work.

Deep and sincere appreciation to my supervisors; Prof. Victor Tsuma, Prof. Henry Mutembei and Dr. John Muthee for their guidance, sound advice, and encouragement at all stages of my work. Their excellent supervision, constructive criticism and comments from the initial conception of the study to the end of this work are highly appreciated.

I appreciate the scholarship from the University of Nairobi through the Faculty of Veterinary Medicine of the University of Nairobi that provided my living expenses as I pursued my studies.

My sincere appreciation to Drs. Kipyegon Ambrose, Abuom Okumu, Ngetich Wycliffe, Dickson Machira, Maurice Karani and Mrs. Alice Kinyua for assisting me in data collection and analysis.

This research project received financial support from Diagnostic For All Company (DFA). I therefore wish to extend my appreciation to the DFA company and its secretariat.

I also thank the University of Nairobi Veterinary farm staff for their support during data collection at the farm.

All respondents of the questionnaires are highly appreciated for their support and willingness to provide the required information.

Anyone else who might have helped in any other form is also acknowledged. Asanteni sana wote!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AHA	Animal Health Assistants
AHP	Animal Health Practitioners
AHT	Animal Health Technicians
AI	Artificial Insemination
CCI	Calving to conception Interval
CI	Calving Interval
CL	Corpus Luteum
EIA	Enzyme Immunoassay
ELISA	Enzyme Linked Immunosorbent Assay
EPF	Early Pregnancy Factor
ET	Embryo Transfer
FAO	Food and Agriculture Organization
FSH	Follicle Stimulating Hormone
GDP	Gross Domestic Product
GnRH	Gonadotropin Releasing Hormone
GoK	Government of Kenya
KAP	Knowledge, Attitude and Practices
LFIA	Lateral Flow Immunoassay

LH	Luteinizing Hormone
MOL&FD	Ministry of Livestock and Fisheries Development
NSC	Number of Services per Conception
P4	Progesterone
PV+	Positive predictive value
PV-	Negative predictive value
RIA	Radio Immunoassay
USA	United States of America
USAID	United States Agency for International Development
UNEP	United Nations Environmental Programme
VWP	Voluntary Waiting Period

ABSTRACT

The livestock sector in Kenya contributes about 10% of the Gross Domestic Product (GDP) with the dairy subsector accounting for 4%. Eighty percent of this contribution is from small scale dairy farmers currently challenged by low reproductive efficiency caused by poor estrus detection, delayed determination of unsuccessful artificial insemination (AI) and sub-optimal calving to conception interval. Blood levels of progesterone (P4) are a valid indicator of the reproductive status of an animal. The current study sought to document P4 levels that could be used to determine the estrous phase of the cows to enable prompt remedial action where it was unsuccessful to optimize reproductive efficiency.

The P4 levels were determined using the lateral flow immuno assay (LFIA) and also the knowledge, attitude and practices of various stakeholders in the dairy industry on the use of P4 as a reproductive management tool was evaluated.

Blood was collected from 46 animals to establish the P4 profiles at the various stages of the reproductive cycle using enzyme linked immunosorbent assay (ELISA). Subsequently, P4 levels analyzed by LFIA and ELISA in blood from 100 dairy cattle were compared for the degree of agreement. Questionnaires were administered to various stakeholders in the dairy industry to determine their knowledge, attitude and practices on use of P4 as a reproductive management tool. Animal Health Practitioners (n=127) and dairy farmers (n=25) were interviewed in the present study. There was limited knowledge amongst dairy cattle industry stakeholders on the LFIA P4 kit and lack of its use in reproductive management of dairy cattle.

The P4 profiles of dairy cattle ranged from 0.2-10ng/ml. Pre-pubertal animals and those in the follicular phase of the cycle had P4 levels of 0.2 to 2.8ng/ml. Non-pregnant luteal phase and

pregnant animals had higher ($p<0.05$) P4 levels ranging between 4 to 10ng/ml. LFIA P4 scores ranged from 1 to 2 for pre-pubertal and follicular phase animals. The scores were higher ($p<0.05$) ranging from 2.5 to 3 for non-pregnant luteal phase and pregnant animals. The semi-quantitative P4 levels as determined by the LFIA were highly correlated (r 0.95; kappa=0.93) with the quantitative ELISA P4 levels of 0-4 ng/ml and 4-10 ng/ml, respectively for low and high P4 concentrations. The breed, body condition and weight did not influence the concentration of P4 levels. Progesterone levels as determined by ELISA were higher ($p<0.05$) for pregnant than non-pregnant luteal phase animals.

Forty two percent of Animal Health Practitioners (AHP) and none of dairy farmers were aware of P4 kits for heat detection whereas 46% of AHP and 4% of dairy farmers were aware of P4 kits for pregnancy diagnosis. Overall, a larger proportion of both the dairy farmers and AHP indicated that the P4 kits would be important in reproductive management and they would use them in reproductive management of dairy cattle.

These findings show that LFIA is a simple, rapid, reliable method for determination of P4 levels in whole blood in cattle and can be used by dairy cattle industry stakeholders for reproductive management of dairy cattle for improved productivity. However, awareness creation is required for the stakeholders for enhanced utilization of this decision support tool.

CHAPTER ONE

1.0 INTRODUCTION

Agriculture plays an important role in the economy of Kenya contributing about 27% of the Gross Domestic Product (GDP). The livestock subsector contributes about 10% of the GDP (GoK, 2012). Dairy farming is traditionally one of the major sub-sectors of agriculture in the East African region. However, estimating the size of the dairy industry in Kenya is a challenge since most of the sector is informal, and official statistics capture only a small portion that is formal (USAID, 2008). It is however, estimated that dairy farming contributes at least 4% of the GDP and directly supports the livelihoods of close to one million people (FAO, 2011). Over 70% of the dairy output in the country is from cattle and more than 80% of the estimated 4.2 million dairy cattle are reared by small-scale farmers in medium to high potential areas (GoK, 2012).

Over the last two decades, small-holder dairy farming has gained momentum in most of the East African countries, stimulating a demand for dairy animals that is a challenge to meet. Small-holder dairy in Kenya has been described as one of the most successful in Africa (Staal *et al*., 2008) and other countries in the region rely on Kenya for the supply of good quality dairy animals, yet the country cannot satisfy even its own needs. A strategy to fast-track the availability of locally adapted high-yielding dairy animals would go a long way in enhancing the performance of the sector and improving the livelihoods of millions of people. This notwithstanding, the dairy sector in the country faces a number of challenges including inadequate quantity and quality of feeds, animal diseases, low uptake of technology, and inefficient breeding practices (Muia *et al.,* 2011).

Reproductive efficiency is the key to achievement of economic productivity in dairy farms. Detection and correct interpretation of estrus, fertility at service and early pregnancy diagnosis are

critical in attainment of optimum reproductive efficiency (Posthuma-Trumpie *et al*., 2009). Calving interval (CI) is the parameter commonly used to assess reproductive efficiency in dairy farms and is composed of the calving to conception interval and gestation period (Nebel and Jobst, 1998). Since the gestation period is fixed, the calving to conception interval is the critical variable and is influenced by the time to post-partum resumption of ovarian cyclicity, the occurrence and detection of estrus, and fertility at service. Accurate determination of estrus is therefore central to optimization of reproductive efficiency, especially where artificial insemination is used. Apart from visual observation, various heat detection aids such as pedometers, teasers, chin-ball markers amongst others, can be used to enhance the accuracy of heat detection on dairy farms (Dalton, 2011). However, most of these may not be practical in small holder dairy systems, in addition to being unaffordable by the resource-poor farmers. Besides observation of heat signs, proper timing of AI is another factor that is critical in determination of fertility at service and is determined by when the animal was confirmed to have been seen on heat. Additionally, early determination of the outcome of an AI before the next expected estrus would inform decision support for remedial action to improve the reproductive performance and consequently target the recommended CI of 12-13 months. For optimum reproductive efficiency, there is therefore need for a rapid, easy and affordable method for timely assessment of reproductive status of cows.

Progesterone levels have been used to indicate the various stages of the reproductive cycle in dairy cattle (Nebel *et al*., 1987). Various methods such as radioimmunoassays (RIA), enzyme immunoassays (EIA), and chemiluminescence assays, have been used to determine P4 in blood and/or milk, to indicate the estrous phase and also pregnancy status in cattle. However, these methods have limitations ranging from requirement of laboratory facilities, are time consuming, are costly, and maybe hazardous (Posthuma-Trumpie *et al.*, 2009). Lateral flow immuno assays (LFIA) are currently used for qualitative, semi quantitative and quantitative tests in resource poor

or non laboratory environments, similar to those found in the small holder dairy systems in Kenya. The assays have been used to test for pathogens, drugs, metabolites and hormones and have been designed for single use at point of care i.e. outside the laboratory. The LFIAs are easy to use, results are obtained within a few minutes, and the sensitivity and specificity of the tests are high (Posthuma-Trumpie *et al.,* 2009). In dairy cattle LFIA have been used to detect P4 in milk for determination of estrus and pregnancy (Waldmann and Raud, 2016; Samsonova *et al*., 2015). However, there is paucity in data on use of LFIA for determination of P4 in whole blood in cattle and also at point of care for decision support in reproductive management of dairy cattle. Use of milk for P4 determination may be limiting as only lactating animals can be used, whereas whole blood would enable evaluation of even the non-lactating animals.

This study therefore sought to characterize the blood P4 profiles of dairy cattle in Kenya, evaluate the efficiency of the LFIA in detection of P4 in whole blood in cattle, and determine the knowledge, attitude and practices (KAP) of dairy industry stakeholders in the utilization of P4 in reproductive management of dairy cows.

1.1 OBJECTIVES

1.1.1 Overall objective

To utilize progesterone levels in reproductive management of dairy cattle for improved productivity.

1.1.2 Specific objectives

1. To determine the blood progesterone profiles of dairy cattle in Kenya.

2. To evaluate the efficiency of the lateral flow immuno assay in detection of progesterone in whole blood in dairy cattle.

3. To assess the knowledge, attitude and practices of dairy industry stakeholders in the utilization of progesterone test kits for reproductive management of dairy cows in Kenya.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 THE DAIRY INDUSTRY IN KENYA

2.1.1 Historical background

Commercial dairy farming in Kenya was started in the early 20th Century by white settlers who imported the dairy breeding herd from Europe (Ngigi, 2004). Indigenous Kenyans were not allowed to carry out commercial dairy farming until 1954 when the Swynnerton plan allocated them a production quota (Wakhungu, 2001). Following independence, rapid subdivision of land and transfer of dairy cattle from white settlers to small holder farmers was done and this led to a rapid decline of large farms (MOL&FD, 2006). The post-independence government established favorable policies that subsidized input services for animal health, production and breeding in order to encourage small holder dairy production (MOL&FD, 2006). This government support, shifted production from large scale to small scale (Muriuki *et al.,* 2004; Owen *et al*., 2005). Presently, the small scale farmers contribute 80% of the dairy production in Kenya.

2.1.2 Contribution of livestock sub sector and dairy industry to Kenya's economy.

The livestock sub-sector accounts for about 10% of the entire GDP and about 42% of the agricultural GDP (UNEP, 2014). It also supplies the domestic requirements of meat, milk, dairy products and other livestock products while accounting for about 30% of the total marketed agricultural products (GoK, 2012). Kenya has close to a million small scale dairy farmers keeping 80% of the 6.7 million dairy cattle and producing an estimate of 5 billion litres of milk every year. Apart from providing milk for consumption, hence improving the household nutritional status, the

dairy enterprise has been estimated to earn farmers over one hundred billion shillings annually from milk sales as well as providing employment to over 350,000 people at farm level and over 400,000 people in the informal and close to 50,000 people in the formal marketing sector. The true contribution by the sub-sector to the economy is likely to be even higher if unrecorded slaughter and home consumption is taken into account (GoK, 2012). Estimating the size of the dairy industry however, is a challenge since most of the sector is informal, and the official statistics capture only a small portion that is formal (Thorpe *et al.*, 2000; USAID, 2008).

Kenya has one of the largest dairy industry in the Sub-Saharan African region. According to a survey conducted by the Smallholder Dairy Project 6.7 million dairy cattle were in Kenya. The dairy industry is the single and largest agricultural sub-sector in Kenya and contributes to more than 15 percent of the agricultural GDP and 4 % percent of total GDP (FAO, 2011).

Kenya's cattle breeds range from local breeds, their crosses with the exotic breeds to pure exotic breeds. Exotic dairy breeds comprise of Holstein-Friesian, Ayrshire, Jersey and Guernsey. The indigenous *Bos indicus* breeds include: Boran and Small East African Zebu (Bebe *et al*., 2003; Lanyasunya *et al*., 2006). High producing exotic dairy breeds are preferred under zero grazing systems whereas cross breeds dominate free grazing herds (Lanyasunya *et al*., 2006). The cattle breeds are kept for numerous reasons: milk production, meat production, manure production and income generation. Other reasons include: animal traction, reproduction/breeding, symbol of wealth, security, dowry payment, employment, prestige, and as a shield against inflation (Mwacharo and Drucker, 2005; Murage and Ilatsia, 2011). Dairy production is concentrated within medium to high potential areas in Kenya, 48% in the former Rift Valley province, 30% in Central province, 15% in Nyanza province, 4% in Eastern province and 3% in Western province (MOL&FD, 2006).

Small holder dairy production accounts for about 80% of the total milk production from 2 to 3 cows on 1-2.5 hectares of land and supports about 400,000 small holder farmers (GoK, 2012). Smallholder dairy in Kenya has been described as one of the most successful in Africa (Staal *et al.*, 2008). Despite the plausible performance, Kenya's dairy industry is still bedeviled by several challenges. Inadequate quantity and quality of animal feeds, is one of the major challenges facing small holder farmers. Feeding is mainly rain fed pasture dependent therefore weather fluctuations determine the quantity and quality. Supplementation with concentrates is minimal (Techno-serve, 2008; GoK, 2010). Animal diseases is another challenge. Diseases reduce the productivity of the cattle and increase the cost of production. Common diseases in dairy farms include metabolic diseases, mastitis, lameness and tick borne diseases (MOL&FD, 2006). Lastly, there is low uptake of technology and inefficient breeding practices among dairy farmers in Kenya.

Technology such as estrus synchronization and embryo transfer among others are not widely used. Although AI for cattle breeding has been adopted by a sizeable number of dairy farmers, low heat detection rates, inappropriate timing of AI and delayed determination of unsuccessful insemination are among the challenges that result to reproductive inefficiency thus, reducing the overall productivity and profitability of dairy farming (Muia *et al*., 2011).

2.2 REPRODUCTIVE MANAGEMENT FOR OPTIMAL PRODUCTIVITY.

Reproductive management is an important aspect of dairy farming which affects daily milk production of a cow, milk production during different lactations, number of calves produced and the culling rate (Esselmont and Peeler, 1993; Esslemont *et al*., 2001; Van-Arendonk and Liinamo, 2003). All these factors influence the profits made by the farmers. The productivity of dairy cattle depends mainly on their reproductive performance. Among reproductive performance indices, age at puberty, age at first service, conception rates and calving interval, form the base of profitable

production for the dairy farm enterprise (Mukasa-Mugerwa, 1989). The reproductive performance level is well below the optimum in most countries (Moore and Thatcher, 2006). Previous studies in Kenya, have reported the reproductive efficiency of small holder dairy systems to be poor due to inadequate and poor quality feeding, prolonged postpartum anestrus periods, poor estrus detection skills, poor breeding techniques and lack of proper breeding records (Bebe *et al*., 2003; Owen *et al*., 2005). Significant economic returns can be achieved by improving reproductive performance (Heuwieser and Oltenacu, 1997).

2.2.1 Measures of reproductive performance

2.2.1.1 Age at puberty

This refers to the age when an animal gains the capacity to release gametes and manifest complete sexual behavioral sequences. The reproductive organs become functional in the female. It is also the time when the first functional estrus, associated with ovulation and formation of the CL occurs and thus reproduction can take place. Puberty is associated with maturation of the hypothalamus so as to set in motion sequential increase in GnRH release and hence action on the pituitary gland to release LH and FSH (Short and Bellows, 1971; Curley *et al*., 2008).

Balakrishan *et al*. (1986) defined puberty in heifers as the age at which plasma P4 levels reach 1.0 ng/ml. Other authors have suggested P4 levels of 2.0 ng/ml to be used as a puberty criteria (Cooke and Arthington, 2009). Most heifers, especially *Bos taurus,* reach puberty and breed satisfactorily at one year of age. However, age at puberty varies among species, breeds and even within families. *Bos taurus* cattle reach puberty earlier than their crosses with *Bos indicus* or pure *Bos indicus* cattle. This is due to genetic and environmental factors, including nutrition, climate and season of birth. These factors affect heifer growth rates (Short and Bellows, 1971). Heifers that are fed on a

high-protein diet often reach puberty earlier than those on a low-protein diet and are more fertile after puberty. Under-nutrition delays puberty, however, overfeeding does not necessarily result in earlier puberty than adequate levels of feeding (Wiltbank *et al.,* 1969; Billings, 2002).

The major factors controlling the onset of puberty are body weight and growth rather than age. Until heifers reach a particular critical weight, estrus is unlikely to occur (Hafez and Hafez, 2000). Taurine breeds of dairy cattle in temperate climates reach puberty at 30-40% of their adult body weight, compared to Boran heifers which attain puberty at 60% of their adult bodyweight. *Bos taurus* breeds attain puberty between 12-18 months while *Bos indicus* breeds reach puberty at the age between 18-24 months (McDowell *et al*., 1976; Hafez and Hafez, 2000).

2.2.1.2 The bovine estrous cycle

The estrous cycle is the period from one estrus to the next. Estrus occurs, on the average, every 18–24 days in sexually mature, non-pregnant female cattle, when they are receptive to a male. The estrous cycle is under control of the reproductive hormones: GnRH, LH, FSH and gonadal hormones estrogen and P4. The estrous cycle is divided into four distinct but continuous stages: proestrus, estrus, metestrus and diestrus (Dobson *et al*., 2008). Proestrus is the period between regression of the CL of the previous cycle and estrus. It is also the period of follicular development. Ovarian activity during proestrus is initiated by regression of the CL of the previous cycle, P4 levels are low and growth of the ovulatory follicle takes place. Estrogens are produced by granulosa cells that form the wall of the developing follicle and the high estrogens are responsible for the behavioral signs of estrus. The estrus period ranges from lasts 8-30 hours and is the period of sexual receptivity (Dalton, 2011).

The continued estrogen production by the developing follicle results in a LH and FSH surge which stimulates more estrogen production by the follicle. Final maturation of the ovum and follicle also occurs during the estrus period. The surge in LH and FSH during estrus leads to ovulation about 10-14 hours after the end of estrus. The granulosa and theca cells lining the collapsed follicle become sensitive to LH and form the CL which begins to produce P4 at around day 4 post estrus. The 3-4 days immediately after estrus are referred to as metestrus, which is followed by diestrus. Diestrus lasts 12-15 days and is the period of active P4 production by the CL. This hormone is responsible for preparing the uterus for pregnancy and inhibiting estrous cycle activity. The CL reaches maximum size 8-10 days after ovulation. The production of P4 increases as the CL grows until maximum levels at around day 10. These levels are maintained until day 16 of the cycle, in cows that are not pregnant, the CL is induced to regress by the release of prostaglandin F2α from the uterus. On the other hand, if the cow is pregnant, the CL is maintained, blood levels of P4 remain high, and resumption of cyclic activity is blocked. During this period follicles grow but they do not ovulate (Dalton, 2011). Synchrony of estrus, mating/insemination and ovulation is critical for successful fertilization. The ovum is only viable for 10-12 hours post ovulation, and the spermatozoa could be viable for up to 48 hours since deposition into the female reproductive tract. The spermatozoa must spend 4-6 hours in the female tract for capacitation to take place before they are capable of fertilizing an egg. This explains why conception rates are higher when cows are inseminated from mid to late estrus rather than after the end of estrus (Dinskin and Sreenan, 2000).

2.2.1.3 Behavioral signs of estrus

The best indicator or the primary sign of a cow or heifer in estrus is when she allows other herd mates to mount her while she remains standing. The animal in estrus has also increased physical

activity and may mount other animals in the vicinity. However, only animals that remain standing for mounting are in estrus (Negussie *et al.,* 2002).

Secondary signs of heat that a farmer may observe to determine whether an animal is in heat include a roughened tail-head due to being mounted and mounting others. The animal may also be nervous, restless, frequently bellow and it may ride or mount other animals. If there are many animals coming into estrus together, usually they will congregate in small groups called the sexually active group. Estrus is easy to detect when a sexually active group is in the herd. Another important sign to observe is clear mucus discharge from the vulva. This discharge may be smeared on her rump by her tail. Swelling and reddening (bright cherry pink color) of the vulva lips may also be an indicator that the cow is in estrus (Yoshida and Nakao, 2005). Cows should be checked for heat at least three times a day for 30 minutes (Roelofs *et al.,* 2005).

2.2.1.4 Estrus detection

One of the major factors potentially influencing pregnancy rates within dairy farms is estrus detection. Estrus detection can be described as either estrus detection efficiency or estrus detection accuracy. Estrus detection efficiency is a measure of the cows that are predicted to come into estrus over a given period of time that actually come into estrus. Estrus detection accuracy refers to the number of cows thought to be in estrus over a certain period of time that are truly in estrus with low blood or milk P4 (Heersche and Nebel, 1994). These two estrus detection parameters affect herd performance and losses have been reported due to suboptimal estrus detection (Esslemont *et al*., 2001; Dinskin and Sreenan, 2000). Estrus detection efficiency is obtained by listing all the cows expected to be in estrus in a given period and then comparing this with cows found in estrus over the next 24-day period. At the end of the 24-day estrous cycle period, the number of cows detected in estrus are divided by the total number of cows on the list. The value obtained would be

the estrus detection efficiency. Estrus detection accuracy is estimated by examining the number of animals returning to estrus (18-24 days considered normal interval), conception rates, and by blood or milk P4 levels (Esslemont *et al*., 2001). The high accuracy rates are obtained in a system where cows are inseminated based on standing estrus and low blood or milk P4 (Heersche and Nebel, 1994).

Improving estrus detection can have major economic benefits. One such benefit is the reduction in the number of reproductive culls due to failure to conceive of infertile cows and repeat breeders (Senger, 1994; Walker *et al.,* 1996). Poor estrus detection efficiency has been documented as one of the costly problems of AI programs (Walker *et al*., 1996; Xu *et al*., 1998). Estrus detection programs based solely on visual observation have been shown to have low estrus detection rates. Use of hormones to induce estrus and mechanical aids to supplement visual detection of estrus have been shown to improve heat detection efficiency and accuracy (Holman *et al*., 2011).

Various mechanical aids currently exist or are being developed to aid in estrus detection. They include: Heat expectancy charts, breeding wheels, heat watch transponders, mount detectors, pressure sensitive heat detectors, pedometers, and implantable sensors to measure changes in vaginal and uterine secretion electrical conductivity (Holman *et al*., 2011). Heat expectancy charts are organized on a 21- day cycle so that future heats can be anticipated. Expected next heat date is marked and therefore anticipated in advance. Breeding wheels consist of wall-mounted reproductive record systems that use color-coded pins or markings to indicate reproductive events for each cow on a daily basis, therefore future heats and reproductive events can be anticipated. Mounting activity can be monitored using chalk or paint which are marked on the tail head or the rump and rubbing off is evidence of mounting.

Pedometers are used to record increased physical activity of the animal in estrus. This may not be reliable since increase in physical activity may be due to other factors other than estrus (Rao *et al*., 2013). Additionally, pressure sensitive tags are glued on the topline of the rump and incase the animal is mounted, sustained pressure by the mounting cow will expel fluid from a small storage chamber into a larger visible plastic chamber, thus providing evidence for mounting. The aids may be altered by other means, such as the cow activating the detector by rubbing on a tree or other items (Dalton, 2011). Mount detectors which detect and record legitimate mounts have also been developed. Each detector is coded with the cow's identification number, and the information is transmitted to a computer and stored. The information can be accessed by the farmer at any time (At-Taras and Spahr, 2001). These systems reduce the labor requirement, but there is a significant chance of false detection and require heavy initial financial investment.

Temporary intra-vaginal probes have also been developed to measure the chemical, physical and electrical conductivity changes in the vagina during estrus. Measurement of vaginal conductivity requires repeated insertion and measurement of the intra vaginal probe which could produce inflammation that affects the reading (Morais *et al*., 2006). Heat watch devices detect mounts through a pressure-sensitive pad placed on the tail head of the cow that radio transmits information. The farmer can therefore access the information on the computer. The devices are reliable but expensive. Chin-ball markers may also be used with a hormone-treated cow, steer or bull with an amputated or deflected penis therefore he cannot breed the cow during mounting. The animal on heat will be marked and identified by the farmer (Holman *et al*., 2011). Unfortunately, such technologies are too expensive to use on a wide-scale basis in a developing country. These mechanical detection aids require both time and other supplies, including sometimes very expensive computer-related items. All these methods are not reliable since the parameters they measure, can change due to other reasons other than estrus (Orchard, 2007). None are substitutes

for visual observation. If a farmer is not able to observe the herd at least two or three times a day, the heat detection aid may not be helpful in detecting standing heat.

Use of hormones (prostaglandins, P4 and GnRH) to synchronize estrus or ovulation reduce the need for observing heat signs and tend to increase the number of animals being inseminated (LeBlanc and Leslie, 1998; Nebel and Jobst, 1998; Higgins *et al*., 2013). Progesterone concentration in milk and blood is associated with events of the estrous cycle. Progesterone levels are normally low (below 2ng/ml) at estrus (Nebel *et al*., 1987). Low milk or blood P4 cannot be used alone to indicate heat, it must be supported by observable heat signs. Consequently, heat detection accuracy may be monitored periodically using P4 levels (Rao *et al*., 2013).

2.2.1.5 Artificial insemination (AI)

Artificial insemination is the technique that involves the transfer of semen from a bull into the reproductive system of a female in order to get the female impregnated (Eklundh, 2013). This technology has been adopted in livestock breeding systems and it is a necessary tool in sustainable farm animal breeding (Rodriguez-Martinez, 2012). AI in cattle was developed in the 1940's and has since then been used widely in the dairy industry all over the world. AI has been the most successful and important assisted reproductive technology in developing countries (Rodriguez-Martinez, 2012). The use of AI can be seen as a chain of events; from the collection of semen from a bull to the birth of a calf. Therefore, for AI to be successful, no failures can be tolerated anywhere since each link of this chain of events is of equal importance (Althouse, 2007). Farmers must therefore detect estrus accurately to ensure that insemination is done at the right time, preferably using the AM/PM rule (Dransfield *et al*., 1998; Dalton *et al*., 2001). Fertility at service which influences success of fertilization is dependent on correct estrus detection (Maatje *et al*., 1997). Other factors that affect the outcome of AI are: semen quality and handling. Finally, the

inseminator must have adequate training and skills in the procedures for handling semen and performing inseminations (Nordin *et al*., 2007).

2.2.1.6 Conception rate

Conception rate is the proportion of cows inseminated which actually become pregnant. This index is significantly influenced by estrus detection because farmers must be able to correctly detect estrus to know when to inseminate for maximum fertility (Maatje *et al*., 1997; Nebel, 2001). Physiological stress from increased milk production, heat stress, and reproductive diseases such as retained placenta, metritis, mastitis, and cystic ovaries affect the conception rates (Radostits, 1985). Assuming a voluntary waiting period of 45-60 days, optimal number of inseminations per conception to achieve a 13-month calving interval is around 1.8, based on a 55% success rate (Hernandez *et al.,* 2001).

Conception rate is also adversely affected by many other factors including: improper AI technique, reproductive pathology, lameness, and heat stress (Radostits, 1985). This parameter has been shown to be higher under uncontrolled natural breeding and low where hand-mating or artificial insemination is used. Number of inseminations per conception values greater than 2.0 should be regarded as poor (Althouse, 2007).

2.2.1.7 Pregnancy diagnosis methods in bovine

Non return to estrus method has been traditionally used to assume pregnancy in cows that were inseminated. Regular estrous cycle of a cow usually takes 18-24 days and an inseminated cow is assumed to be pregnant if she does not come on heat after 24 days. This happens during pregnancy because the CL of the previous cycle persists and the next cycle is inhibited. The limitation of this pregnancy detection technique is that the animal may not return to estrus due to other reasons other

than pregnancy such as a cystic CL or anestrous (Purohit, 2010). Moreover, difficulty in estrus detection and silent estrus render this method of pregnancy diagnosis unsuitable (Purohit, 2010; Lucy *et al.*, 2011).

The common method used in the field for pregnancy diagnosis is trans-rectal genital palpation, which involves palpation of the reproductive organs of a cow/heifer with the hand through the rectum. It is based on the principle that the reproductive organs lie on the pelvic floor beneath the rectum in early pregnancy in cattle. The growth of the conceptus in either of the uterine horns leads to increase in the size of the horn, tenseness and palpable characteristics of the gravid uterine horn. Thus, the palpator can feel these changes in the uterus of a pregnant animal. This method is based on the detection of the cardinal signs of pregnancy during palpation.

Confirmation of pregnancy is at about Day 35 post-insemination onwards, and the practitioners rely on the palpation of the amniotic vesicle and slipping of the chorio-allantoic membranes between the thumb and forefinger (Purohit, 2010). Other cardinal features of pregnancy include: palpation of the placentomes, and the fetus. This method of pregnancy diagnosis is the cheapest and easiest but limitations include labor required to restrain the animals and skilled personnel. Rectal palpation can also induce early embryonic loss if not done correctly (Orchard, 2007). Since the pregnancy is seldom detected earlier than 35 days post breeding, at this time a non-pregnant cow will have ovulated at about 21 days post insemination. This method cannot therefore be used to detect non pregnancy before the next ovulation after service (Romano *et al*., 2007).

Ultrasonography can be used to detect pregnancy as early as 25 days post insemination. This method involves use of high frequency sound waves to produce an acoustic image of internal body organs. Images of the uterine horns with their contents are visualized on the monitor. This method is accurate and early diagnosis of pregnancy as well as viability of the fetus can be determined.

The limitation is the high cost of the machine and the expertise required to operate the machine and also to interpret the image (Safronova *et al*., 2012).

Pregnancy diagnosis can also be based on detection of conceptus specific substances from the maternal body fluids such as pregnancy associated glycoproteins and early pregnancy factor. Early pregnancy factor (EPF) can be detected in maternal blood as early as 3 days after conception using a rosette inhibition test (Lucy *et al*., 2011). Although EPF is secreted in early pregnancy, it is not pregnancy specific since it can also be secreted from tumors and transformed cell lines (Cavanagh, 1996) which makes it an erroneous pregnancy detection method and it has also been proved to be unreliable (Ambrose *et al*., 2007).

Pregnancy specific protein B and pregnancy associated glycoprotein have been identified in the maternal blood during pregnancy (Sheldon *et al*., 2006). These can be detected by immunoassay in a laboratory and can be reliably used as indicators of pregnancy. Pregnancy can be detected at Day 26 and confirmed at any later stage in the pregnancy (Sheldon *et al*., 2006; Silva *et al*., 2007). This method can therefore not be used for pregnancy diagnosis before the next estrous cycle begins and its reliability is also in doubt (Fricke *et al*., 2012).

Oestrone sulphate (E_1-S) hormone levels can be used for pregnancy diagnosis although it can only reliably indicate pregnancy from Day 120 of pregnancy (Henderson *et al*., 1994).

Measuring P4 in blood or milk as a method to identify open cows was perhaps the first true example of chemical pregnancy testing. If a cow is not pregnant then she will theoretically have low P4 levels at approximately 21 days after insemination, or a period equivalent to her estrous cycle length. If she is pregnant then her P4 concentrations will remain elevated. There is excellent physiological underpinning for the P4 test because cows cannot be pregnant if they have low (less

than 1 ng/ml) P4 21 days after insemination. If a cow tests high for P4 18-24 days post breeding then she may be pregnant (Nebel *et al*., 1987; Lucy *et al*., 2011).

2.2.1.8 Calving interval (AI)

Calving interval is the period of time between successive parturitions usually reported in months or days at the herd level (Nebel and Jobst, 1998). This is the parameter mostly used to assess reproductive efficiency and is composed of the calving to (successful) conception interval and gestation period (French and Nebel, 2003). The gestation period is biologically determined and therefore the critical variable is the calving to conception interval (CCI). The calving to conception period consists of the voluntary waiting period, time of postpartum resumption of ovarian cyclicity, the occurrence and detection of estrus and fertility at service (Ill-Hwa and Hyun-Gu, 2006). Estrus detection is a factor of emphasis during this period. Failures in estrus detection efficiency can result from either delayed resumption of ovarian cyclicity or failure to detect heat once it occurs (Eicker *et al.*, 1996; Nebel, 2003). Additionally, the likelihood that a service or insemination will result in pregnancy is also very critical.

Biological and managerial factors that may affect CCI include: delayed resumption of postpartum ovarian activity, silent heat, sub estrus, poor heat detection skills, poor heat detection efficiency and accuracy, postpartum diseases such as endometritis, suboptimal voluntary waiting period, increased cases of failed insemination, and inadequate feeding (Heersche and Nebel, 1994; Hare *et al*., 2006; Ill-Hwa and Hyan- Gu, 2006). To attain the recommended calving interval of 365-390 days, a calving conception interval of 85-110 days should be targeted (Hare *et al*., 2006). Studies in Kenyan dairy farms have reported long CCI (Bebe *et al*., 2003; Ojango and Pollot, 2004; Owen *et al*., 2005). As the CCI increases, fewer calves are produced, breeding costs increase due to higher number of inseminations per conception and veterinary costs increase due to more repeat

breeders. As calving interval increases, days in milk increases and lifetime milk yield decreases (Heuwieser and Oltenacu, 1997; Barnes, 2001).

2.3 PROGESTERONE (P4)

Progesterone is a progestogen steroid hormone, the first biologically active compound in the steroid biosynthesis pathway. This hormone was first isolated by Corner and Allen, (1929). Progesterone is synthesized in the corpus luteum of the ovary and also by the placenta during pregnancy (Mondal and Prakash, 2003). Low levels of P4 are also produced by the adrenal glands (Cookie and Arthington, 2009). Constant secretion of P4 is essential to maintain the circulating levels since it has a short physiological half-life. Progesterone is metabolized by breaking of the double bonds and hydroxylation at the C-16 and C-21 atoms. The conjugation products are glucuronides and sulphates which are then excreted in bile (Mondal and Prakash, 2003).

Progesterone plays an important role in preparation of the uterus for implantation, pregnancy maintenance, expression of estrus, normal ovarian cyclic function and in hypophyseal gonadal interrelationship (Mondal and Prakash, 2003). Progesterone decreases gonadotropin secretion by negative feedback mechanism and prevents behavioral estrus from occurring during pregnancy (Spencer *et al.,* 2009). Progesterone is also essential for blastocyst development, maintenance of the fetus and nullifying the uterine tone during pregnancy (Parr *et al*., 2013). The growth, development and survival of the embryo require the action of P4 on the uterus to regulate endometrial function, including conceptus-maternal interactions, pregnancy recognition, and uterine receptivity to implantation (Lonergan *et al*., 2013). The outcome of the P4 induced changes in the cyclic and pregnant uterus is to modify the intrauterine milieu, such as an increase in select amino acids, glucose, cytokines and growth factors in histotroph, for support of blastocyst growth (Dorniak *et al*., 2013).

2.4 PROGESTERONE AS A REPRODUCTIVE MANAGEMENT TOOL

Levels of P4 in blood or milk can be used to monitor the reproductive cycle in cattle therefore used in reproductive management (Nebel *et al*, 1987). Progesterone measurements taken every 3-4 days during the estrous cycle would be useful in establishment of P4 profiles that could be used in assessing reproductive status to identify estrus, fertility status and also disorders in cattle (Safronova *et al*., 2012). Maximum P4 levels in milk or blood are recorded 10-15 days post estrus. If an inseminated animal is pregnant, the P4 levels remain elevated throughout pregnancy. If the inseminated animal is not pregnant, P4 levels will drop from day 18 post insemination as the animal will be coming back to estrus (Nebel *et al*, 1987; Orchard, 2007).

High levels of P4 in serum or milk between days 18 and 24 after insemination form the basis of establishment of pregnancy in cattle and low levels at estrus form a basis for establishment of estrus status (Orchard, 2007). Progesterone concentrations vary with the stage of the estrous cycle which makes it one of the most commonly studied reproductive hormones in bovine ruminants for pregnancy detection and ovarian activity (Posthuma-Trumpie, 2008). Low progesterone levels 18-24 days post insemination accurately indicate non pregnancy. However, high P4 at the same period may be not a specific indicator of pregnancy due to length of estrous cycle variations as well as the incidence of early or late embryonic mortality (Muhammd *et al*., 2000). The advantages of using P4 assays for pregnancy diagnosis include, the feasibility to conduct the test on the farm and early indication of the outcome of an insemination (Nebel *et al*., 1987). The main limitation of practically using P4 assay is that in cases where only a single sample has been taken, breeding dates must be known for accurate interpretation of the results.

2.5 METHODS OF MEASURING PROGESTERONE LEVELS

Analytical methods of P4 determination in blood and milk were developed several years ago, the first being in the 1940s. These were based on extraction of the solvent, spectrophotometric quantification and chromatographic analysis (Edgar, 1953; Short, 1958). These techniques were expensive, labor intensive, time consuming and sample pretreatment was required (Short, 1958). The development of radio assays for steroids in the 1960s led to development of P4 radio assays and radio-immunoassays (Holdsworth *et al.,* 1980). The development of ELISAs in the 1960s (Engvall *et al.,* 1971) led to many ELISAs for determination of P4 in blood and milk (Chang and Estergreen, 1983). These ELISA formats allowed widespread use of P4 analysis. An immunoassay using chemiluminescence technology for P4 analysis was also developed (Miller *et al*., 1988).

Other technologies such as the surface plasma resonance assays have also been used for laboratory P4 analysis (Gillis *et al*., 2006). Progesterone sensors in milk both online and offline were also developed (Pemberton *et al*., 2001). However, none of these technologies has been successfully commercialized, mainly because they are complex and/or expensive (Orchard, 2007). Most cow-side tests are simplified enzyme immunoassays, with several in a lateral flow format (Laitinen and Vuento, 1996; Sananikone *et al*., 2004; Posthuma-Trumpie *et al*., 2009).

2.5.1 Enzyme-linked immunosorbent assay (ELISA)

The development of the ELISA format in the 1970s was a major breakthrough in assay technology. Progesterone analysis in ng/ml can be determined in 2-3 hours and there is no use of hazardous material as in RIA. Furthermore, the technology also led to the development of portable test kits such as home pregnancy tests and cow-side P4 tests (Pemberton, *et al*., 2001). All ELISA formats involve binding an enzyme to an antigen or antibody and some method of binding the conjugate to

a solid surface to allow detection by the addition of a substrate. In a typical P4 assay, anti-P4 antibody is coated on the micro plate surface. The sample is exposed to this surface and the P4 in the sample which is the antigen binds to the antibody. A P4 enzyme conjugate is then exposed to the surface and the P4 already bound limits the amount that can bind to the immobilized antibody. A substrate is then exposed to the surface and the color change depends on the amount of bound enzyme, which is inversely correlated with the P4 concentration in the sample (Elderet *et al*., 1987). The assay can be competitive or non competitive. In a competitive enzyme immunoassay, the wells of the micro plate are coated with antibodies for the specific analyte. The analyte in the sample and the analyte conjugated to an enzyme (horseradish peroxidase or alkaline phosphatase), compete for binding sites on the antibody.

The excess of conjugate is washed away, and after addition of a substrate, the bound enzyme catalyzes the formation of a colored product (Simersky *et al*., 2007). The analyte could also be conjugated to a protein which competes with free analyte in the sample for the added antibody (Elderet *et al*., 1987). In a non-competitive enzyme immuno assay format, antibodies are pre incubated with the sample or standard and unoccupied binding sites are then available for binding to the analyte conjugate. Competitive assays usually have a higher sensitivity but with addition of one extra step in the procedure (Simersky *et al*., 2007).

Enzyme linked immunosorbent assays are normally carried out on many samples simultaneously in a 96-well micro-titre plate. Reagents are pipetted into the wells and manually discarded. The optical density is measured using a plate reader, which measures the transmission through every well (Simersky *et al*., 2007). Enzyme linked immunosorbent assays are advantageous due to the relative simplicity of the detection system. They measure the optical density by shining light through the liquid. The disadvantages of the plate format include: non flow-through nature of the

assay, takes a longer time due to addition of substrate and the washings make the assay complex (Simersky *et al*., 2007).

2.5.2 Lateral flow (immuno) assay (LFIA)

The LFIA technology has been widely applied in qualitative, semi quantitative and to some extent quantitative diagnostic purposes such as detection of hormones, drugs, pathogens and metabolites in biomedical, phytosanitary, veterinary, feed/food and environmental settings (Safronova *et al.*, 2012). The strips are made up of a carrier material containing dry reagents that are activated by applying the fluid sample. They are especially designed for single use at point of care/need, i.e. outside the laboratory. The best known application is the human pregnancy test (Posthuma-Trumpie *et al*., 2009). The current generation of LFIAs have high sensitivity, high specificity, are easy to use and results usually are obtained within 10–20 minutes (Posthuma-Trumpie *et al*., 2009).

Important in the LFIA formats is the flow of a liquid sample containing the analyte of interest, along a strip of polymeric membrane thereby passing various zones where molecules have been attached that exert specific interactions with the analyte (Posthuma-Trumpie, 2008). The LFIA test is made up of a porous polymeric membrane whereby one end of the strip is provided with a sample application pad and the other end with a conjugate release pad. Labelled analyte is dried on the conjugate pad and after addition of the sample, this material interact with the fluid flow, initiating specific interactions to give required response. Both sample pad and conjugate pad are connected to one another and to the polymeric membrane (Sananikone *et al.*, 2004). Labels are made of colored or fluorescent nanoparticles with sizes of 15–800 nm, allowing an unobstructed flow through the membrane. They are often made of colloidal gold or latex (Al-Yousif *et al*., 2002). At least two lines are sprayed on the strip: a test line and a control line. The response is read

at the testline. A response at the control line confirms a proper flow of the liquid through the strip. More test lines can be applied allowing for multianalyte testing or for semi quantitative evaluation of the response(Al-Yousif *et al*., 2002). When exclusively antibodies are used as recognition elements, the tests are called lateral flow immunoassays (LFIA).

The LFIA set-up is designed to confirm the presence or absence of an analyte. Antibodies to the analyte are used for recognition (Laitinen and Vuento, 1996). There are two layouts of application of the antibody. In the first method, the antibody is be sprayed at the test line and a mixture of sample analyte and labelled analyte is applied at the conjugate pad. The two compete for binding sites on the antibody at the test line (Laitinen and Vuento, 1996). In the second method, an analyte conjugate is sprayed at the test line, and a mixture of labelled antibody and sample analyte is applied at the conjugate pad, giving the sample analyte a head start for binding to the antibody (O'Keeffe *et al.,* 2003). The control line is made up of immunoglobin G raised against the animal species of the labeled antibody. In the competitive LFIA format the response is negatively correlated to the analyte concentration.

One of the weakness of the LFIA is the subjective nature of the visual interpretation of the semi quantitative results. This can be overcome by using a measuring device to evaluate the signal such as a hand held reflectometer (Zaytseva *et al*., 2004), or when a more precise evaluation is required, the signal can be digitized using a flatbed scanner and dedicated to a software for analysis (Van-Amerongen and Koets, 2005) however, costs and analysis time will increase.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY AREA

The study was carried out at the University of Nairobi Veterinary farm from January to April, 2016. The farm is located on a 375 acre piece of land in Kanyariri sub location of Kabete constituency, Kiambu County and West of Nairobi city. It is 15 kilometers from Nairobi city centre (Figs 3.1 and 3.2). Kiambu county is located in the highlands of Kenya and lies between latitudes 00 25' and 10 20'South of the equator and longitude 360 31' and 370 15' East. The whole of this region experiences a warm climate, with temperatures ranging between 12 and 20 degrees Celsius and an average annual rainfall of 1200mm. The region experiences long rains between March to May followed by a cold season with drizzles from June to August and short rains between October and November. The warm to cool climate makes the county conducive for dairy farming.

This farm was purposively selected due to its high population of dairy cattle and therefore cattle at different stages of the reproductive cycle would be available at any one point, which was convenient for this research. The animals are reared in an extensive system where they graze all day and supplementation is done with concentrates (dairy meal) and silage in the morning and evening. Water and mineral licks are provided ad libitum. Animals on the farm are bred by artificial insemination.

Figure 3. 1: Map of Kenya showing position of Kiambu County (red)-source Wikipedia

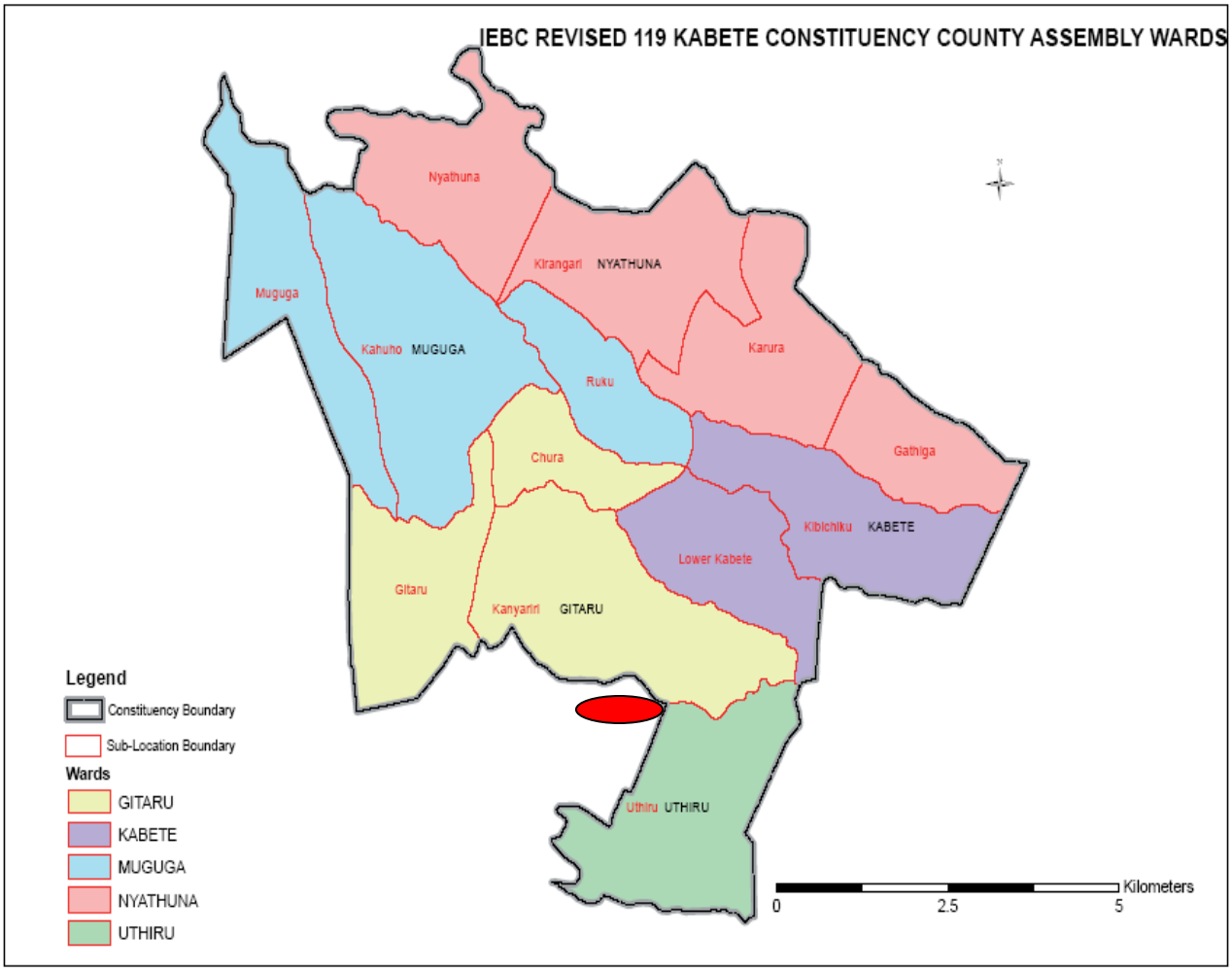


Figure 3. 2: Administrative map of Kabete Constituency showing position of Kanyariri sub-location-courtesy of IEBC

3.2 SAMPLING DESIGN AND SAMPLE SIZE

This was a cross-sectional study in which the study animals were purposively selected. The study at the farm was done in two phases. Phase one evaluated blood P4 profiles in the dairy cattle in Kenya. Phase two determined P4 levels in whole blood in the dairy cattle using LFIA and compared the findings with those by the standard ELISA test.

A multistage sampling criteria of the animals was followed. A sampling frame which was the list of all the dairy cattle kept at the farm was obtained. This list had a total of 180 female animals of

the Friesian, Ayrshire, Jersey and Guernsey breeds at different stages of the reproductive cycle. Records of these 180 animals were examined. From this, 160 animals ranging from 5 months to 12 years of age were included based on their health status and good reproductive history. The Friesian breed had the highest proportion at 59% (94), followed by Ayrshire at 23% (38), Guernsey at 10% (16), Jersey at 5% (8) and the lastly the crosses 2% (4). The important reproductive information obtained from the records included age, cyclicity status, pregnancy status, data of last calving, chronic infertility disorders and health status. The animals were categorized as pregnant or non pregnant using the reproductive records. Further stratification of the pregnant animals was done based on the estimated age of pregnancy as calculated from the date of insemination as first, second and third trimester of pregnancy. Based on estimated age, non-pregnant animals were categorized as either pre-pubertal or pubertal. Subsequently, subject pregnant and non-pregnant animals were selected by generation of random computer numbers. The different breeds of cattle were represented in the sample size. The reproductive status of the selected animals was confirmed by rectal palpation and trans- rectal ultrasonography. The aim of the confirmation of the reproductive status was to enable grouping of the animals into expected high P4 and low P4 groups prior to sampling. An additional group of animals whose reproductive status was not clinically confirmed was used in phase two of the study (unknown P4 level group). The unknown group was to used as a control to assess the agreement of ELISA and LFIA findings without prior knowledge of the expected P4 levels of the animals. Trans-rectal ultrasonography was carried out using a real time B mode ultrasound scanner (Aquila vet Esaote Europe B.V.Philipsweg 1 6227 AJ Maastricht the Netherlands) equipped with a 6–8 MHz linear endo-rectal transducer. Ultrasonography was performed on non pregnant pubertal cattle and those that were expected to be ≤3 months pregnant. Trans-rectal palpation was done to confirm pregnancies that were ≥3 months. Positive diagnoses of pregnancy by trans-rectal ultrasonography were dependent on the detection of anechoic fetal fluids

and/or the embryo proper/fetus in the uterine lumen (Lucy *et al.*, 2011). The ovaries were scanned for presence of the corpus luteum and/or follicles. The animal protocols used were approved by the biosafety, animal use and care committee, of the Faculty of Veterinary Medicine, University of Nairobi.

The body condition score of all the selected animals was obtained using a 5 scale grade according to Edmonson *et al.* (1989) and bodyweight in Kg was estimated using a weighing band (Dingwell *et al*., 2006). A field evaluation form (Appendix i) with details of the animal bio-data, reproductive history, feeding regime, body condition score and bodyweight of each selected animal was filled.

The mean weight of the animals was 380±129.36Kg with the minimum bodyweight of 172 Kg and maximum of 600Kg. The median score of body condition was 3 in a scale of 1 to 5 where 1 was emaciated animals and 5 was obese animals. All the animals were apparently healthy during selection and sample collection.

In addition to the two phases of the study, the knowledge, attitude and practices of the dairy industry stakeholders on the use of P4 test kits in reproductive management of dairy cattle was also assessed. The dairy industry stakeholders targeted were Animal Health Practitioners (AHP) from different parts of Kenya (Veterinary Surgeons, Animal Health Assistants and AI Technicians) and dairy farmers who were part of the University of Nairobi Large Animal Clinic Clientele.

Semi-structured questionnaires, one for the dairy farmers (Appendix iii) and the other for the Animal Health Practitioners (Appendix iv) were prepared and pretested before the study commenced. The questionnaire had both open and closed-ended questions. Majority of the questions were closed ended (yes/no response or selection from a list of options) and few of them were open ended. The questions covered aspects of dairy herd reproductive management including: breeding techniques, heat detection methods and interpretation, artificial insemination

timing, pregnancy diagnosis, management of postpartum period and management of poor reproductive performance. Additionally, the questionnaires also captured profile details of the respondents. Sample size was calculated based on the formula $Z^2\alpha pq/L^2$ by Martin *et al*. 1987 where the expected proportion was 50%. At least 100 respondents were required for the study.

A total of 152 dairy industry stakeholders in Kenya were interviewed comprising 127 Animal Health Practitioners (AHP) and 25 dairy farmers. Of the 127 AHP, 36 were Artificial Insemination Technicians (AI technicians), 28 Animal Health Assistants (AHA) and 63 Veterinary Surgeons (Vets).

Questionnaires were administered to AHP from Kikuyu subcounty in Kiambu County. A list of all the practising AHP was obtained from the Subcounty Veterinary office in Kikuyu. The AHP were contacted and interviewed. The questionnaire were also administered during the 50th Kenya Veterinary Association Scientific Conference which is the largest convergence of Animal Health Practitioners. Lastly, questionnaires were administerd during the Estrus Synchronization and Fixed time Insemination Animal Health Practitioners training in Migori, and Siaya Counties. All the questions were clear and it took about 10 minutes to administer the questionnaire.

3.3 BLOOD SAMPLES COLLECTION, HANDLING AND PROCESSING

The subject animals were physically restrained in a crush, and 10 ml of blood collected from the coccygeal vein into heparinized tubes (BD Vacuitainer sodium heparin, Franklin Lakes USA) after swabbing the coccygeal area with a cotton swab soaked in surgical spirit (Fig 3.3). The heparinized tubes were labelled with the animal identification number and date of collection. In phase one of the study, a total of 46 samples were collected, stored in a cool box packed with ice packs and immediately transported to the laboratory where the P4 ELISA test was carried out. In phase two of the study, a total of 100 samples were collected and a portion of it was used for the

LFIA test in the field. The remaining portion of blood was transported to the laboratory where ELISA and LFIA were carried out sisde by side. In the laboratory, blood samples were inspected for any coagulation and placed on the bench for 30 minutes to allow for adjustment of the temperatures to room temperatures.

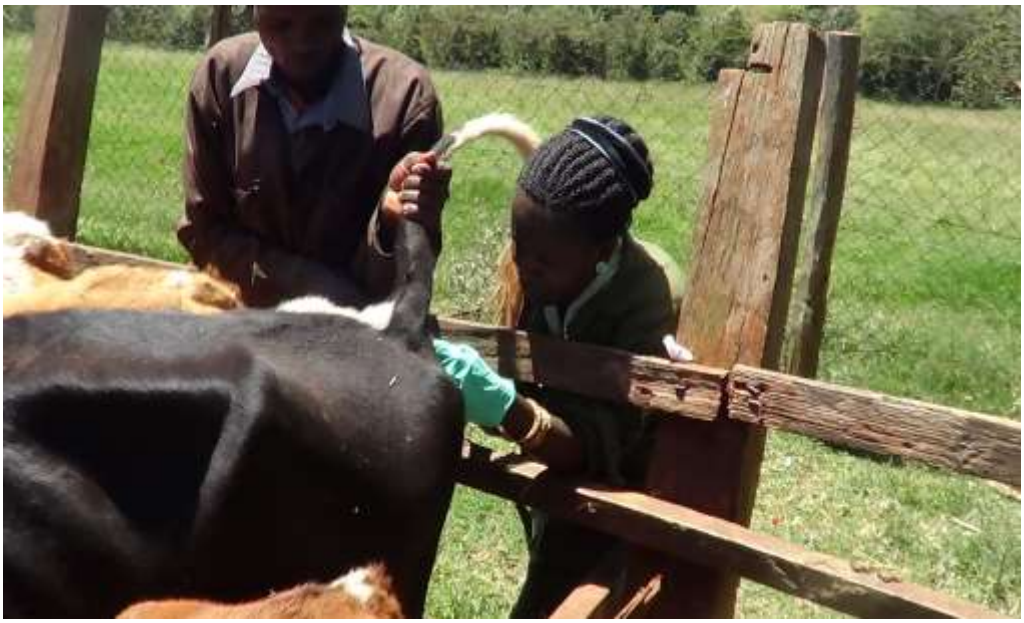


Figure 3. 3: Blood collection from a non pregnant cow tag no. 831 on 23/2/2016 at the University of Nairobi Veterinary farm.

3.4 PROGESTERONE ANALYSIS

3.4.1 ELISA

A volume of 1.5ml of whole blood was pippeted into 2 separate vials and centrifuged at 1000-2000 x g for 15 minutes to obtain plasma for ELISA. The remaining blood was stored in a refrigerator at 4 degrees Celsius until all analysis for the day was complete. Following the centrifugation, the plasma which sits at top of the packed cells was removed by gently lowering a micropipette tip fitted onto a micropipette into the plasma of each sample, being careful not to disturb the cells

below. Slowly the plasma was aspirated into the pipette tip and transferred into new labeled vials. Excess plasma was aliquoted into separate tubes and stored in a refrigerator until completion of the ELISA run. Animal numbers and dates of collection were labelled on each vial. Following completion of ELISA, excess aliquots of plasma were stored at -20 degrees Celsius. Progesterone analysis was done using Ovucheck®, Biovet kit as described by Samsonova *et al*. (2015). Briefly, aliquots of 10µl of standard solutions in duplicate, controls and samples in triplicate were added to appropriate micro plate wells followed by 200µl of conjugate. After incubation for 30 minutes at room temperature and washing, 200µl of substrate was added to each well. The color reaction was stopped after 30 minutes room temperature incubation with 100µl stop solution. The results were evaluated on the ELISA plate reader (SpectraMax Micro plate reader LLC, USA) at 405nm wavelength. A standard curve was drawn using the optical density values for the standards at 1,2.5, 5, 10 ng/ml of P4. Subsequently, the equation for calculation of the corresponding P4 levels of the samples was derived from the standard curve.

The assay works based on the principle that P4 found in the sample will compete with horseradish peroxidase labeled P4 conjugate for binding sites to the antibody coated on the plate. The substrate will be converted to a yellow color in proportion to the amount of P4 horseradish peroxidase labeled P4 bound to the antibody on the plate.

The intra-assay coefficient of variation for the assay was 7.5% and inter-assay coefficient of variation was 15%. The assay calibration curve ranged from 0 ng/ml to 10 ng/ml. The limit of the detection of the assay using a 10µl blood sample was below 0.2ng/ml. The cut off value used for the ELISA was 4 ng/ml similar to what was used by Friggens *et al*. (2008).

3.4.2 LFIA test

The lateral flow assay for each sample was done in triplicate in the field and in the laboratory. The LFIA strips used were manufactured by Diagnostics For All Company, USA. A drop of heparinized blood (35µl) was pippeted into the sample well and 35 µl of diluent added. In a second assay well, 75 µl of chase buffer was added. The LFIA strip was then inserted into the sample well that contained the blood and diluent and timed for 5 minutes, and then moved to the second well with the chase buffer for 10 minutes. After incubation in the chase buffer, the strip was removed from the assay well and results which were in the form of purple color development at the test line and control line on the strip interpreted using the read guide chart. Each of the test was run in triplicate.

The guide chart (Appendix ii) was a sheet of paper made up of three columns of LFIA strips with different reference color intensities of the test lines. The three columns were labelled as score 1, 2 and 3 in which column one (score 1) consisted of reference LFIA strip test lines with high color intensity (dark purple). Column two (score 2) consisted of the reference LFIA strip test lines with a low color intensity (faint purple color) and Column 3 (score 3) was made up of reference test lines with very low color intensity to a non visible test line. Score 1 and 2 were interpreted as low P4 levels while a score of 2.5 (interface between score 2 and 3) and 3 was interpreted as high P4 concentration. Scoring of the lateral flow assay strip results was done visually with the naked eye by Veterinary Medicine students at the University who were not aware of the status of the subject animals.

Lateral flow assay strip images were digitized by scanning using Doxie flip scanner (Apparent Corporation, 121 Dry Ave, Cary, NC 27511 USA) and quantification in optical units of the color intensity of both the test and control lines of the digital images done by a software image analysis

program (image j), so as to obtain true intensity of the color development of the test line. The intensity for each individual strip of each sample was obtained and an average of the intensity of the three strips per sample was calculated. The overall intensity of the LFIA test in animals at different stages of the reproductive cycle was obtained. The obtained overall mean intensity for each category of animals was also used to draw graphs against the corresponding lateral flow assay strip scores and also corresponding quantitative P4 levels by ELISA. In the laboratory, LFIA was carried out side by side with ELISA.

This LFIA test is based on the principle of P4 in the sample competing with immobilized labelled analyte for P4 antibody binding sites. There is binding of P4 in the sample to P4 antibody sites conjugated to gold particles at the conjugate pad zone. Any unoccupied P4 antibody sites will be bound by labelled P4 analyte at the test line resulting to a color development at the test line. The level of P4 in the sample is therefore inversely proportional to the strength of color development of the test line. High levels of P4 are indicated by low color intensity test line signal and low levels of P4 will be indicated by more intense dark test line signal (Waldman and Raud, 2016).

3.5 DATA MANAGEMENT AND STATISTICAL ANALYSIS

The data was entered in Microsoft excel spreadsheet program (Excel, Microsoft Corp 2010, Redmond WA) and then transferred to STATA statistical software (STATA Corp., Version 12, College station USA) for analysis. Significant differences in mean P4 levels in animals at different stages of the reproductive cycle were obtained by Analysis of Variance (ANOVA) and Student t test statistics. The effect of different variables on the P4 levels was assessed by ANOVA. Logistic regression model was applied to determine potential predictors of P4 concentration. Statistical significance was set at probability values of ≤ 0.05.

Lateral flow assay strip images were digitized by scanning using Doxie flip scanner (Apparent Corporation, 121 Dry Ave, Cary, NC 27511 USA) and quantification in optical units of the color intensity of both the test and control lines of the digital images done by a software image analysis program (image j), so as to obtain true intensity of the color development of the test line.

The diagnostic parameters of LFIA test were calculated based on formulas by Karen *et al*. (2015) and Martin *et al*. (1987). The LFIA results were classified as either correct positive (a), false negatives (b), or false positives (c), correct negatives (d). The following diagnostic parameters were calculated: Sensitivity [(a/a + b) x 100], specificity [(d/c + d) x 100], positive predictive value [(a/a + c) x 100], negative predictive value [(d/b+ d) x 100], overall accuracy [(a + d/a + b + c + d) x 100]. A 95% confidence interval of each accuracy parameter of the diagnostic tests was determined.

Sensitivity was defined as the ability of the LFIA test to correctly detect low P4 (positives, <4ng/ml) in concurrence to ELISA. Specificity was defined as the ability of the test to correctly identify high P4 animals (negatives,>4ng/ml) determined to have high P4 by ELISA. The positive predictive value (PV+) was the probability of a positive diagnosis by the LFIA test being further corroborated by the ELISA. The negative predictive value (PV-) was the probability of negative results by the LFIA test being corroborated the ELISA results. Accuracy was defined as the ability of the LFIA test to correctly diagnose high and low P4 cows among those diagnosed as high and low by ELISA test.

The correlation coefficient (r) and Kappa statistics were used to assess the agreement between the LFIA strip scores (semi quantitative analysis) and quantitative analysis of P4 (Martin *et al*., 1987).

A 95% confidence interval of each accuracy parameter of the diagnostic tests was determined.

Data from the questionnaires was coded and entered into Microsoft excel spreadsheet program (Excel, Microsoft Corp 2010, Redmond WA), double checked with the questionnaire information to avoid errors in input and then exported in to STATA statistical software version 12.0 (STATA Corp., College Station, USA) for cleaning and analysis. Descriptive tables were generated and descriptive statistics computed from the questionnaires. Answers that contained continuous variables were summarized as means with their 95% confidence intervals. Pearson Chi square test was used to test for association between categorical variables at $p < 0.05$ significance level. Student t test was used to test for difference in various means of continuous variables. All tests were done at 95% confidence interval. The data was also summarized as graphs and pie charts where applicable. All analysis was done for the overall results as well as based on category of the respondents.

CHAPTER FOUR

4.0 RESULTS

4.1 STUDY ANIMALS

4.1.1 Phase one animals

Forty six animals were used for phase one and they were selected from the 160 animals as shown in Figure 4.1. The high P4 group had pregnant (12) and non pregnant luteal phase (15) animals while the low P4 group comprised of non pregnant follicular phase (7) and non cycling pre-pubertal animals (12).

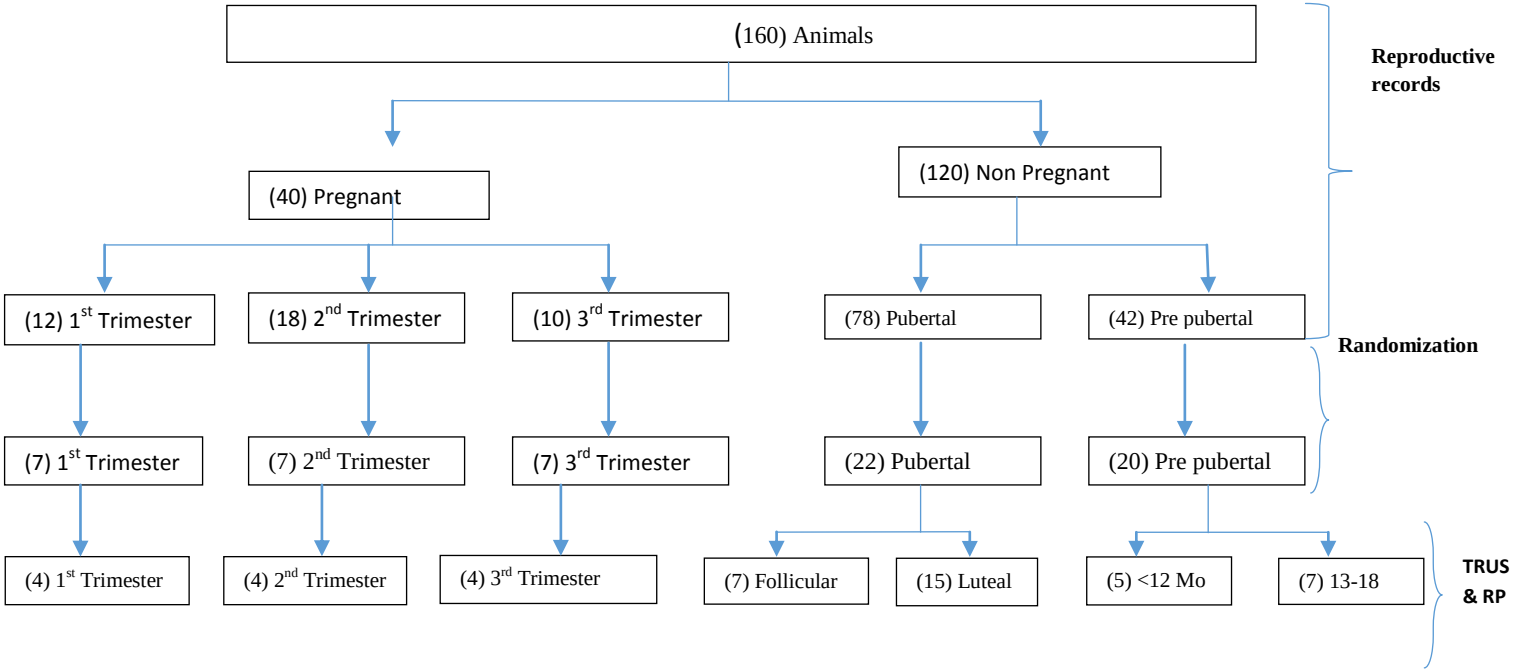


Figure 4. 1: Selection criteria of 46 animals that were sampled in phase 1 of the study

Key: TRUS- Trans-rectal ultrasonography

RP- rectal palpation

4.1.2 Phase two study animals

The selection procedure for subject animals was as shown in Figure 4.2. A total of 100 animals were used for this activity. Based on rectal palpation and ultrasonography, 20 animals confirmed as pregnant and 10 non pregnant luteal phase were categorized as the high P4 group; 29 animals based on ovarian findings categorized as low P4 group comprising of 8 follicular phase, 6 pre-pubertal heifers, 2 in estrus, 5 at one week postpartum and 8 animals at Day one post insemination. A further 41 animals whose reproductive status was not clinically established were categorized as the P4 unknown group.

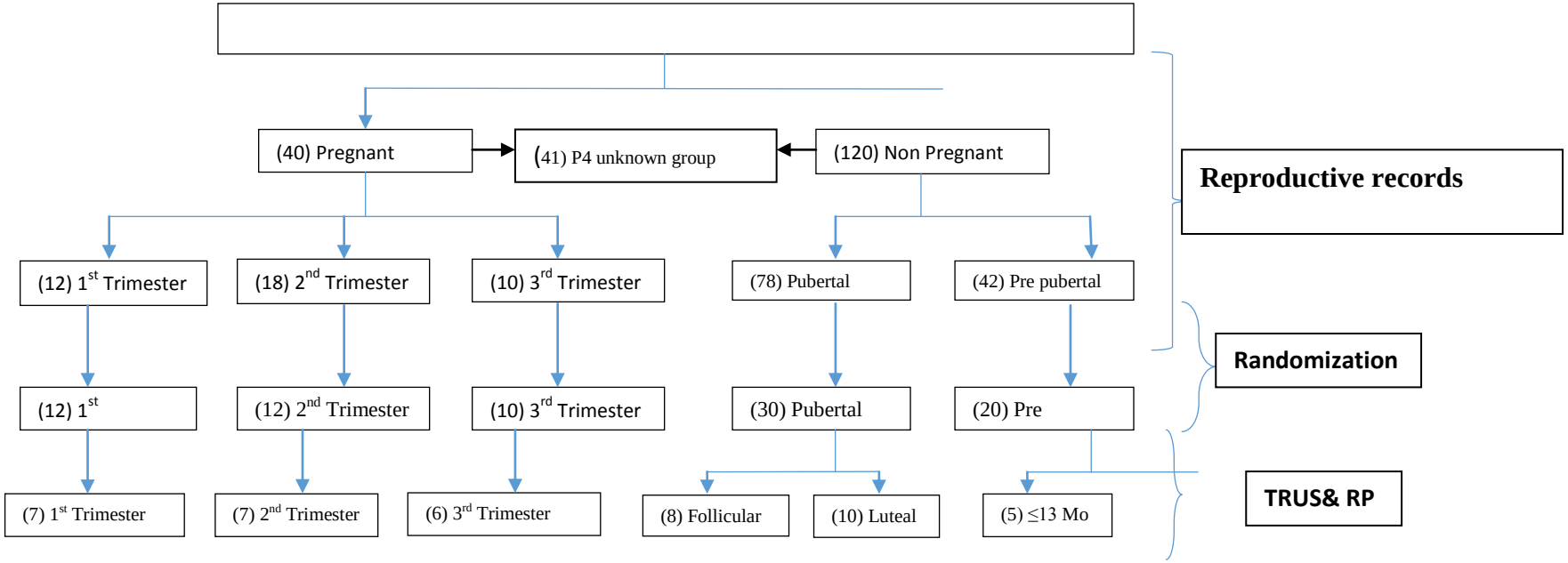


Figure 4. 2: Selection criteria for 100 animals that were sampled in phase 2 of the study

Key: TRUS- Trans-rectal ultrasonography RP- rectal palpation

4.2 PROGESTERONE PROFILES OF DAIRY CATTLE IN KENYA

The P4 profiles across of the 46 animals across different stages of the reproductive cycle ranged from 0.2 ng/ml to 10ng/ml. Non cycling animals aged 18 months and below had progesterone levels of less than 3 ng/ml. Animals above 18 months of age were cycling therefore P4 levels varied depending of the estrous cycle or pregnancy status (Table 4.1).

Table 4. 1: Mean P4 levels of groups of animals in phase one

Group of animals	N	Mean P4 levels (ng/ml)
≤12 months	5	0.8519 ± 0.7817^{a}
Follicular phase	7	1.234 ± 1.0623^{a}
13-18 months	7	2.6257 ± 1.2108^{a}
luteal phase non pregnant	15	7.9820 ± 2.2571^{b}
Pregnant	12	9.5280 ± 0.8498^{c}

Values with different superscripts within the same column differ significantly ($p<0.05$).

The mean P4 levels of high P4 group (pregnant and non pregnant luteal phase) was higher ($p<0.05$) than that of low P4 group of animals (≤12 months of age, follicular phase, 13-18 months of age) (Table 4.1).

4.2.1 The effect of age, pregnancy age, weight, breed and body condition score on progesterone levels.

The effect of age, weight, breed and body condition score on P4 levels in animals not significant ($p>0.05$). There were small significant differences between the early pregnany ELISA P4 levels and advanced pregnancy P4 levels ($p=0.06$).

4.3 PROGESTERONE LEVELS AS DETERMINED BY LFIA AND ELISA

4.3.1 LFIA and ELISA results

A total of 100 samples were analyzed for P4 levels using the LFIA and ELISA tests (Table 4.2). The stages of reproductive cycle of the P4 unknown group were based on P4 analysis results (LFIA and ELISA) and reproductive status from the records. They were also classified as either high P4 or low P4 group.

The LFIA scores were classified into two classes: strip scores of 1-2 were classified as low P4 (0 - 4ng/ml). The low P4 had a high intensity colour testline. The score of 2.5-3 were classified as high P4 (4.1-10 ng/ml).

Low P4 group of animals (pre-pubertal heifers, animals in estrus, Day one post insemination, one week postpartum cows and follicular phase animals) had LFIA scores of less than 2 and ELISA P4 levels of less than 2 ng/ml. The high P4 group of animals (pregnant and non preganant luteal phase) had LFIA scores of 3 with corresponding ELISA P4 levels of above 7 ng/ml (Table 4.2).

Table 4. 2: **Progesterone levels of cattle across the reproductive cycle by LFIA and ELISA**.

Sample size	Category of animals	LFIA score in the field	LFIA score in the lab	Mean P4 levels by ELISA (ng/ml)
19	Pre-pubertal heifers 13 months and below	1.6±0.47 [a]	1.6±0.47 [a]	1.453±0.950 [a]
14	follicular phase	1.25±0.31 [a]	1.25±0.31 [a]	1.234±1.0623 [a]
2	Estrus	1.0 [a]	1.0 [a]	0.3046±0.151 [a]
5	One week postpartum	1.3±0.45 [a]	1.3±0.45 [a]	1.066±0.5242 [a]
8	Day one post insemination	1.3±0.70 [a]	1.3±0.70 [a]	1.041±0.642 [a]
17	Non pregnant luteal phase	3.0 [b]	3.0 [b]	7.972±1.852 [b]
35	Pregnant	3.0 [b]	3.0 [b]	8.876 ± 0.7823 [c]

Values with different superscripts within the same column differ significantly ($p<0.05$).

The LFIA scores and mean P4 levels by ELISA were significantly different between the low P4 group (pre-pubertal heifers, animals in estrus, Day one post insemination, one week postpartum cows and follicular phase animals) and the high P4 group of animals (pregnant and non preganant luteal phase) ($p<0.05$) (Table 4.2).

4.3.2 Correlation between LFIA scores with corresponding P4 levels by ELISA

LFIA scores from 1 to 1.5 had corresponding mean ELISA P4 levels of below 4 ng/ml. Score of 2 had upper limit corresponding to P4 values of above 4 ng/ml although most values were below the cut off of 4ng/ml. A score of 2.5 had lower limit values corresponding to P4 levels of below 4ng/ml although most of the values were above 4ng/ml. The score of 3 had corresponding mean ELISA P4 levels of above 5ng/ml (Figure 4.3).

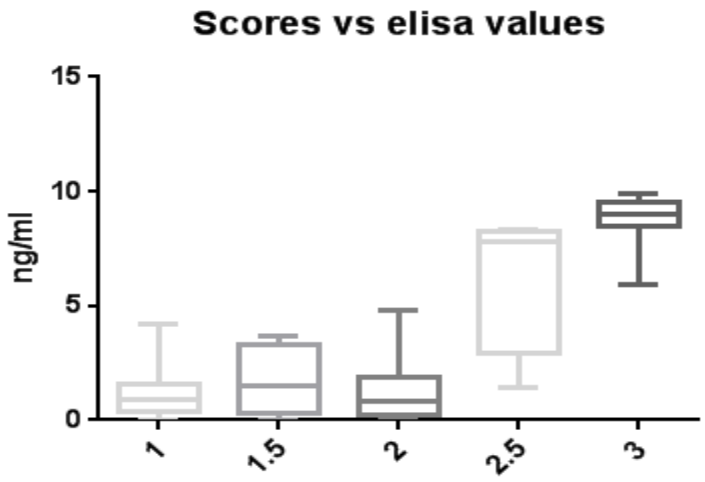


Figure 4. 3: Quantitative ELISA P4 levels against corresponding LFIA scores.

4.3.3. LFIA score at different stages of the reproductive cycle.

The low P4 group of animals (pre-pubertal heifers, Day one post insemination and one week postpartum animals) had LFIA strip scores of less than 2 while high P4 group of animals (pregnant and non pregnant luteal phase) had LFIA score of 3 (Figure 4.4).

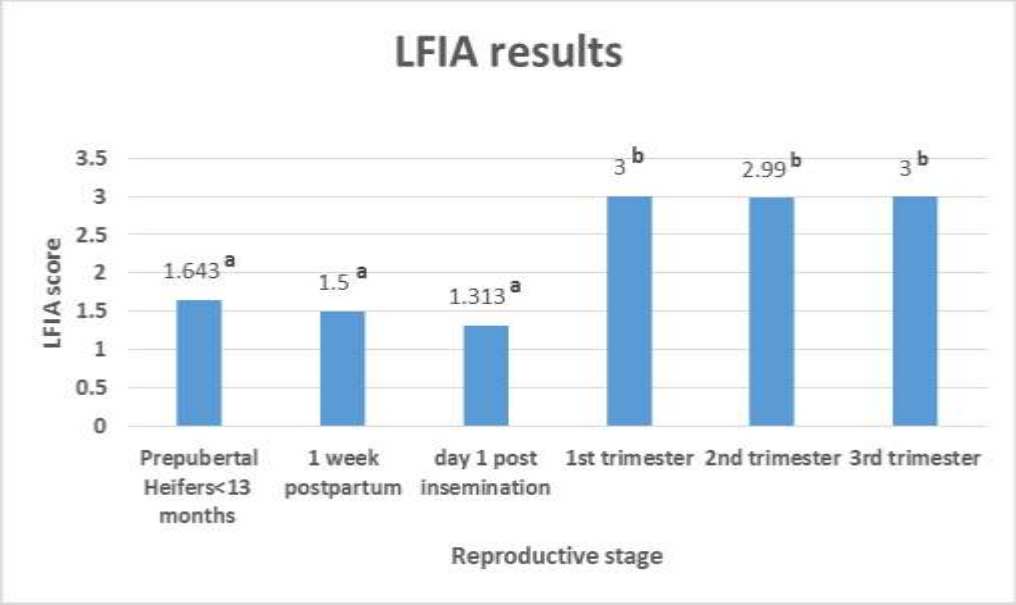


Figure 4. 4: Lateral flow immunoassay strip scores at different stages of the reproductive cycle.

Bars with different superscripts have significantly different LFIA scores.

The LFIA scores of the high P4 animals (pregnant animals) was higher ($p<0.05$) than that of low P4 animals (Day one post insemination, one week postpartum and prepubertal heifers (Figure 4.4).

4.3.4. Quantitative intensity in optical units of the LFIA strips

High testline intensity of the LFIA strips corresponded to low P4 levels while low intensity of the LFIA strips testlines corresponded to high P4 levels. The high intensity in optical units was recorded in pre-pubertal heifers, Day one post-insemination and one week postpartum wheres the low intensity in optical units was recorded in pregnant animals (Figure 4.5).

Figure 4. 5: Lateral flow immunoassay strip intensity values at different stages of the reproductive cycle.

Bars with different superscripts have significantly different intensities.

The intensity values for the high P4 animals (pregnant animals) was higher ($p<0.05$) than that of low P4 animals (pre-pubertal heifers, Day one post-insemination and one week postpartum animals) (Figure 4.5).

4.3.5 Comparison of the color intensity of the LFIA and the corresponding LFIA scores

The LFIA strips with scores of 2 and below had high intensity values of above 18698.73 optical units while LFIA strips with scores of 2.5-3 had low intensity values of 16659.17 and below (Figure 4.6).

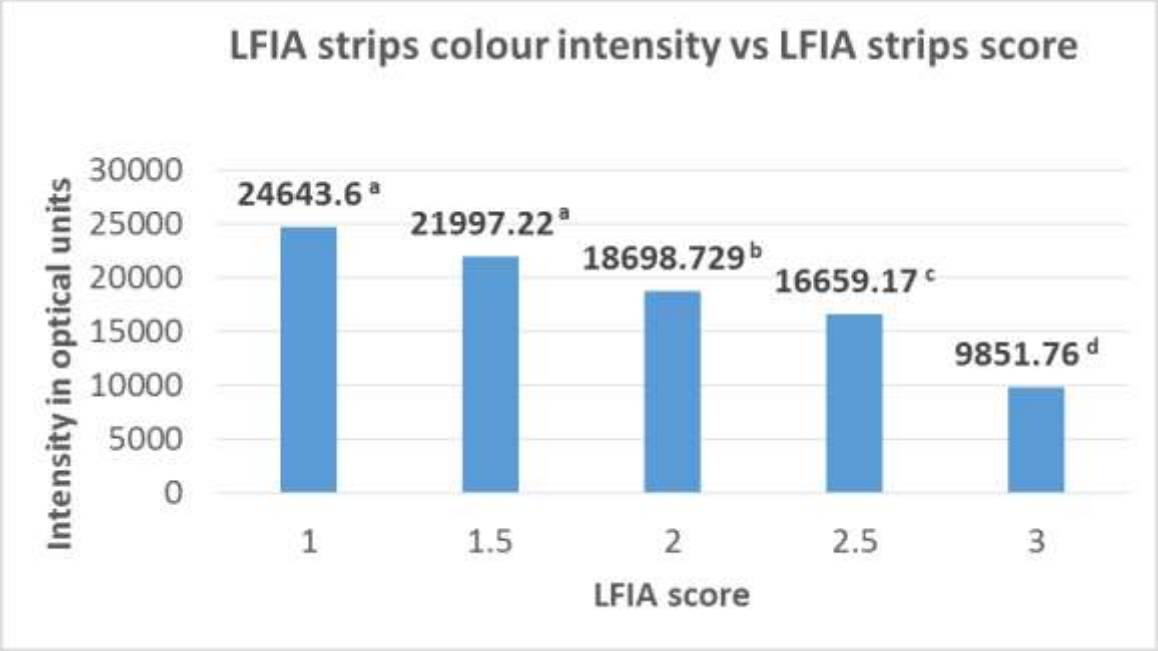


Figure 4. 6: Lateral flow immunoassay color intensity and corresponding LFIA scores.

Values depicted by bars with different superscripts differ significantly ($p<0.05$).

In summary, the intensity of LFIA strips with scores of 3 was significantly different from the intensity of LFIA strips with score of 1, 1.5 and 2 ($p<0.05$). However, there was an overlap in the intensity of the LFIA strips with scores of 2 and 2.5 (Figure 4.6).

4.3.6 Calculation of diagnostic values of LFIA.

The LFIA test diagnostic values sensitivity, specificity, predictive values and accuracy were calculated with the ELISA as the standard test (Table 4.3).

Table 4. 3: Diagnostic values for the LFIA test

	LFIA positive (score 1-2)	LFIA negative (score 3)	Total
ELISA positive	49	1	50
ELISA negative	4	46	50
Total	53	47	100

			Confidence interval
Specificity	0.92	46/50	(0.87-0.97)
Sensitivity	0.98	49/50	(0.95-1.00)
PV-	0.98	46/47	(0.95-1.00)
PV+	0.92	49/53	(0.87-0.98)
Accuracy	0.95	46+49/100	(0.92-0.99)

4.3.7 Lateral flow assay strip results for the high and low P4 animals.

The LFIA score for high P4 animals for example pregnant animals was 3 and the testline was non visible while that of low P4 animals such those in estrus animals was 1 and testline colour was dark purple (Figure 4.7 and 4.8) respectively. The blue arrow points to the control line while black arrow points to the testline.

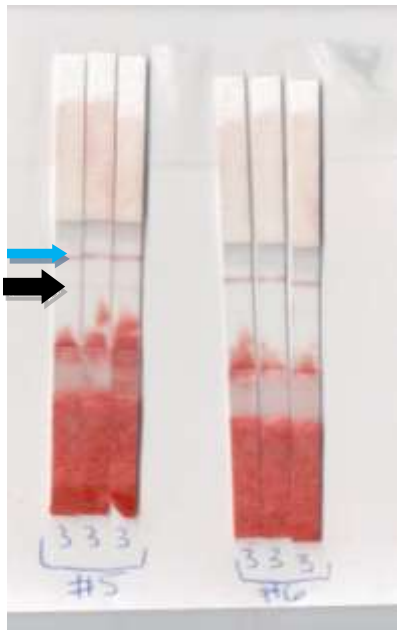


Figure 4. 7: LFIA strip showing non visible test-line in high progesterone levels

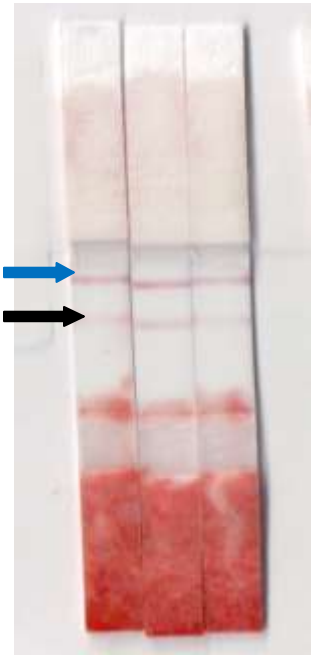


Figure 4. 8: LFIA strip score results showing dark test line for low P4 levels.

4.4 KNOWLEDGE, ATTITUDE AND PRACTICES OF THE DAIRY INDUSTRY STAKEHOLDERS ON THE USE OF PROGESTERONE AS A REPRODUCTIVE MANAGEMENT TOOL.

4.4.1 Demographic characteristics of AHP.

The respondents aged 18-30 years of were 19%, 31-40 years were 40%, 41-50 years were 19% and those aged above 50 years were 22%. Most of them were males (87%). Almost half (49%) of the AHP had been practitioners in the livestock industry for more than ten years with those having practiced for less than one year being the least at 5%. A third of the practitioners were certificate and University degree holders at 33% each whereas 13% and 21% respectively were Diploma and postgraduate degree holders. Most of the practitioners (73%) were engaged full time in the livestock sector (Table 4.4).

Table 4. 4: **Demographic characteristics of AHP**

Characteristic		n	Percentage (%)
Profession	AI technicians	36	28
	Animal Health Assistant	28	22
	Veterinary Surgeon	63	50
	Cumulatively	127	100.00
Age	18-30 years	24	19
	31-40	51	40
	41-50	24	19
	Above 50	28	22
	Cumulatively	127	100.00
Gender	Male	110	87
	Female	17	13
	Cumulatively	127	100.00
Highest level of education	Certificate	42	33
	Diploma	17	13
	Degree	42	33
	Postgraduate	26	21
	Cumulatively	127	100.00
Period of practice	Less than 1 year	6	5
	1-5 years	34	26
	6-10 years	25	20
	More than 10 years	62	49
	Cumulatively	127	100.00
Part time/full time practice	Full time	93	73
	Part time	34	27
	Cumulatively	127	100.00

4.4.2 Demographic characteristics of the dairy farmers

A total of twenty five dairy farmers in Kiambu County were interviewed in this study. Farmers aged below 30 years were 12%, those between 31-40 years were 40% and older farmers aged above 40 years were 48%. Males made up the majority of respondents with 56% and females 44%. Fifty six percent of the dairy farmers indicated that they had attained college diploma education but not in livestock farming. Graduates from the University were 32% and 12% had secondary school education as the highest level of education. Majority of farmers (72%) had kept dairy cattle for more than 7 years, while 20% had been involved in dairy farming for a period between 4-7 years and 8% had been in dairy farming for between 1-3 years (Table 4.5).

Table 4. 5: **Demographic characteristics of dairy farmers.**

Characteristic		n	Percentage
Age	Below 30	3	12%
	Between 31-40 years	10	40%
	Above 40 years	12	48%
Gender	Male	14	56%
	Female	11	44%
Highest level of education	Secondary	3	12%
	College diploma	14	56%
	Degree and above	8	32%
Duration as a dairy farmer	1-3 years	2	8%
	4-7 years	5	20%
	More than 7 years	18	72%

4.4.2.1 Farm enterprise characteristics

About half (52%) of dairy farmers kept less than 5 animals with the other half divided between those keeping 6-10 animals (40%) and those keeping more than 20 animals (8%). Of the animals

kept by the farmers about half of them on each farm were mature breeding cows and the rest heifers and calves. Dairy farming was a part time activity for 76% of the farmers. Dairy farming contributed up to 50% of the livelihood to majority (88%) of the farmers (Table 4.6).

Table 4. 6: Farm enterprise characteristics of the dairy farmers.

Farmers enterprise	Response	n	Percentage of farmers
Total number of animals	< 5 animals	13	52%
	6-10 animals	10	40%
	11-20 animals	0	0
	>20 animals	2	8%
Nature of enterprise	Full time	6	24%
	Part time	19	76%
Contribution of livestock to livelihood	0-25%	0	0
	26-50%	22	88%
	51-75%	3	12%
	76-100%	0	0
Other livelihood sources	Livestock farming	4	16%
	Crop farming	12	48%
	Other businesses	4	16%
	Employment	5	20%
Caretaker of the dairy animals	Husband	6	24%
	Wife	9	36%
	Workers	10	40%
Did they seek services of animal health practitioners?	Yes	25	100%
	No	0	0
If yes which one	Animal Health Technician	21	84%
	Vets	25	100%
Do they cull cows in your farm?	Yes	25	100%
	No	0	0
Reason for culling cows?	Old age	25	100%
	Mastitis	25	100%
	Other diseases	17	68%

4.4.3 Knowledge of Animal Health Practitioners on the use of P4 in reproductive management (Table 4.7)

Only 42% (53/127) of Animal Health Practitioners (AHP) were aware about use of P4 kits for heat detection (31% AI Technicians, 46% AHA and 46% Vets). Forty six percent (59/127) were aware that P4 kits could be used for pregnancy diagnosis. Of this, 17% were AI technicians, 25% AHA and 58% Vets. The proportion of Vets knowledgeable about use P4 kits for pregnancy diagnosis was higher ($p<0.0001$) than that of AI Technicians and AHA. All the AHP irrespective of type of qualification had limited knowledge at 26% (33/127) on the use of P4 in diagnosis of ovarian associated fertility disorders. The younger AHP were more knowledgeable ($p<0.002$) than the older ones about application of P4 kits in diagnosis of fertility disorders.

Table 4. 7: Knowledge of AHP on the use of P4 in reproductive management of dairy cattle.

	Answers given	AI Technicians (n=36)		AHA (n=28)		Vets (n=63)	
Question							
Which methods of breeding are you aware of?	AI	36	100%	28	100 %	63	100%
	Bull	35	97%	26	93%	63	100%
	ET	8	22%	10	36%	53	84%
What factors affect success rate of AI?	Reproductive status of the cow	30	83%	25	75%	56	89%
	Semen handling	31	86%	16	57%	57	90%
	Timing of AI	34	94%	26	93%	60	95%
Methods of heat detection you are aware of?	Observation of heat signs	36	100%	28	100 %	63	100%
	Heat detection aids	10	18%	5	18%	30	48%
	Hormone levels	7	20%	5	18%	33	54%
Are you aware of P4 kits for heat detection?	Yes	11	31%	13	46%	29	46%
	No	25	69%	15	54%	34	54%
What is the optimum time of serving cows after heat detection?	Immediately	0	0	4	4%	3	5%
	After 6 hours	3	6%	3	11%	14	22%
	Use AM/PM rule	34	94%	24	86%	47	73%
At what time should cows be checked for heat	In the morning	36	100%	26	93%	59	94
	In the afternoon	6	16%	5	18%	16	25%
	In the evening	15	42%	8	29%	32	51%
	Mor_aft_eve	6	17%	4	14%	14	22%
How many times in a day should cows be checked for heat?	Once	0	0	1	4%	2	3%
	Twice	19	53%	15	54%	31	49%
	Thrice	17	47%	12	43%	30	48%

How does an inseminator determine if a cow is ready for service?	Clear mucus from the vulva	6	17%	9	32%	33	52%
	Standing to be mounted	28	78%	18	64%	33	52%
	Palpation of graafian follicle	2	6%	2	7%	13	21%
How many times should an inseminator serve a cow per estrus?	Once	27	75%	24	86%	56	89%
	Twice	6	17%	3	11%	5	8%
	Thrice	3	8%	1	4%	2	3%
How soon after calving should cows be served?	Immediately	0	0	0	0	0	0
	45 days	9	25%	10	35%	24	38%
	60 days	13	36%	9	32%	34	54%
	90 days	14	38%	9	32%	5	8%
After how long post insemination is it possible to know the outcome?	1 month	6	17%	9	32%	27	43%
	2 months	4	11%	0	0	16	30%
	3 months	23	64%	17	60%	19	25%
	4 months	3	8%	2	7%	1	2%
Which methods of confirming pregnancy are you aware of?	Non return to estrus	13	36%	14	50%	43%	68%
	Rectal palpation	36	100	26	98%	63	100%
	P4 kits	1	3%	8	29%	34	54%
	Ultrasound	9	25%	7	25%	41	65%
Are you aware of P4 kits used for pregnancy diagnosis?	Yes	10	28%	15	54%	34	54%
	No	26	72%	13	46%	29	46%
Are you aware of P4 kits for diagnosis of fertility disorders?	Yes	9	25%	6	21%	18	29%
	No	27	75%	22	79%	45	71%
Are you aware of AHP using P4 kits for pregnancy diagnosis	Yes	1	3%	3	11%	7	11%
	No	35	97%	25	89%	56	89%
Do you know any methods that can be used to improve reproductive performance?	Yes	27	75%	19	68%	54	86%
	No	8	22%	9	32%	8	13%
	No response	1	3%	0	0	1	1%
Methods of improving reproductive performance?	No response	12	33%	11	39%	14	22%
	Use of ART's	15	42%	10	36%	25	40%
	Improved Nutrition	9	25%	7	25%	24	38%

On reproductive management aspects about heat detection and time of service, all AHP were aware of observable heat signs for heat detection, 34% (43/127) were also aware of use of heat detection aids and 35% (45/127) had heard about use of hormone levels for estrus detection. The

majority of AHP (95%; 121/127) indicated that heat should be checked in the morning only, while 46% (59/127) indicated that heat should be checked thrice a day, in the morning, afternoon and evening. Eighty three percent (105/127) of AHP indicated that animals that are seen on heat in the morning should be served in the evening (AM/PM rule) and majority agreed that animals should be served once per estrus period (84%; 107/127). The knowledge about the AM-PM rule of artificial insemination in relation to start of estrus was higher among AI Technicians ($p<0.05$) compared to other practitioners. Majority of AHP (62%; 79/127) were aware that standing to be mounted was a primary sign for determination estrus before and 38% (48/127) indicated that clear mucus discharge from the vulva was the sign to be used in determination of estrus.

A bigger proportion of AHP (75%) were in agreement that reproductive status of the cow at service, semen handling and timing of AI were important determinants of success of AI. Artificial insemination and natural mating using the bull were the methods of breeding that the AHP were aware of at 100% and 98% respectively. Embryo transfer as a method of breeding was known to 56 % (71/127) of the AHP, most of them ($p<0.0001$) being Vets.

Seventy eight percent (99/127) of AHP indicated that a calving to conception period of 45-60 days was optimal to achieve a calving interval of 13 months. Of this 22% were AI Technicians, 19% were AHA and 59% Vets. The Vets were more knowledgeable that calving to conception interval period should be between 45-60 days ($p<0.001$) as compared to the other practitioners. A slightly larger proportion of AHP (46%; 59/127) indicated that earliest pregnancy diagnosis could be done after 3 months post insemination while 33% (42/127) indicated after one month.

The Vets were more aware ($p<0.001$) of pregnancy diagnosis after one month whereas most of those that stated pregnancy diagnosis should be 3 months, were AI technicians ($p<0.001$). The most common method of pregnancy diagnosis known to the AHP at 98% (125/127) was rectal

palpation. Use of ultrasonography was the second most common method at 45 % (57/127) and lastly, was P4 kits at 34% (43/127). The proportion of Vets among those aware of P4 kits for pregnancy diagnosis was higher than that of AHA and AI Technicians ($p<0.003$).

Most AHP were aware (79%; 100/127) that there were methods available for improving reproductive performance of dairy cattle. However, when told to list those methods only a small proportion (20%; 25/127) were able to do so, indicating adoption of reproductive technologies, continuous training of farmers on proper reproductive management and improved nutrition as possible options (Table 4.7).

4.4.4 Knowledge of dairy farmers on use of P4 in reproductive management of dairy cattle.

Table 4.8 summarises the knowledge of farmers on use of P4 kits in reproductive management. None of the dairy farmers were aware of P4 kits that can be used for heat detection and only 4% were aware of the existence of P4 kits that can be used in early pregnancy diagnosis. Majority of dairy farmers were aware of various aspects of reproductive efficiency; 88% (22/25) indicated that they knew what reproductive efficiency is and they took measures to ensure reproductive efficiency was achieved in their farms. The farmers listed various methods of measuring reproductive efficiency; 60% (15/25) indicated calving interval of 365-400 days, 36% (9/25) indicated that the number of services per conception of should be 2 and the rest (4%) indicated that at least 50% of breeding females should be pregnant at any one time. All the dairy farmers (100%; 25/25) were aware of artificial insemination and use of the bull as breeding methods but only 16% were aware of the embryo transfer technology. All the dairy farmers (100% 25/25) were aware of use of the observable signs of heat for detecting estrus.

Sixteen percent said that they had heard about heat detection devices in developed countries. None were aware of the use of neither P4 kits nor teaser animals for heat detection. Majority of farmers (92%: 23/25) indicated that they knew that if cows or heifers started showing signs of heat in the morning, they should be served in the evening while (8%; 2/25) stated that service should be done 6 hours after the start of estrus.

Farmers were aware of several signs of heat that should be used to determine if a cow was ready to be served. Signs listed by all farmers (100%; 25/25) included, restlessness of the animal, frequent bellowing and clear vulvar mucus discharge. An animal standing to be mounted was listed by only 64% (16/25) of the dairy farmers, who indicated that the animal on heat would attempt to mount others but also when others mounted on her she would stand still, therefore this showed she was in standing heat and ready for service.

Majority of farmers (64%) indicated that the earliest an inseminated animal could be confirmed pregnant was at 3 months usually by AHP using rectal palpation method, although all of them also knew that a cow that did not return to estrus after insemination could be pregnant. Rectal palpation and non return to estrus were the methods of pregnancy diagnosis that were known by all farmers with 40% and 4 % of them, also aware of ultrasonography and P4 kits respectively (Table 4.8).

Table 4. 8: Knowledge of dairy farmers on use of P4 in reproductive management of dairy cattle.

Question	Response	n=25	
Do you know what reproductive efficiency is?	Yes	22	88%
	No	3	12%
Which methods of measuring reproductive efficiency are you aware of?	One year calving interval	15	60%
	Number of services per conception	9	36%
	Number of pregnant animals in the herd	1	4%
Which methods of breeding are you aware of?	AI	25	100%
	Bull	25	100%
	Embryo transfer	4	16%
Which heat detection methods are you aware of?	Observation of heat signs	25	100%
	Use of heat detection aids	4	16%
	Use of P4 kits	0	0
	Use of teaser animals	0	0
What is duration from heat detection to insemination?	Immediately	0	0
	After 6 hours	2	8%
	After 12 hours	23	92%
How do you determine that a cow is ready to be served?	Increased physical activity and bellowing	25	100%
	Clear mucus discharge from the vulva	25	100%
	Standing to be mounted	16	64%
Are you are aware of P4 kits for heat detection?	Yes	0	0
	No	25	100%
After how long is it possible to know the outcome of an insemination?	After 1 month	9	36%
	After 3 months	16	64%
Methods of pregnancy diagnosis that you are aware of?	Non return to estrus	25	100%
	Rectal palpation	25	100%
	Use of P4 kits	1	4%
	Ultrasonography	10	40%
Are you aware of P4 kits used for pregnancy diagnosis?	Yes	1	4%
	No	24	96%

4.4.5 Willingness of Animal Health Practitioners on the use of P4 for reproductive management (Table 4.9)

Majority of AHP (79%; 100/127) believed that P4 kits could be used as a reproductive management tool to improve reproductive performance. Of these, 62% (62/100) indicated that P4 kits would improve reproductive performance since estrus and pregnancy could be detected early. The other practitioners did not give a reason why they thought P4 measurement could be an important reproductive management tool.

Most of the AHP (82%; 104/127) indicated that they would use P4 kits in reproductive management of dairy cattle, 12% (16/127) would not use and 6% (7/127) were not sure whether they would use. Of those that would use P4 kits 29% were AI Technicians, 24% AHA and the majority ($p<0.05$) at 47% were Vets. Inaccuracy of P4 kits was indicated as the reason for unwillingness to use them for reproductive management by 19% (3/16) of the respondents who indicated they would not use them. All the AHP that did not know if they would use P4 kits indicated that they did not have knowledge them. Majority of AHP (72%; 91/127) were positive that the P4 technology for reproductive management would be embraced by Animal Health Practitioners. The reasons they gave for this were improved accuracy and efficiency in heat detection and pregnancy diagnosis using the P4 kits. Most of the AHP themselves stated that they would use P4 kits for pregnancy diagnosis (80%%; 101/127) and estrus detection (67%; 85/127). Ninety one percent (116/127) of AHP stated that they thought P4 kits would be important in early pregancy diagnosis. More Vets as compared to the other practitioners thought that P4 kits would be important in early pregnancy diagnosis ($p<0.05$) (Table 4.9).

Table 4. 9: The willingness of AHP on the use of P4 in reproductive management of dairy cattle.

Question	Response	AHP n=127		AI technicians n=36		AHA n=28		Vet surgeons n=63	
Do you think use of P4 kits will improve reproductive performance?	Yes	100	79%	27	75%	23	82%	50	79%%
	No	20	16%	8	22%	2	7%	10	16%
	I don't know	7	5%	1	3%	3	8%	3	5%
Would you use P4 kits to improve reproductive performance?	Yes	104	82%	30	83%	25	89%	49	78%
	No	16	12%	5	14%	3	11%	8	13%
	I don't know	7	6%	1	3%	0	0	6	10%
Would other AHP use P4 kits in reproductive management?	Yes	91	72%	26	72%	22	79%	43	68%
	No	27	21%	9	25%	5	18%	13	21%
	I don't know	9	7%	1	3%	1	4%	6	10%
Would you use P4 kits for heat detection?	Yes	85	67%	23	64%	18	64%	44	70%
	No	37	29%	12	33%	10	36%	15	24%
	I don't know	5	5%	1	3%	0	0	4	6%
How soon would you like to know the outcome of an insemination?	After 21 days	90	71%	29	81%	20	71%	41	65%
	After 1 months	29	23%	6	17%	6	21%	17	27%
	After 2 months	4	3%	0	0	0	0	4	6%
	After 3 months	4	3%	1	3%	2	7%	1	2%
Do you think it's important to know the pregnancy status early?	Yes	127	100%	36	100%	28	100%	63	100%
	No	0	0	0	0	0	0	0	0
Do you think P4 kits will be important in detecting the outcome of an insemination early?	Yes	116	91%	33	92%	27	96%	56	89%
	No	11	9%	3	8%	1	4%	7	11%
Would you use P4 kits in pregnancy diagnosis?	Yes	101	80%	28	78%	23	82%	50	79%
	No	24	19%	8	22%	5	18%	11	17%
	No response	2	1%	0	0	0	0	2	3%

Seventy one percent (90/127) of AHP would have liked to know the outcome of an insemination after 21 days before the next estrous cycle began while 23% (29/127) stated that they would have liked to know the outcome one month after insemination. The other 6% (5/127) would have liked to know the outcome two months after insemination. All the AHP (100%; 127/127) stated that it was important to know the pregnancy status of an animal as soon as possible (table 4.10). Most (75%; 94/127) indicated that this was important so that non pregnant animals would be re-inseminated promptly to reduce the calving interval and 17% (22/127) indicated that the information would be used by the farmer for early planning.

Generally, the animal health practitioners were in agreement that the use of P4 kits in various aspects of reproductive management would improve the reproductive efficiency in dairy farms (Figure 4.9).

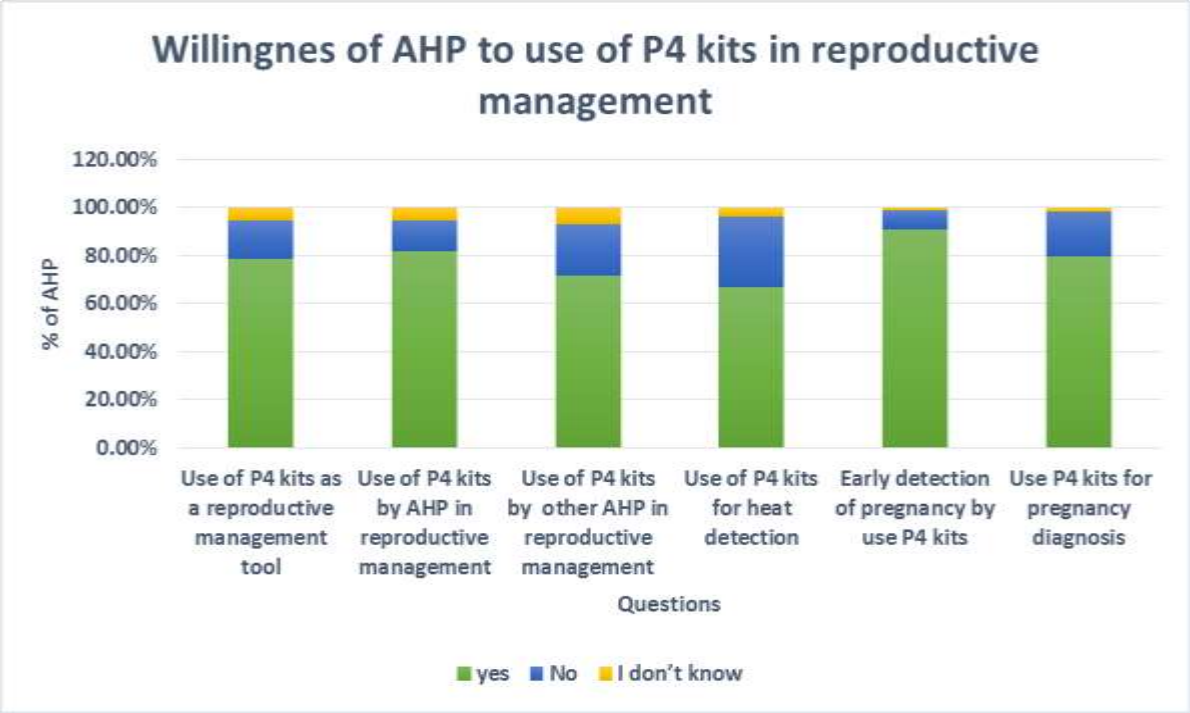


Figure 4. 9: Willingness of AHP to use P4 kits as a reproductive management tool

4.4.6 Willingness of dairy farmers to use of P4 in reproductive management.

After being made aware of the possible applications of P4 kits in reproductive management during the interview, majority of the farmers (76%; 19/25) indicated that they thought the kits would improve reproductive efficiency especially through early detection of pregnancy and 72% (18/25) would use them in general reproductive management in their farms. Additionally, 56% (14/25) indicated that other farmers would also use the kits since they would improve reproductive efficiency in farms.

A small percentage of farmers (12%; 3/25) would use P4 kits for heat detection. Contrary to heat detection, 88% of the farmers were willing to use P4 kits for pregnancy detection to reduce the open days of cows in cases of failed insemination. Most farmers (88%; 23/25) indicated that they would have liked to know the outcome of an insemination after one month, while 12% would have liked to know the outcome after 2 months (Figure 4.10).

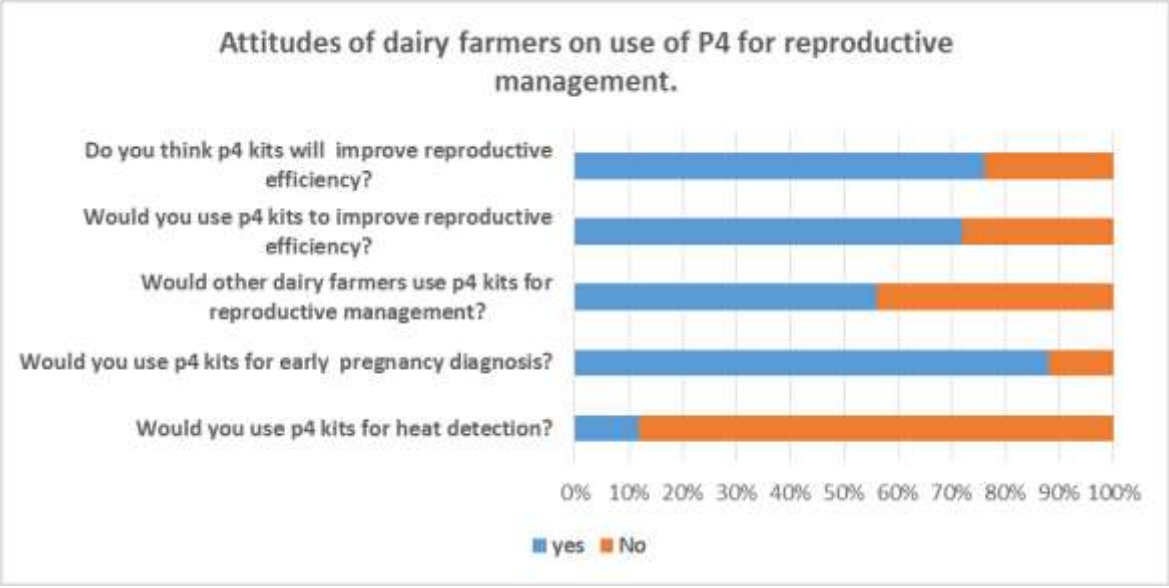


Figure 4. 10: Willingness of dairy farmers on the use of P4 kits in reproductive management.

4.4.7 Reproductive management practices of AHP on the use of P4 for reproductive management.

The majority of AI Technicians offered AI services (94%), followed by AHAs (79%) and then Vets (33%). The proportion of AI Technicians offering AI services was higher ($p<0.0001$) than that of AHS and Vets (Figure 4.11).

All the AHP that offered artificial insemination services used signs of heat to detect heat and none of them used neither heat detection aids nor P4 kits. Majority of the practitioners (74%; 57/127) used the AM-PM rule for determination of the time for insemination. At least 26% (20/77) of the AHP that offered AI services had served a cow not in standing estrus. A large proportion of those (80%; 16/20) attributed this to reliance on farmers on when heat was observed and that by the time they realized the cow was not in standing estrus they had already thawed the semen. The remaining 20% (4/20) attributed this to long distance to the farm, unfavorable weather conditions and challenges of going to the farms at night.

Pregnancy diagnosis was carried out by 77% (98/127) of the AHP. They all used the trans-rectal palpation method at 4 months post insemination (60%), 2 months post insemination (26%) and 11% after 1 month post insemination (11%) (Figure 4.12).

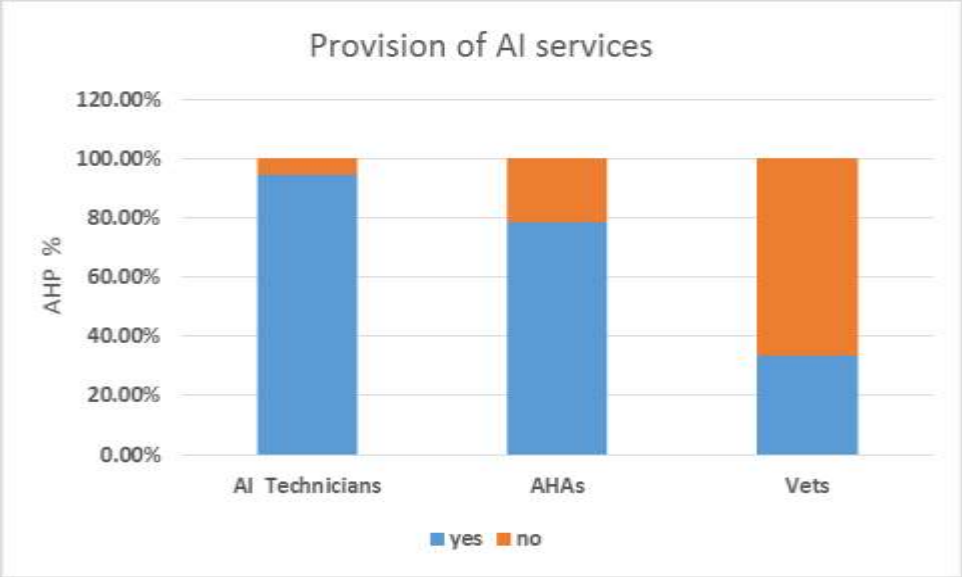


Figure 4. 11: **Proportion of AHP that offer AI services.**

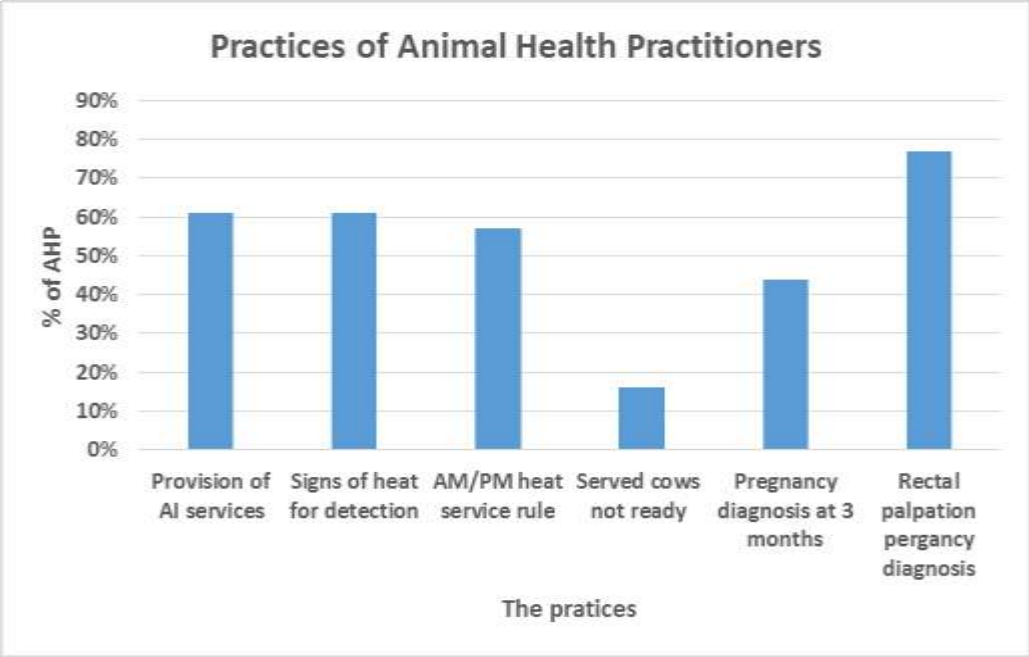


Figure 4. 12: Reproductive management practices carried out by AHP

4.4.8 Reproductive management practices of dairy farmers on the use of P4 in reproductive management.

All the dairy farmers interviewed used only artificial insemination for breeding. They mainly used Animal Health Technicians (AHT) (92%) and seldom Vets (8%). The method for heat detection used by all the farmers was observable signs of heat as exhibited by the animals. The signs used by all farmers included: the restlessness of the animal, frequent bellowing and decreased milk production. In addition to these, 52% (13/25) of farmers used standing to be mounted and 58% (14/25) clear vulval mucus discharge as the signs that the cow was on heat. Majority of farmers (88%; 22/25) reported that their cows were usually served 12 hours after start of heat and the rest indicated that their animals were usually served 6 hours after heat detection.

All farmers assumed that the inseminated animals were pregnant if they did not return to estrus, whereas a small percentage (16%) also indicated that if an inseminated animal had a bloody discharge from the vulva within 7 days after estrus, insemination was assumed to have failed. Eighty eight percent of dairy farmers usually called AHP 3 months after insemination to confirm pregnancy while the rest waited until the inseminated animal returned to heat (Figure 4.13).

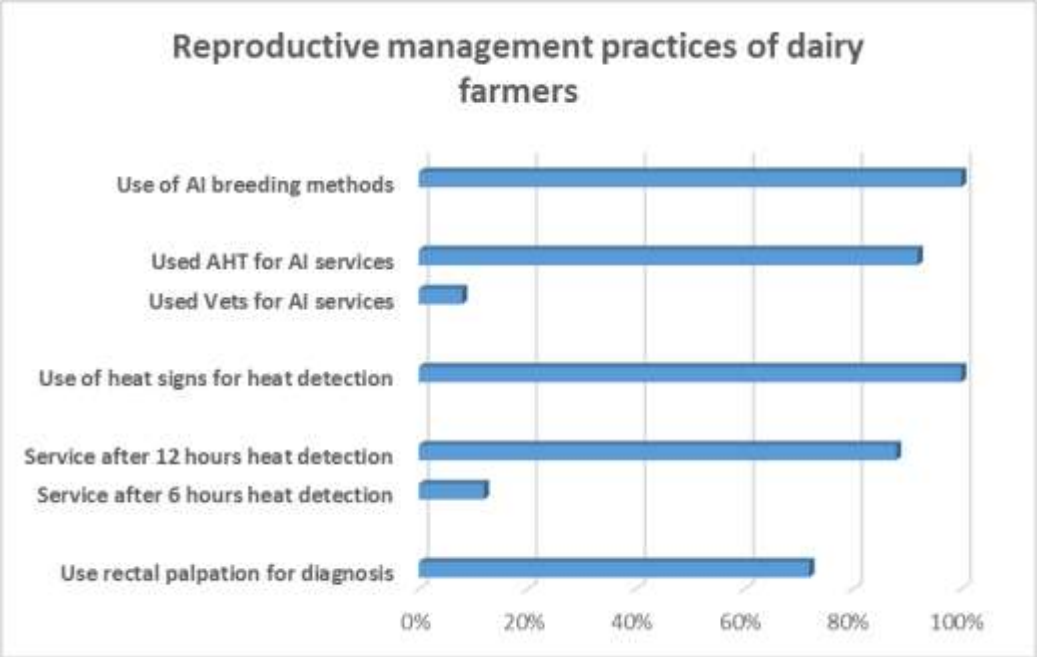


Figure 4. 13: Reproductive management practices of dairy farmers

4.4.9.1 Measures of reproductive efficiency used by farmers

Dairy farmers listed the parameters they used to measure reproductive efficiency in their farms and the values they considered optimum (Figure 4.14). These included: an optimum calving to conception interval of 90 days (44%; 11/25), 2 services per conception (24%; 6/25), 50% of breeding females at any one time pregnant (16%; 4/25) and an optimum age at first calving of 3 years (16%; 4/25) (Figure 4.14).

All farmers indicated that they culled cows with poor reproductive performance after seeking intervention from AHP. They culled these animals since they were incurring costs on management yet the animals were infertile and unproductive.

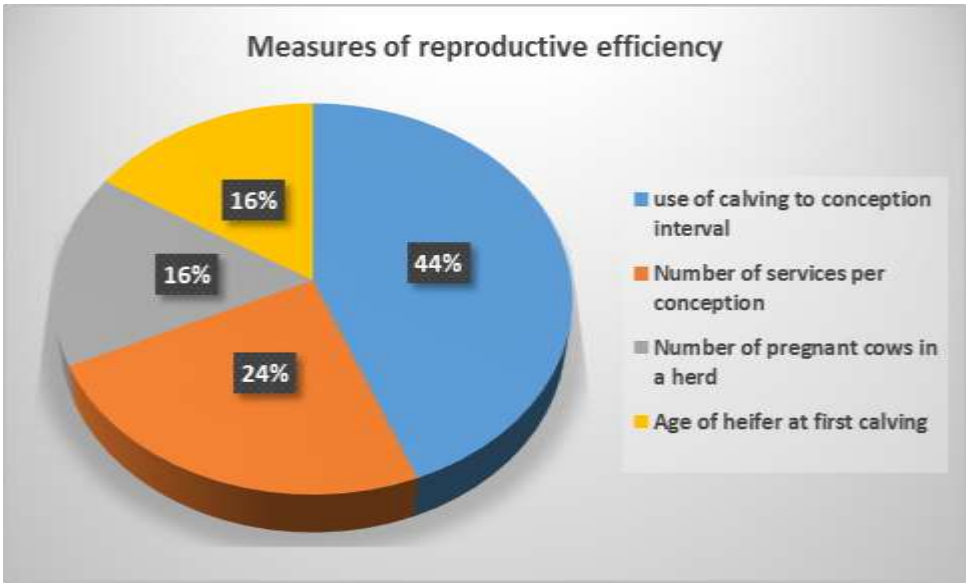


Figure 4. 14: Reproductive indices used by farmers as indicators of reproductive efficiency

4.4.9 Comparison of the knowledge, attitude and practices of AHP on the use of P4 kits for estrus detection and pregnancy diagnosis

None of the AHP used P4 kits for heat detection, however, 42% were aware of these kits and 67% would use them if they were availed to them. None of the AHP used P4 kits for pregnancy diagnosis, although 46% were aware of the use of these kits and 80% would use them if they were availed to them (Figure 4.15).

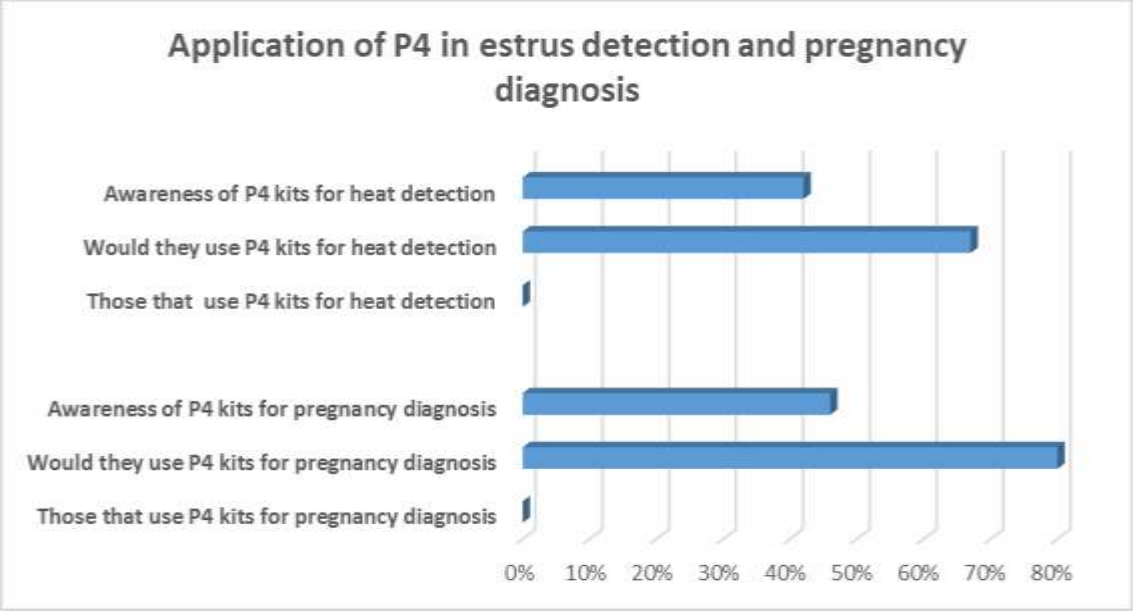


Figure 4. 15: Knowledge, attitude and practices of AHP on the use of P4 kits in estrus detection and pregnancy diagnosis.

4.4.10 Knowledge, attitude and practices of dairy farmers on the use of P4 kits for estrus detection and pregnancy diagnosis.

None of the dairy farmers were aware of P4 kits for heat detection. Only 12 % of them indicated that they would use P4 kit for estrus detection. Only a small percentage (4%) of the dairy farmers were aware of P4 kits for pregnancy diagnosis and 88% indicated that they would use the technology for pregnancy diagnosis especially if the outcome of the insemination was to be known within 24 days post service. None was presently using this technology for pregnancy diagnosis (Figure 4.16). There was no association between the level of education of the farmer and the attitude on the use of P4 kits for estrus detection and pregnancy diagnosis ($p>0.05$).

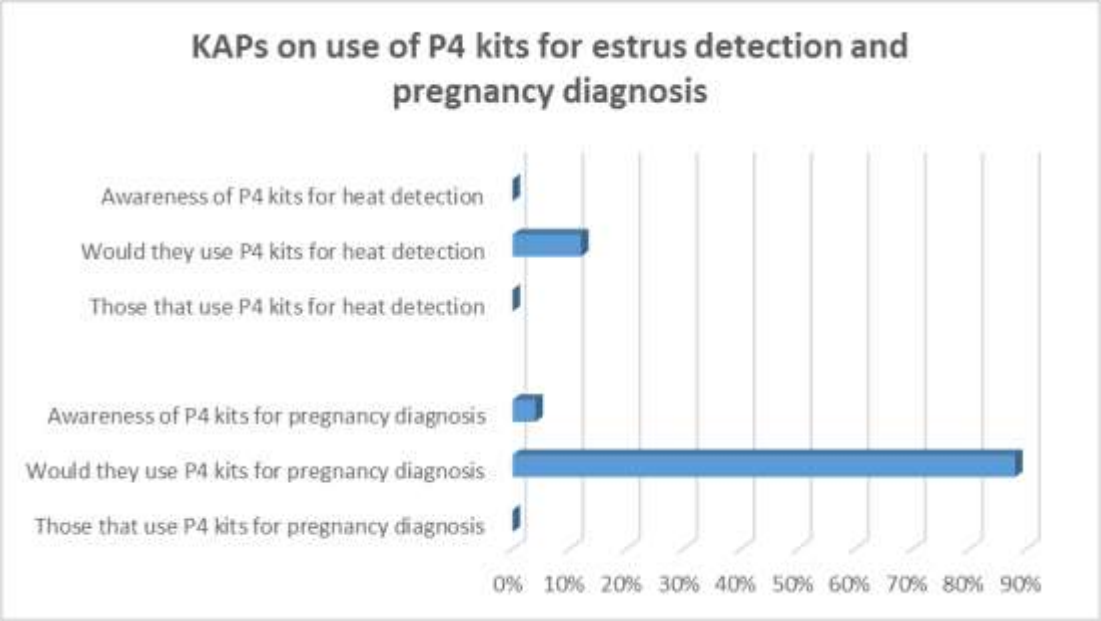


Figure 4. 16: Knowledge, attitude and practices of dairy farmers on the use of P4 kits for estrus detection and pregnancy diagnosis.

CHAPTER FIVE

5.0 DISCUSSION

The dairy industry in the country has experienced tremendous growth and development over the last decade or so. This trend is likely to continue, with the emerging farmers being more knowledgeable and animals kept expected to produce optimally. Consequently, adoption of various technologies to help in management decisions on farms will become more and more common. Blood progesterone levels were determined by ELISA in various categories of cattle in the current study to establish baseline data of progesterone profiles in dairy cattle in Kenya.

Progesterone levels have previously been used in decision support in reproductive management of dairy cattle, with circulating levels indicating what is happening in the reproductive cycle of the cow (Friggens *et al*., 2008). The levels determined will be the indicator of the reproductive status, and can therefore be used in decision making. It is likely that the progesterone values determined, and hence the inference levels for decision making, could be influenced by various factors. Indeed, studies have indicated that breed, body weight, plane of nutrition and body condition can affect the levels of circulating P4 levels (Folman *et al*., 1973; Battochio *et al*., 1998; Mann *et al*., 2005; Rodrigues *et al*., 2010). It is thus plausible that the levels determined elsewhere may not be representative of Kenyan conditions. Due to the potential use of circulating P4 levels in decision support for reproductive management of dairy cattle, in order to enhance the accuracy of interpretations made, the current study determined plasma P4 levels in dairy cattle in the country. Similar to other studies (Schomberg *et al*., 1967; Stabenfeldt *et al*., 1969; Donaldson *et al*., 1970; Henricks *et al*., 1970, Hill *et al*., 1970, Shemesh *et al.*, 1971; Folman *et al.*, 1973; Agarwal *et al*., 1980; Nebel *et al*., 1987; Friggens and Chagunda, 2005; Friggens *et al*., 2008; Naik *et al*., 2013), P4 levels were less than 2 ng/ml in follicular phase, estrous and anestrous heifers and ranged from

5 to 9 ng/ml in luteal phase non pregnant and pregnant cows. Follicular phase and estrus animals are deficient in luteal tissue, the main source of P4, as the CL of the previous cycle had undergone lysis (Nebel *et al*., 1987; Orchard, 2007). Once the animal is in estrus and ovulates, the CL develops and grows, the luteal phase of the estrous cycle, with production of P4 until luteolysis occurs and a new cycle begins (Nebel *et al*., 1987; Orchard, 2007). On the other hand, if the animal is successfully mated, maternal recognition of pregnancy occurs, extending the lifespan of the CL of the estrous cycle, which becomes the CL of pregnancy and continues producing P4 for pregnancy maintenance (Spencer *et al*., 2009).

A previous study in Kenya (Hoka *et al*., 2008), found P4 levels almost twice as high as those found in the current study in both luteal phase and first trimester pregnant animals. That study measured P4 levels in milk using RIA. Higher P4 levels are detected in milk because of the steroid hormones high solubility in the fat fraction of milk , and the findings of that study are supported by those of Ginther *et al*. (1976) and Marcos *et al.* (2008), who found almost twice as high P4 levels in milk than in plasma in samples taken at the same time in the same animal. However, blood plasma P4 profiles determined in the current study are comparable to those seen elsewhere and can therefore be used reliably for decision support in reproductive management of dairy cattle for improved productivity.

The value, adoption and application of diagnostic tools for management decisions to a large extent depends amongst other things on the availability, cost, and ease of use of the tool. Various methods such as radioimmunoassay, enzyme immunoassays, and chemiluminescence assays, have been used to determine P4 in blood and/or milk, to indicate the estrous phase and also pregnancy status in cattle. However, these methods have limitations ranging from requirement of laboratory facilities, are time consuming, are costly, and maybe hazardous (Posthuma-Trumpie *et al.*, 2009). Lateral flow immuno assays, on the other hand, are qualitative, semi quantitative and quantitative

tests that can be used in non-laboratory environments, similar to those found in the small holder dairy systems in Kenya. The LFIAs have been used for various diagnostic purposes including determination of P4 in milk to assess estrus and pregnancy status in dairy cattle (Samsonova *et al*., 2015; Waldmann and Raud, 2016). Information on use of LFIAs in assessment of circulating P4 levels in blood in dairy cattle, and especially in whole blood in dairy cattle is scarce. Progesterone levels were determined in the current study in whole blood across various reproductive phases in dairy cattle in Kenya, and the values compared with those detected at the same time in the same sample by ELISA. Progesterone levels are biologically expected to be low or high at different stages of the reproductive cycle (Rioux and Rajotte, 2004; Otava *et al*., 2007; Cooke and Arthington, 2009; Osman *et al.,* 2012). Using LFIAs, determination of P4 levels in a sample is based on the color intensity of the test line on the LFIA strip, and the amount of P4 in the sample is inversely proportional to the color intensity of the test line. Low P4 levels are expected in pre-pubertal, follicular phase, estrus, one week postpartum and day one post-insemination animals (Nebel *et al*., 1987; Safronova *et al*., 2012). In the current study, these categories of animals corresponded to a high color intensity of the test-lines on the LFIA strips, indicative of low P4 levels. The LFIA strip color intensity was low, indicative of high P4 levels, in luteal phase and pregnant animals, as is expected physiologically (Nebel *et al*., 1987; Safronova *et al*., 2012).

Low P4 levels were represented by LFIA scores of 1 to 2 and high P4 levels by scores of 2.5 to 3. The LFIA strip scores for P4 levels were compared with quantitative P4 values as determined by ELISA. There was a high concordance in LFIA and ELISA P4 findings (r 0.95; kappa 0.93) with scores 1 to 2 representing 0 to 4 ng/ml and scores 2.5 to 3 indicating P4 levels of 4 to 10 ng/ml. These results indicate that LFIA is reliable in detection of P4 levels in whole blood. Additionally, the high statistical difference between all the LFIA scores (1, 2 and 3; $p<0.0001$), further affirms that the LFIA can be used to determine P4 levels in whole blood in dairy cattle at various phases of

the reproductive cycle. Waldmann and Raud, (2016) and Safronova *et al.* (2012) also found that high LFIA strip color intensity scores of 1 to 2 corresponded to P4 levels ranging from 0-4ng/ml while the low color intensity scores of 2.5 to 3 corresponded to P4 levels ranging from 4.1 to 10ng/ml.

In spite of the ability of the LFIA to reliably differentiate high and low P4 levels in whole blood, interpretation of findings at the transition point between high and low P4 may not be concise. The cut off value for high and low P4 in the current study was 4ng/ml as recommended by Friggens *et al*. (2008) which corresponded to LFIA score of 2 or below. However, in some cases LFIA scores of 2.5, which should have represented high P4 levels according the cut off value, were commensurate with low P4 levels as determined by ELISA. This indicates that the interpretation of scores in the interface between high and low P4 can give a low proportion of false positive results. The discrepancy initially was thought to be due to overlap in the reading of the color intensities for low and high P4 levels at LFIA scores of 2 and 2.5. Color intensity readings in the LFIA is done visually, and accuracy may consequently be subjective. However, images of the LFIA strips were made and digitized and the color intensity of the test- and control lines quantified by an image analyzer to rule out subjectivity of visual scoring. Since this quantification of strips confirmed the discrepancy of the readings, it was speculated that there could be cross reactivity of individual animal blood components in some animals with P4 antibody sites on the LFIA strips (personal communication). This therefore indicates that an LFIA score of 2.5 may not be a reliable indicator of relative blood P4 levels and such samples should be rechecked for confirmatory diagnosis of the reproductive status. This notwithstanding, the sensitivity of the LFIA was 98% with an accuracy of 95%. The Kappa agreement between the LFIA and ELISA was 0.93 with a correlation coefficient of 0.95. The specificity of the LFIA test was 92%. These LFIA diagnostic parameter values compare favorably with those obtained previously using milk for P4 determination by LFIA

(Samsonova *et al.,* 2015; Waldmann and Raud, 2016). Lateral flow immunoassay is therefore reliable in determination of P4 levels in whole blood during different phases of the reproductive cycle in dairy cattle.

Progesterone levels as determined in the current study by ELISA were higher in the pregnant animals compared to the luteal phase non-pregnant animals. However, the P4 levels as detected by the LFIA did not differ between the pregnant and non-pregnant luteal phase animals. Although an explanation for these ELISA findings was not available, the LFIA findings in the current study are supported by those of Ghanem and Nishibori, (2015) who reported similarity in P4 levels of pregnant and non-pregnant cows with normal luteal function. The most advanced stage of pregnancy in the animals in the current study was 230 days and P4 levels ranged from 7.9 ng/ml to 10.5 ng/ml. Although not statistically different, the lower P4 levels were recorded in earlier pregnancies and higher levels in more advanced pregnancies. Probably had the sample size been bigger, and more late pregnancy animals included in the sample such differences in P4 levels at different gestational stages may have been seen. These results were similar to those of Mukasa-Mugerwa and Tegegne, (1989) who documented significant variation of P4 levels across the gestation period, the levels of plasma P4 increase significantly in the second and third trimester of pregnancy.

A limiting factor of the LFIA strip is that the scoring with the naked eye could be subjective. To address this the current study digitized the intensity of the test lines using a scanner and the intensity signals quantified by image software analyzer to obtain objective results, but this increases the time required to obtain results.

The adoption of diagnostic tools in reproductive management decisions in dairy cattle depends greatly on the willingness of the dairy industry stakeholders to acquire and use the tools. It was therefore paramount to assess the knowledge, attitude and practices (KAP) of Animal Health

Practitioners and dairy farmers on the use of P4 in reproductive management. Such information may help in identification of knowledge gaps that need to be filled, behavioral patterns on the use of diagnostics tools and dairy reproductive management practices that would influence the adoption the LFIA technology. Based on the information from the KAP, both the farmers and AHP were knowledgeable about appropriate reproductive management practices although, they could not apply some of them due to various challenges mainly, the financial cost involved. However, their knowledge and practices of assisted reproductive technologies other than AI was limited.

These dairy industry stakeholders had adequate knowledge on the observable signs that animals on heat exhibit. Nevertheless, only about half of the farmers and AHP used the standing to be mounted as a primary sign to determine if an animal was in estrus. The challenge of dairy farmers' not using standing to be mounted as the primary sign of heat has also been reported previously (Eklundh, 2013). In the current study, failure of observation of mounting behavior was speculated to be due to the intensive nature of small holder dairy systems which is common in Kenya in which the animals are confined each in its own stall with limited space, therefore this compromises the natural expression of heat signs (Staal *et al.,* 2008; Muia *et al*., 2011). Use of rapid P4 kits together with the signs of heat will help increase the heat detection efficiency and accuracy in this production systems. Additionally, the knowledge and practices of the recommended times for checking heat in a herd which is at least three times a day for 30 minutes each day (Negussie *et al*., 2002; Galloway and Perera, 2003) was limited among the dairy farmers. This was thought to be due to inability of the farmers to partition the requisite labor for heat detection due to cost limitations. The farmers themselves may also not be able to invest that required time in heat detection as dairying was not the primary activity for majority of them as seen in the study, in addition to many being away from the farm for a significant amount of time during the day

attending to other activities. Therefore use of the P4 kits will go a long way in helping these farmers to increase the heat detection efficiency in their farms at an affordable cost.

Artificial insemination was being used for breeding widely as indicated by the large number of AHP offering these services across the country and also from the practices of dairy farmers. In contrast, the knowledge and adoption of other technologies such as embryo transfer (ET) and use of P4 kits was very low. Artificial insemination has been documented as the most successful and widely adopted assisted reproductive technology (Rodriguez-Martinez, 2012) as also reported in the current study. The dairy industry in Kenya still has some way to go in terms of improvement of the genetic base, and currently AI remains the most viable and affordable breeding technology to achieve this. Although the dairy sector is undergoing rapid growth and transformation, adoption of other technologies such as ET remains low and expensive. Majority of AHP and farmers also knew about the AM/PM rule of serving animals in relation to start of estrus. However, definitive determination of the time of the start of estrus was the main challenge that hindered the use of this AM/PM rule. Animals that start estrus late in the evening or at night may be reported the next day by farmers to have started heat that same morning. Such information results in timing of AI being inaccurate. Consequently, adoption of P4 kits for estrus detection would enable confirmation of estrus status of the animals presented for AI.

Animal Health Practitioners were knowledgeable that the estrus status of the cow and timing of AI were critical determinants of the success of AI. The conception rates are usually high when these two factors are accurate, and this could be increased further by use of rapid P4 kits which would be used to accurately determine estrus status before AI. Determination of standing estrus status by use of P4 kits would also ensure that only animals that are in estrus are served, thereby reducing the cost incurred by farmers due to repeated inseminations caused by serving animals that are not in standing estrus as reported by some AHP.

Calving to conception interval is a parameter of importance in achievement of the optimum calving interval of 365-400 days (Hernandez *et al.,* 2001) of which a larger proportion of both dairy farmers and AHP were knowledgeable about. At least a quarter of them indicated that they use a voluntary waiting period of 90 days in their farms since that was the period within which most postpartum animals show heat for the first time. Ninety days is the recommended cut off point if one is too achieve a CI of 365 days. Otherwise, breeding animals earlier than this would be the desirable practice. Delayed resumption of ovarian cyclicity and suboptimal estrus detection have been listed as the main challenges causing long calving to conception intervals (Ill-Hwa and Hyun-Gu, 2006). This can be overcome by use of P4 kits 45 days post calving or earlier to determine if the animal has resumed cyclicity and monitor her cycle to know when to expect estrus.

There was limited knowledge among the AHP and dairy farmers on the possibility of determination of pregnancy before the next estrous cycle starts, although all of them indicated they would have liked to know the outcome of an insemination one month post-insemination. Rapid P4 kits can therefore be used to indicate outcome of an insemination 18-24 days (the period equivalent to the estrous cycle length of an animal, if it is known) post insemination before the next estrous cycle begins so that corrective measures can be taken early enough.

LFIA kits are a new technology in Kenya as indicated by the low levels of knowledge as well as the fact that none of the dairy industry stakeholders was presently using them in reproductive management of dairy cattle. However, a larger proportion of both the dairy farmers and AHP indicated that they would be willing to use these rapid kits in reproductive management of dairy cattle. The willingness of the dairy industry stakeholders to use lateral flow assay kit and high levels of education of the dairy industry stakeholders are indicators that the adoption of the LFIA

would be high. With more training and awareness of the use and importance of these kits, their use for decision support in reproductive management of dairy cattle may increase.

5.1 CONCLUSIONS

1. The study established baseline data for P4 profiles in Kenyan dairy cattle which were found to be similar to those reported in other studies elsewhere.

2. The findings of the current study show that lateral flow immunoassay P4 kit is a reliable method for determination of P4 levels in whole blood at different stages of the reproductive cycle in dairy cattle.

3. Due to its ease of use, the LFIA kit can be used at point of care to determine blood P4 levels for reproductive management of dairy cattle.

4. Stakeholders in the dairy cattle industry are willing to adopt decision support tools such as LFIA for reproductive management of their farms for improved productivity.

5.2 RECOMMENDATIONS

1. Create awareness on use of decision support tools such as LFIA in reproductive management of dairy cattle.

2. Further studies on use of the LFIA at the point of care for reproductive management of dairy cattle to assess the actual impact on reproductive efficiency.

REFERENCES

Agarwal S.P., Agarwal V.K. and **Ahmad A**. (**1980**): Serum progesterone concentration in Zebu cows during pregnancy. Indian Journal of Animal Science **50:** 706-709.

Althouse G. (**2007**): Artificial Insemination. In: Schatten, H. and Constantinescu, G.M. Comparative Reproduction Biology, 5th edition pp.159-169. Oxford: Blackwell Publishing Limited.

Al-Yousif Y., **Anderson J., Chard-Bergstrom C**. and **Kapil S.** (**2002**): Development, evaluation and application of lateral flow immunoassay (immunochromatography) for detection of rotavirus in bovine fecal samples. Journal of Immunology **9:** 723–724.

Ambrose D.J., Radke B., Pitney P.A. and **Goonewardene L.A.** (**2007)**: Evaluation of early conception factor lateral flow test to determine non-pregnancy in dairy cattle. Canadian Veterinary Journal **48**: 831-835.

At-Taras E.E. and **Spahr S.L.** (**2001**): Detection and characterization of estrus in dairy cattle with an electronic heat mount detector and an electronic activity tag. Journal of Dairy Science **84:** 792-798.

Balakrishnan M., Chinnaiya G.P., Nair P.G. and **Jaganadha A. R.** (**1986**): Studies on serum progesterone levels in Zebu crossed Holstein heifers during pre and peri-pubertal periods. Animal Reproduction Science Journal **11:** 11-15.

Barnes M.G. (**2001**): Principles and Practices in Bovine Reproduction: Reproduction and Lactation, 5th edition pp. 500-533, Blacksburg Publishers.

Battochio M., Gabaia G., Mollo M.C., Veronesi F., Soldano G.B. and **CairolI F.** (**1998**): Agreement between ultrasonography classification of the corpus luteum and plasma progesterone concentration in dairy cows. Theriogenology Journal **51:** 1059-1069.

Bebe B.O., **Udo H.M.J., Rowlands G.J.** and **Thorpe W.** (**2003**): Smallholder dairy systems in the Kenya highlands: cattle population dynamics under increasing intensification. Livestock Production Science **82:** 211-221.

Billings R.A. (2002): Factors influencing the reproductive efficiency of dairy herds in Dominican Republic. MSc. Thesis. Virginia State University.

Cavanagh A.C. (**1996**)**:** Identification of early pregnancy factor as chaperonin 10: implications for understanding its role. Reviews of Reproduction **1**: 28–32.

Chang, C.F. and **Estergreen V.L.** (**1983**): Development of a direct enzyme immunoassay of milk progesterone and its application to pregnancy diagnosis in cows. Journal of Biochemistry **41:** 95-173.

Cooke R.F. and **Arthington J.D. (2009**): Plasma progesterone concentrations as puberty criteria for Brahman-crossbred heifers. Journal of Livestock Science **123:**101–105.

Corner G.W. and **Allen W.M**. (**1929**): The physiology of corpus luteum. Journal of Physiology **92:** 174-188.

Curley K.O., Neuendorff D.A., Lewis A.W., Cleere J.J., Welsh T.H. and **Randel R.D.** (**2008**): Functional characteristics of the bovine hypothalamic–pituitary–adrenal axis vary with temperament. Hormone Behaviour Journal **53**: 20-27.

Dalton C.J. (**2011**): Strategies for success in heat detection and artificial insemination. Advances in Dairy Technology Journal **23**: 215-229.

Dalton C.J., Nadir S., Bame J.H., Noftsinger M., Nebel R.L. and **Saacke R.G.** (**2001**): Effect of time of insemination on number of accessory sperm, fertilization rate, and embryo quality in non lactating dairy cattle. Journal of Dairy Science **84**: 2413-2418.

Dingwell R.T., Wallace M.M., Mclaren C.J., Leslie C.F. and **Leslie K.E.** (**2006**): An evaluation of two indirect methods of estimating bodyweight in Holstein calves and heifers. Journal of Dairy Science **89**: 3992-3998.

Dinskin M.G and **Sreenan J. M.** (**2000**): Expression and detection of oestrus in cattle. Journal of Reproduction, Nutrition and Development **40**: 481-491.

Dobson H., Walker S.L., Morris M.J., Routly J.E. and **Smith R.F.** (**2008**): Why is it getting more difficult to successfully artificially inseminate dairy cows? Journal of Animal Science **90:** 1104–1111.

Donaldson L.E., Bassett J.M. and **Thorburn D.G.** (**1970**): Peripheral plasma progesterone concentration of cows during puberty, estrous cycle, pregnancy and lactation, and the effects of under-nutrition or exogenous oxytocin on progesterone concentration. Journal of. Endocrinology. **48:** 599-600.

Dorniak P., Bazer F.W. and **Spencer T.E.** (**2013**): Biological role of interferon tau in endometrial function and conceptus elongation. Journal of Animal Science **91**:1627–1638.

Dransfield M.B.G., Nebel R.L., Pearson R.E. and **Warnick L.D.** (**1998**): Timing of insemination for dairy cows identified in estrus by a radio telemetric estrus detection system. Journal of Dairy Science **81**: 1874-1882.

Edgar D.G. (**1953)**: The chemical assay of progesterone. Biochemistry Journal **54**:50-55.

Edmonson A.J., Lean I.J., Weaver L.D., Farver T. and **Webster G.** (**1989**): A body condition scoring chart for Holstein Friesian. Journal of Dairy Science **72:** 68-78.

Eicker S.W., Grohn Y.T. and **Hertl J.A** (**1996**): The association between cumulative milk yield, days open and days to first breeding in New York Holstein Cows. Journal of Dairy Science **79:** 235-241.

Eklundh C. (**2013**): The use of artificial insemination in dairy farms Peri-urban and Urban areas of Uganda: A study of knowledge, attitude and practices. Swedish University of Agricultural Sciences (Available at http://epsilon.slu.se. Accessed 20/5/2016).

Elderet P.A., Yeo K.H.J., Lewis J.G. and **Clifford J.K**. (**1987**): An enzyme linked immunosorbent assay for plasma progesterone: immobilized antigen approach. Journal of Clinical Chimica Acta **162:** 199-206.

Engvall E., Jonsson K. and **Perlmann P**. (**1971)**: Enzyme-linked immunosorbent assay: Quantitative assay of protein antigen, immunoglobulin g, by means of enzyme-labelled antigen and antibody-coated tubes. Biochimica Biophysica Acta Protein Structure and enzymology Journal **251:** 427-434.

Esslemont R.J., Kossaibati M.A. and **Allcock J.** (**2001**): Economics of fertility in dairy cows. British Society of Animal Science Journal **26:** 19-29.

Esslemont R.J. and **Peeler E. J**. (**1993**): The scope for raising margins in dairy herds by improving fertility and health. British Veterinary Journal **149:** 537-547.

Folman Y., Miriam Rosenberg., Herz Z. and Davidson M. (**1973**): Relationship between plasma progesterone concentration and conception in postpartum dairy cows maintained on two levels of nutrition. Journal of Reproduction and Fertility **34:** 267-278.

Food and Agriculture Organization (FAO) (2011): Dairy development in Kenya. (www.fao.org/docrep/013/.../al745e00.pdf. Accessed on 20/3/2016).

French P.D. and Nebel R.L. (**2003**): The simulated economic cost of extended calving intervals in dairy herd and comparison of reproductive management programs. Journal of Dairy Science **86:** 54-56.

Fricke P.M., Alessandro R., Paulo D. C. and **Mason C**. (**2012**): Milk vs. Blood which is best for PAG Pregnancy Prediction. Department of Dairy Science University of Wisconsin-Madison (https://dysci.wisc.edu/Fricke. pdf. Accessed 10/4/2016).

Friggens N.C. and Chagunda M.G. (**2005**): Prediction of the reproductive status of cattle on the basis of milk progesterone measures: model description. Theriogenology Journal **64:** 155–190.

Friggens N.C., Bjerring M., Ridder C. S., Hojsgaard L. and **Larsen T**. (**2008**): Improved detection of reproductive status in dairy cows using milk progesterone measurements. Reproduction in Domestic Animals **43**: 113-121.

Galloway D. and **Perera O.** (**2003**): Guidelines and recommendations for improving artificial breeding of cattle in Africa. A working document of the AFRA Project III-2-RAF/5/046. (http://www.afra.org/animal breeding. Accessed on 20/4/2016).

Ghanem M.E. and **Nishibori M.** (**2015**): Effects of season on plasma progesterone profiles in repeat breeding cows**.** Journal of Veterinary Medicine **60**: 227–234.

Gillis E.H., Gosling J.P., Sreenan J.M. and **Kane M.** (**2006**): Development and validation of a biosensor-based immunoassay for progesterone in bovine milk. Journal of Immunology **267:** 131-138.

Ginther O.J., Nuti L.C., Garcia M.C., Wentworth B.C. and **Tyler W.J.** (**1976)**: Factors affecting progesterone concentration in cow‘s milk and dairy products. Journal of Animal Science **42:** 58-60.

Government of Kenya (GoK) (**2010**): Kenya National Dairy Master Plan: Ministry of Livestock Development (www.dairy africa.com/African dairy portal/…/Kenya dairy master plan. Accessed on 20/5/2016).

Government of Kenya (GoK) (**2012**): National Dairy Farming Strategy: Making Kenya’s Dairy Farming sector a competitive driver of growth. (www.commsconsult.org/.../National dairy strategy. Accessed on 21/3/2016).

Hafez E.S.E. and **Hafez B**. (**2000**): Reproduction in Farm Animals, 7th Edition. Lippincott Williams and Wilkin, London.

Hare E., Norman H.D. and **Wright J.R.** (**2006**): Trends in calving ages and calving intervals for dairy cattle breed in United States. Journal of Dairy Science **89:** 365-370.

Heersche J.G and **Nebel R.L.** (**1994**): Measuring efficiency and accuracy of detection of estrus. Journal of Dairy Science **77:** 2754-2761.

Henderson K.M., Camberis M., Simmons M.H., Starrs W.J. and **Hardie A.H.** (**1994**): Application of enzyme immunoassay to measure oestrone sulphate concentrations in cow's milk during pregnancy. Journal of Steroid Biochemistry and Molecular Biology **50:** 189-196.

Henricks D.M., Dickey J.F. and **Niswender G.D.** (**1970**): Serum luteinizing hormone and plasma progesterone levels during the oestrus cycle and early pregnancy in cows. Journal of Biology and Reproduction **2:** 346-347.

Hernandez J., Shearer J.K. and **Webb D.W.** (**2001**): Effect of lameness on the calving to conception interval in dairy cows. Journal of the American Veterinary Medical Association **218**: 1611-1614.

Heuwieser W. and **Oltenacu P.A.** (**1997**): Evaluation of different protocols for prostaglandin synchronization to improve reproductive performance in dairy herds with low estrus detection efficiency. Journal of Dairy Science **80**: 2766-2774.

Higgins H.M., Ferguson E., Smith R.F. and **Green M.J.** (**2013**): Using hormones to manage dairy cow fertility: The Clinical and Ethical Beliefs of Veterinary Practitioners. PLoS ONE **8**: 1371-1378.

Hill J.R., Lamond D.R., Henricks D.M., Dickey J.F. and **Niswender G.D. (1970**): The effects of under nutrition on ovarian function and fertility in beef heifers. Journal of Biology and Reproduction **2**: 78-80.

Hoka A.I., Indetie D., and Ng'ang'a Z. and **Gicheru M.** (**2008**): Use of milk progesterone profiles to determine causes of reproductive deficiencies in Friesian dairy cows. East African Agricultural and Forestry Journal **74**: 71-76.

Holdsworth R.J., Booth J.M., Sharman G.A. and **Rattray E.A.** (**1980**): Measurement of progesterone levels in whole and fore-milk from dairy cows. British Veterinary Journal **136:** 546-554.

Holman A.N.J., Thompson J. E., Routly J., Cameron D. N., Jones, D.W., Smith R.F. and **Dobson H.** (**2011**): Comparison of oestrus detection methods in dairy cattle**.** Journal of Veterinary Record **169:** 47-49.

Ill-hwa K. and **Hyun-Gu. K.** (**2006**): Risk factors for delayed conception in Korean herds. Journal of Veterinary Sciences **7:** 381-385.

Karen A.N.M., Beckers J.F., Bajcsy A.C., Tibold J., Mádl I. and **Szenci O**. (**2015**): Comparison of a commercial bovine pregnancy associated glycoprotein ELISA test and a pregnancy associated glycoprotein radioimmunoassay test for early pregnancy diagnosis in dairy cattle. Animal Reproduction Science Journal **10:** 1016-1020.

Laitinen M.P.A. and **Vuento M.** (**1996**): Immunochromatographic assay for quantitation of milk progesterone. The Journal Acta Chemica **50:** 141–145.

Lanyasunya P.T., Rong H.W., Mukisira A. E. and **Abdulrazak A.S.** (**2006**): Perfomance of dairy cows in different livestock production systems on smallholder farms in Bahati Division, Nakuru District, Kenya. Pakistan Journal of Nutrition **5:** 130-134.

LeBlanc S.J. and **Leslie K.E.** (**1998)**: Measures of estrus detection and pregnancy in dairy cows after administration of Gonadotropin-Releasing Hormone within an Estrus Synchronization Program based on Prostaglandin f2α. Journal of Dairy Science **81:** 375-381.

Lonergan P.L., O'Hara P. and **Forde N. (2013**): Role of diestrus progesterone on endometrial function and conceptus development in cattle. Journal of Animal Reproduction **10:** 223-227.

Lucy M., Green J. and **Poock S. (2011**): Pregnancy determination in cattle: A review of available alternatives. In: Proceedings of Applied Reproductive Strategies in Beef Cattle, Joplin, MO, pp. 367–375.

Maatje K., Loeffler S.H. and **Engel B.** (**1997**): Predicting optimal time of insemination in cows that show visual signs of estrus by estimating onset of estrus with pedometers. Journal of Dairy Science.**80:** 1098-1105.

Mann G.E., Mann S.J., Blache D. and **Webb R.** (2005): Metabolic variables and plasma leptin concentrations in dairy cows exhibiting reproductive cycle abnormalities identified through milk progesterone monitoring during postpartum period. Journal of Reproduction and Fertility **90:** 330-335.

Marcos G.C., Divakar J.A., John P.K. and **Julie A.S.** (**2008**): Comparison of 2 enzyme immunoassays and a radioimmunoassay for measurement of progesterone concentrations in bovine plasma, skim milk, and whole milk. Canadian Veterinary Journal **72:** 32-36.

Martin S.W., Meek A.H. and **Willeberg P. (1987)**: Veterinary Epidemiology, Principles and Methods. Lowa State University Press, Ames, IA, pp.63–71.

McDowell R.E., Hollon B.F., Camoens J.K. and **Van Vleck L.D.** (**1976**): Reproductive efficiency of Jerseys, Red Sindhis and crossbreds. Journal of Dairy Science **59:** 127-136.

Miller S., Morton M.S. and **Mote A.T.** (**1988**): Chemiluminescence immunoassay for progesterone in plasma incorporating acridinium ester labelled antigen. Journal of Clinical Biochemistry **25:** 27-34.

Ministry of Livestock and Fisheries Development MOL&FD (**2006)**: Dairy draft policy: Towards a competitive and sustainable dairy industry for economic growth in the 21st century and beyond. Annual report.

Mondal S. and Prakash B.S. (**2003**): Peripheral plasma progesterone concentration in relation to estrus expression in Sahiwal cows. Indian Journal of Physiology and Pharmacology **47:** 80-86.

Moore K. and **Thatcher W.W.** (**2006**): Major advances associated with reproduction in dairy cattle. Journal of Dairy Science **89:** 1254-1266.

Morais R., Valente A., Almeida J.C., Silva A.M., Soares S., Reis, M.J., Valentim R. and **Azevedo J.** (**2006**): Concept study of an implantable microsystem for electrical resistance and temperature measurements in dairy cows, suitable for estrus detection. Sensors Actuators, **132:** 354-361.

Muhammd F., Sarwar A., Hayat C.S. and **Anwar M.I.** (**2000)**: Peripheral plasma progesterone concentration during early pregnancy diagnosis in Holstein pregnant cows. Pakistan Veterinary Journal **20:** 2000-2010.

Muia J.M.K., Kariuki J.N., Mbugua P.N., Gachuiri C.K., Lukibisi L.B., Ayako W.O. and **Ngunjiri W.V. (2011**): Smallholder dairy production in high altitude Nyandarua milk-shed in Kenya: Status, challenges and opportunities. Livestock Research for Rural Development **23:** 108-120.

Mukasa-Mugerwa E. (**1989**): A review of reproductive performance of female *Bos indicus* (Zebu) cattle. In International Livestock Centre for Africa Annual Repor 1989. ILCA and ILRAD Publishers, Ethiopia.

Mukasa-Mugerwa E. and Tegegne A. (1989): Peripheral progesterone concentration in Zebu (Bos Indicus) cows during pregnancy. Journal of Reproduction and Nutritional Development **9:** 303-308.

Murage A.W. and **Ilatsia E.D. (2011**): Factors that determine use of breeding services by small-holder dairy farmers in Central Kenya. Tropical Animal Health and Production **43:** 199-207.

Muriuki H., Omore A., Hooton N., Waithaka M., Ouma R. Staal S. and **Odhiambo P.** (**2004**): The Policy environment in the Kenya dairy sub-sector: Smallholder Dairy Research and Development Project report 2 Nairobi Kenya (http://www.smallholderdairy.org/collaborativereports.htm. Accessed on 10/6/2016).

Mwacharo J.M. and **Drucker A.G.** (**2005**): Production objectives and management strategies of livestock keepers in south-east Kenya: implications for a breeding programme. Tropical. Animal. Health and Production Journal **37:** 635–652.

Naik B.R., Siva Kumar A.V.N., Bramhaiah K.V., Ravi A. and **Praveen V.C. (2013**): Estrogen and P4 hormone levels in Punganur Cattle. Journal of Agriculture and Veterinary Sciences **2:** 50-53.

Nebel R.L. (**2003**): Components of a successful heat detection program. Journal of Advanced Dairy Technology **15:** 191-203.

Nebel R.L. (**2001**): What we know about heat detection. In Principles and Practices in Bovine Reproduction class, pp. 511-540, Blacksburg Publishers.

Nebel R.L. and **Jobst S.M. (1998)**: Evaluation of systematic breeding programs of lactating dairy cows: A Review. Journal of Dairy Science **81:** 1169-1174.

Nebel R.L., Whittier W.D., Cassell, B.G. and **Britt J.H.** (**1987**): Comparison of on-farm and laboratory milk progesterone assays for identifying errors in detection of estrus and diagnosis of pregnancy. Journal of Dairy Science **70**: 1471–1476.

Negussie F., Kassa T. and **Tibbo, M**. (**2002**): Behavioural and physical signs associated with estrus and some aspect of reproductive performance in Fogera cows and heifers. Tropical Animal Health and Production **34:** 319-328.

Ngigi M.W. (**2004**). Building on successes in African Agriculture: Smallholder Dairy in East Africa. IFPRI International Food Policy Research Institute, Washington 2020 Focus Paper **6:** 12.

Nordin Y., Zaini N. and **Zahari W.M. (2007**): Reproductive status following artificial insemination and factors affecting conception rate in dairy cows in smallholder production

systems. In: Improving the reproductive management of dairy cattle subjected to Artificial Insemination. International Atomic Energy Agency **38:** 79-91.

Ojango J.M.K and **Pollot G.K.** (**2004)**: Productivity of Holstein-Friesian dairy cattle in different farming systems in Kenya. International Journal of Agriculture and Rural development **5:** 145-155.

O'Keeffe M., Crabbe P., Salden M., Wichers J., Peteghem C., Kohen F., Pieraccini G. and **Moneti G.** (**2003**): Preliminary evaluation of a lateral flow immunoassay device for screening urine samples for the presence of sulphamethazine. Journal of Immunology **278:** 117–126.

Orchard R.G. (**2007**). A flow through enzyme linked immunoassay for progesterone. University of Waikato. MSc Thesis (Available at http://waikato.researchgateway.ac.nz. Accessed on 25/2/2016).

Osman M.M., El Bayomi K.H.M., Abdoon A.S. and El Nabtiti1 A.S. (**2012**): Using progesterone field test (on farm) as a tool for early detection of pregnancy in dairy farms. Suez Canal Veterinary Medicine Journal **17:** 9-17.

Otava G., Cernescu H.C.M. and **Violeta I.** (**2007)**: Pregnancy diagnosis in cow using progesterone measurements**.** Lucrări Stiinłifice Medicină Veterinară **11:** 1030-1045.

Owen E., Kitalyi A. Jayasuriya N. and **Smith T.** (**2005**): Livestock and wealth creation: Improving the husbandry of animals kept by resource poor people in developing countries. Ist edition. Nottingham university press.

Parr M.H., Mullen M.P., Crowe M.A., Roche J.F., Lonergan P. and **Evans A.C.** (**2013**): The repeatability of embryo survival, and the relationship between plasma P4 in the early luteal phase and embryo survival in dairy heifers. Journal of Dairy Science **90:** 1135-1140.

Pemberton R.M., Hart J.P. and **Mottram T.T. (2001**). An electrochemical immunosensor for milk progesterone using a continuous flow system. Journal of Biosensors and Bioelectronics **16:** 715-723.

Posthuma-Trumpie G.A. (**2008**): Towards a dry reagent immunoassay of progesterone in bovine milk. S, n. Doctor of philosophy, University of Groningen.

Posthuma-Trumpie G.A., Van-Amerongen, A., Korf J. and **Van-Berkel, W.J.H**. (**2009**): Lateral Flow Immunoassay for progesterone detection. Journal of Biotechnology **27:** 652-654.

Purohit G. (2010). Methods of pregnancy diagnosis in domestic animals: The current status. (http://www.webmedcentral.com/wmcpdf/Article_WMC001 305.pdf. Accessed on 19/3/2016).

Radostits O.M. (**1985**): Dairy cattle- Maintenance of reproductive efficiency, in herd health: A Textbook of Health and Production Management of Agricultural Animals. Saunders Company, Philadelphia.

Rao T.K.S, Kumar N, Kumar P., Chaurasia S. and **Patel N.B** (**2013**). Heat detection techniques in cattle and buffalo. Veterinary World Journal **6:** 363-369.

Rioux P. and **Rajotte D.** (**2004**). Progesterone in milk: a simple experiment illustrating the estrous cycle and enzyme immunoassay. Advances in Physiology Education **28:** 64-67.

Rodrigues R.O., Trevisanuto C., Cooke R.F. and **Vasconcelos J.M.L.** (**2010**): Effect of body weight loss on serum P4 concentrations in non lactating dairy cows. Theriogenology Journal **69:** 661-667.

Rodriguez-Martinez H. (**2012**): Assisted reproductive techniques for cattle breeding in developing countries: a critical appraisal of their value and limitations. Reproduction in Domestic Animals **47**: 21-26.

Roelofs J.B., Van Eerdenburg, F.J.C.M., Soede N.M. and **Kemp B**. (**2005**): Various behavioral signs of estrus and their relationship with time of ovulation in dairy cattle. Theriogenology Journal **63:** 1366-1377.

Romano J.E., Thompson J.A., Kraemer D.C., Westhusin M.E., Forrest D.W. and **Tomaszweski M.A**. (**2007**): Early pregnancy diagnosis by palpation per rectum: Influence on embryo/fetal viability in dairy cattle. Theriogenology Journal **67:** 486-493.

Safronova V.A., Samsonova J.V., Grigorenko V.G and **Osipov A.P.** (**2012**): Lateral flow immunoassay for progesterone detection. Moscow University Chemistry Bulletin **67:** 241-248.

Samsonova J.V., Safronova V.A. and **Osipov A.P.** (**2015**): Pretreatment-free lateral flow enzyme immunoassay for progesterone detection in whole cows' milk. Talanta Journal of Analytical Chemistry **132:** 685-688.

Sananikone K., Delwiche M.J., BonDurant R.H. and **Munro C.J**. (**2004**): Quantitative lateral flow immunoassay for measuring progesterone in bovine milk. Transactions of American Society of Agricultural Engineers Journal **47:** 1357-1365.

Savela H., Vahtiala S., Lindeberg H., Dahl E., Ropstad E., Beckers J.F. and **Saarela S.** (**2009**): Comparison of accuracy of ultrasonography, P4, and pregnancy-associated glycoprotein tests for pregnancy diagnosis in semi-domesticated reindeer. Theriogenology Journal **72:** 1229-1236.

Schomberg D. W., Coudert S. P. and **Short R. V.** (**1967**): Effects of bovine luteinizing hormone and human chorionic gonadotrophin on the bovine corpus luteum in vivo. Journal of Reproduction and Fertility **14**: 277.

Senger P.L. (1994): The Estrus Detection Problem: New Concepts, Technologies, and Possibilities. Journal of Dairy Science **77:** 2745-2753.

Sheldon I.M., Wathes D.C. and **Dobson H**. (**2006**): The management of bovine reproduction in elite herds. Veterinary Journal **171:** 70-80.

Shemesh M., Lindner H. R. and **Ayalon N.** (**1971**): Competitive protein-binding assay of SAVE progesterone in bovine jugular venous plasma during the oestrous cycle. Journal of Reproduction and Fertility **26:** 167-169.

Short R .E. and **Bellows R. A.** (**1971**): Relationships among weight gains, age at puberty and reproductive performance in heifers. Journal of Animal Science **32:** 127-131.

Short R.V. (1958). Progesterone in blood: The chemical determination of progesterone in peripheral blood. Journal of Endocrinology **16:** 415-425.

Silva E., Sterry R.A., Kolb D., Mathialagan N., McGrath M.F., Ballam J.M and **Fricke P.M. (2007**): Accuracy of a pregnancy-associated glycoprotein ELISA to determine pregnancy

status of lactating dairy cows twenty-seven days after timed artificial insemination. Journal of Dairy Science **90:** 4612-4622.

Simersky R.J., Swaczynova M., Morris D.A., Franek M. and Stranad M. (**2007**): Development of an ELISA based kit for the on farm determination of P4 in milk. Journal of Veterinary Medicine Czech **52:** 19-28.

Smallholder Dairy Project SDP (2005). The uncertainty of cattle numbers in Kenya. Smallholder Dairy Research and Development Project Policy Brief No.10. (http://www.smallholderdairy.org/collaborativereports.htm. Accessed on 9/6/2016).

Spencer T.E., Sandra O. and **Wolf E.** (**2009**): Genes involved in conceptus–endometrial interactions in ruminants: insights from reductionism and thoughts on holistic approaches. Journal of Reproduction **135:** 165-79.

Staal S.J., Pratt A.N. and **Jabbar M.** (**2008**): Dairy Development for the Resource Poor Part 1: A Comparison of Dairy Policies and Development in South Asia and East Africa. PPLPI. Working Paper No. 44 International Livestock Research Institute.

Stabenfeldt G.H., Ewing L.L. and **Mcdonald L.E.** (**1969**): Peripheral plasma progesterone levels during the bovine oestrous cycle. Journal of reproduction fertility **19:** 433-442.

Techno-serve (2008): Dairy value chain in Kenya. Techno-serve Annual Report. Washington: TNS Publishers.

Thorpe W., Muriuki H.G., Omore A., Owango M.O. and Staal S. (**2000**): Development of smallholder dairying in Eastern Africa with particular reference to Kenya. Paper presented at the annual symposium of the Animal production Society of Kenya. Nairobi Kenya.

(http://www.smallholderdairy.org/publications/conference/Thorpe. Kenya.pdf. Accessed on 10/3/2016).

United Nations Environment Programme UNEP (2014): Green Economy Assessment Report: Kenya. (Available at http://www.unep.org/greeneconomy/portal/88/documents/Kenya assessment.pdf. Accessed on 12/2/2016).

United States Agency for International Development USAID (**2008**): Kenya Dairy farming Sector competitiveness program: Milk shed Assessment and small business organization needs analysis. Land O Lakes/ Fibec Report (http://www.usaid.org/Kenya dairy. Accessed on 12/1/2016).

Van-Amerongen A. and **Koets M.** (**2005**): Simple and rapid bacterial protein and DNA diagnostic methods based on signal generation of colloidal carbon particles. In: Rapid methods for biological and chemical contaminants in food and feed 2nd edition. Wageningen Academic Publishers.

Van-Arendonk J.A.M. and **Liinamo A.** (**2003**): Dairy cattle production in Europe. Theriogenology **59:** 563–569.

Wakhungu J.W. (**2001)**: Dairy cattle breeding policy for Kenyan smallholders: An evaluation based on the demographics stationery state productivity model. Doctor of philosophy Thesis. University of Nairobi.

Waldmann A. and Raud A. (2016): Comparison of lateral flow milk progesterone test with enzyme immunoassay as an aid for reproductive status determination in cows. Veterinary Record **10:** 1136-1150.

Walker W.L., Nebel R.L. and **McGilliard M.L.** (**1996**): Time of ovulation relative to mounting activity in dairy cattle. Journal of Dairy Science **79:** 1555-1561.

Wiltbank J.N. Kasson C. W. and **Ingalls J. E.** (**1969**): Puberty in crossbred and straight-bred beef heifers on two levels of feed. Journal of Animal Science **29:** 602-605.

Xu Z.Z., Macknight D.J., Vishwanath R., Pitt C.J. and **Burton L.J. (1998)**: Estrus detection using Radio telemetry or visual observation and tail painting for dairy cows on pasture. Journal of Dairy Science **81:** 2890-2896.

Yoshida C. and Nakao T. (**2005**): Some characteristics of primary and secondary oestrus signs in high-producing dairy cows. Reproduction in domestic animals. **40:** 150-155.

Zaytseva N.V., Montagna R.A., Lee E.M. and **Baeumner A.J.** (**2004**): Multi analyte single membrane biosensor for the serotype specific detection of Dengue virus. Journal of Analytical and Bioanaytical Chemistry **380:** 46–53.

APPENDICES

Appendix i: Field evaluation form

Date of blood draw: **Method of blood draw:** Jugular Coccygeal

General Information

Animal ID:
Date of Birth:
Breed:
Diet:
Supplements:
Body condition
Body weight
Hydration status

Reproductive Information

How many calvings:
Dates of calvings:
Is the animal undergoing synchronization?

Yes No

If yes, what were last 3 injections and the dates?

Date Injection
Date Injection
Date Injection

Date of last heat:

Signs of heat seen

Date of last AI service:
Confirmed pregnant: Yes No
Reproductive challenges ever reported:

Silent heat Non-cycling Cysts

In field evaluation

Date and Time:

	Test Line	Control line
Strip 1 score		
Strip 2 score		
Strip 3 score		

In lab evaluation

Date and time:

	Test Line	Control Line
Strip 1 score		
Strip 2 score		
Strip 3 score		

.

ELISA results

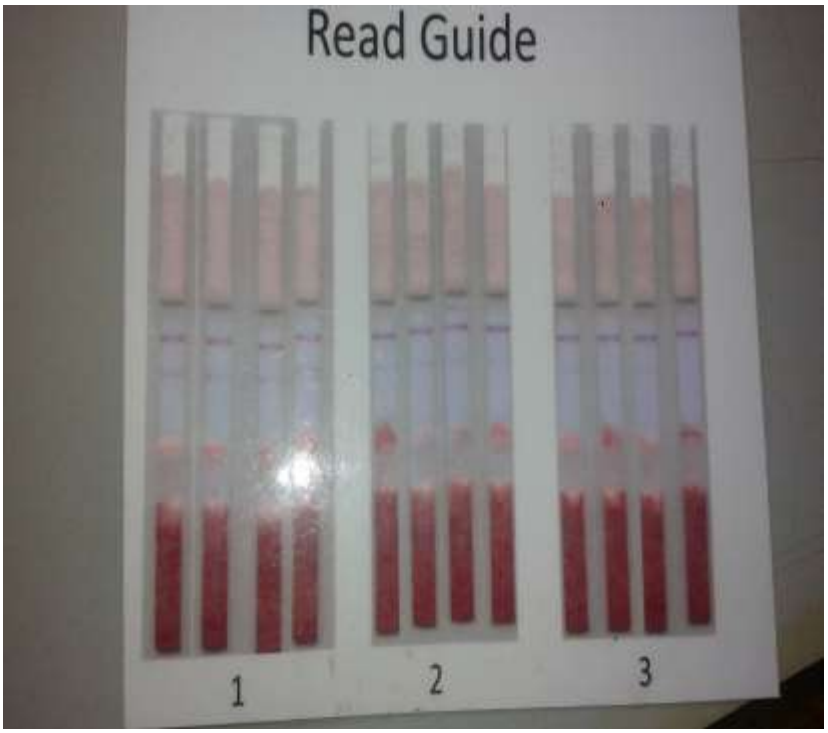
Date and time:

Value 1

Value 2

Value 3

Appendix ii: LFIA read guide



Appendix iii: KAP questionnaire for Animal Health Practitioners

This questionnaire will take approximately 15 minutes to answer. Please, be assured that any information you provide will be anonymous and no personal information collected will appear in any documents or reports based on this survey.

Part 1 – Profiling

1. Which Profession is the interviewee?

Artificial insemination technician ☐

Animal health assistant ☐

Veterinary surgeon ☐

2. Age in years: 18-30 ☐ 31-40 ☐ 41-50 ☐ above 50 ☐

3. What is the gender of the participant? Male ☐ Female ☐

4. What is your highest level of education?

Certificate level ☐

Diploma level

Degree level

Post graduate

Other ……

5. How long have you been an animal health practitioner?

Less than 1 year
1-5 years
6-10 years
More than 10 years

6. Are you a full time or part time animal health practitioner?

Part time
Full time

Part 2: Knowledge

1. Which methods of breeding do you know of?

AI
Bull
Embryo transfer

2. What factors affect the success rate of AI?

Reproductive status of the cow
Semen handling
Timing of AI
Other……

3. Rank the following 5 keys to a successful reproductive management according to importance rank 1-5

1-most important
5-least important

Key 1: Inseminate cows quickly after the end of the voluntary waiting period……

Key 2: Inseminate cows at the correct time in relation to estrus or ovulation…….

Key 3: Improve AI Efficiency……..

Key 4: Identify non pregnant cows early after an insemination……

Key 5: Aggressively re-inseminate non pregnant cows……

4. Which methods of detecting heat in cows are you aware of?

By observing the signs of heat
Use of heat detection aids
Use of hormone levels

4. Are you aware of P4 kits used for heat detection?

Yes

No ☐

5. What is the optimum time to serve a cow after detecting heat?
   - Immediately ☐
   - After 6 hours ☐
   - Use the 6am -6 pm rule ☐

6. At what time should the cows be checked for heat?
   - In the morning ☐
   - In the afternoon ☐
   - In the evening ☐

7. How many times in a day should a cow be checked for heat?
   - Once ☐
   - Twice ☐
   - Thrice ☐

8. For how long should an animal be checked for heat?
   - 1 day ☐
   - 2 days ☐
   - 3 days ☐

9. How should an inseminator determine if a cow is ready to be served?
   - By looking at clear mucus discharge from the vulva of the cow ☐
   - When a cow stands to be mounted ☐
   - By palpating a graafian follicle on the ovary ☐

10. How many times should an inseminator serve a cow?
    - Once ☐
    - Twice ☐
    - Thrice ☐

11. How soon after calving should cows be served?
    - Immediately ☐
    - After 45 days ☐
    - After 60 days ☐
    - After 90 days ☐

12. What factors affect success rate of Artificial Insemination

Semen handling ☐

Timing of artificial insemination in relation to ovulation time ☐

Other……

13. After how long post insemination is it possible to know the pregnancy status of an animal?
    - After I month ☐
    - After 2 months ☐
    - After 3 months ☐
    - After 4 months ☐

14. Which methods of confirming pregnancy are you aware of?
    - No return to estrus ☐
    - Rectal palpation ☐
    - Use of P4 kits ☐

Ultrasound 

15. Are you aware of P4 kits used to detect pregnancy?

Yes 
No 

16. Are you aware of the use P4 levels in blood to detect fertility disorders in dairy cows?

Yes 
No 

17. Are you aware of Animal Health Practitioners that are using P4 kit in detection of pregnancy?

Yes 
No 

Give reason(S)……

18. Do you know of any methods that can be used to improve reproductive performance?

Yes 
No 
If yes, which ones……..

Part 3: Attitude

1. Do you think use of P4 kits (point of care devices) will improve reproductive performance of dairy cows?

Yes 
No 
Give reason…….

2. Would you use P4 kits to improve reproductive performance of dairy cows?

Yes 

No 
Give reason……

2. Would other animal health practitioners utilize the P4 kits in reproductive management of dairy cows?

Yes 

No 
Give reason……

3. Would you use a P4 kit (point of care device) for detecting heat in cows on the farm?

Yes 
No 

4. How soon would you want the know the outcome of an insemination

After 21 days 
After one month 

After two months
After three months

5. Do you think it's important to know the pregnancy status of an inseminated cow early?

Yes
No

Give reason……..

6. Do you think P4 kits will be important in detecting outcome of an insemination early?

Yes
No
Give reason…….

7. Would you use a P4 kit in detection of pregnancy status of an inseminated cow?

Yes
No
Give reason……..

Part 4: Practices

1. Do you provide artificial insemination services for your clients?

Yes
No

2. How do you confirm that a cow is on heat?

Use of signs of heat
Heat detection aids
P4 kits

If signs of heat which one……..

3. After how long do you serve cows after detecting heat (In Hrs.?)

Immediately
After 4 hrs.
After 12 hours
After more than 12 hour

4. Have you ever served a cow when she not ready to be served

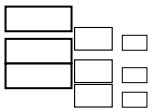
Yes
No
Give reason……

5. After how long do you detect pregnancy after insemination?

1 month
2 months
3 months
4 months

6. Which method do you use for pregnancy detection?

Rectal palpation
Non return to estrus
Use of P4 kits



Appendix iv: KAP questionnaire for dairy farmers

Knowledge, Attitude, Practices of dairy farmers on the use of Progesterone in reproductive management in dairy cows in Kenya

This questionnaire will take approximately 15 minutes to answer. Please, be assured that any information you provide will be anonymous and no personal information collected will appear in any documents or reports based on this survey.

Part 1 – Profiling

1. Name of participant ……..

2. Age category: Below 18 ☐ 19-30 ☐ 31-40 ☐ 41-50 ☐ Above 50 ☐

3. What is the gender of the participant?

Male ☐
Female ☐

4. What is the highest level of education of the participant?

No school ☐
Primary level ☐
Secondary level ☐
Diploma level ☐
Degree level ☐
Post graduate ☐
Other ……

5. How long have you been keeping dairy cows?

Less than 1 year ☐
1-3 years ☐
4-7 years ☐
More than 7 years ☐

Part 2- Farm enterprise

1. How many animals do you have?

Cows ☐
Bulls ☐
Calves ☐

2. is this enterprise a full time or part time?

Part time ☐
Full time ☐

3. Is dairy farming the major source of livelihood for the family?

Yes
No

4. Who takes care of the dairy cows?

Husband
Wife
Workers
Other family members…….

6. What are the challenges of dairy farming?

Feeding
Marketing of milk
Reproductive inefficiency
Animal diseases

Other……

7. How do you overcome these challenges? .....
……..

8. Do you cull cows in you farm?

Yes
No

9. What are the reasons for culling the cows?

Part 3: Knowledge

1. Do know what reproductive efficiency is?

Yes
No

2. What methods of measuring reproductive efficiency are you aware of?
…….
……....

3. Which methods of breeding do you know of?

AI
Bull

4. Which methods of detecting heat in cow you aware of?

By observing the signs of heat
Use of heat detection aids
Use of P4 kits

5. After how long should a cow be served after detecting heat?

Immediately

After 6 hours
Use 6 am-6 pm rule

6. How do you determine when a cow is ready to be served?
…….....
……

6. Are you aware of P4 kits used for heat detection?

Yes
No

7. After how long post insemination is it possible to know the pregnancy status of an animal
After I month
After 2 months
After 3 months
After 4 months

8. Which methods of confirming pregnancy are you aware of?

No return to estrus
Rectal palpation
Use of P4 kits

9. Do you know of P4 kits used to detect pregnancy?

Yes
No

Part 4: Attitude

1. Do you think P4 kits will help improve reproductive efficiency in dairy farms?
Yes
No
Give reason……..

2. Would you use P4 kits to improve reproductive performance of dairy cows?
If yes, how……..

3. Would other dairy farmers utilize the P4 kits in reproductive management of dairy cows?
Yes

No

Give reason……..

4. After how long would you like to know the outcome of an insemination?

1 month ☐
2 months ☐
3 months ☐

5. Would you use a P4 kit for detecting heat in cows on the farm?

Yes ☐
No ☐

6. Would you use a P4 kit in detection of pregnancy status early before the next estrous cycle of the cow begins?

Yes ☐
No ☐

Part 5: Practices

1. Do you seek veterinary services?

Yes ☐
No ☐

2. Which category of Animal health service providers do you engage?

Veterinary Surgeon ☐

Animal health assistant ☐

3. How accessible are veterinary services?

4 .Are the veterinary services beneficial

Yes ☐
No ☐

5. How do you measure reproductive efficiency in your farm?

…….
……..

6. Do you cull cows that have low reproductive performance?

Yes ☐
No ☐
Give reason……..

7. Which method of breeding do you use?

Artificial insemination ☐
Use of a bull ☐

8. Who provides artificial insemination services to you?

AI Technician ☐
AHS ☐
Vet. Surgeon ☐

9. Which method do you use to detect estrus/heat on your cows?

Signs of heat
Heat detection aids
P4 kits

10. After how long are your animals served after detecting heat (In hrs.)

Immediately
After 4 hrs.
After 12 hours
After more than 12 hours

11. Who serves your cows?

Artificial insemination technician
Animal health assistant
Veterinary Surgeon

12. Which method do you use for pregnancy detection?

Rectal palpation
Non return to estrus
Use of P4 kits