

THESIS REF. NO. _____

ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE



**OPTIONS FOR ESTRUS SYNCHRONISATION IN CROSSBRED DAIRY CATTLE: EMPHASIS
ON CONCEPTION RATE TO INSEMINATION ON ESTRUS OR SYNCHRONIZED
OVULATION, EAST SHOA, ETHIOPIA**

BY
ADDISE AMBILO

JUNE, 2019
BISHOFTU, ETHIOPIA

**OPTIONS FOR ESTRUS SYNCHRONISATION IN CROSSBRED DAIRY CATTLE: EMPHASIS
ON CONCEPTION RATE TO INSEMINATION ON ESTRUS OR SYNCHRONIZED
OVULATION, EAST SHOA, ETHIOPIA**



**A thesis submitted to Addis Ababa University, College of Veterinary Medicine and Agriculture in
partial fulfillment of the requirements for the Degree of Master of veterinary Science in Veterinary
Obstetrics and Gynecology (VOG)**

By

Addise Ambilo

Department of Clinical Studies

MVSc in Veterinary Obstetrics and Gynecology (VOG)

Advisors: Tilaye Demissie (Assoc. Professor)

Alemayehu Lemma (Professor)

June, 2019

Bishoftu, Ethiopia

ADDIS ABABA UNIVERSITY

College of Veterinary Medicine and Agriculture

Department of Clinical Studies

OPTIONS FOR ESTRUS SYNCHRONISATION IN CROSSBRED DAIRY CATTLE: EMPHASIS ON CONCEPTION RATE TO INSEMINATION ON ESTRUS OR SYNCHRONIZED OVULATION, EAST SHOA, ETHIOPIA

Submitted by:

Addise Ambilo

Name of student

Signature

Date

Approval for submittal to dissertation assessment committee

Dr. Tilaye Demissie

Major Advisor

Signature

Date

Prof. Alemayehu Lemma

Co-Advisor

Signature

Date

Dr. Fufa Abuma

Department head

Signature

Date

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Clinical Studies

As members of the Examining Board of the final MVSc open defense, we certify that we have read and evaluated the thesis prepared by: ADDISE AMBILO Entitled: OPTIONS FOR ESTRUS SYNCHRONISATION IN CROSSBRED DAIRY CATTLE: EMPHASIS ON CONCEPTION RATE TO INSEMINATION ON ESTRUS OR SYNCHRONIZED OVULATION, EAST SHOA, ETHIOPIA and recommend that it be accepted as fulfilling the thesis requirement for the degree of: Masters of Veterinary Obstetrics and Gynecology.

_____	_____	_____
Chairman	Signature	Date
_____	_____	_____
External Examiner	Signature	Date
_____	_____	_____
Internal Examiner	Signature	Date
1. Dr. Tilaye Demissie	_____	_____
Advisor	Signature	Date
2. Prof. Alemayehu Lemma	_____	_____
Co-Advisor	Signature	Date
3. Dr. Fufa Abunna	_____	_____
Department chairperson	Signature	Date

Final approval and acceptance of the thesis dissertation is contingent upon the submission of its final corrected copy to the candidate's major department.

DEDICATION

I dedicate this thesis manuscript to my late father Ambilo Larebo for his ambition to get his talent through his children and to my mother Ayelech Dobamo for nursing her children with affection and love.

STATEMENT OF AUTHOR

First, I declare that this thesis is my work and that all sources of material used have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced degree (MVSc) at Addis Ababa University, College of Veterinary Medicine and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate. Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however permission must be obtained from the author.

Name: Addise Ambilo Larebo

Signature: _____

College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission: June, 2019

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	IVIII
LISTS OF TABLES	X
LIST OF FIGURES	X
ANNEXES	XII
ABBREVIATIONS	XIII
ABSTRACT	XV
1. INTRODUCTION	1
2. LITERATURE REVIEW	5
2.1. Current status of dairy farming in Ethiopia	5
2.2. Estrous Cycle in Bovine	7
2.3. Principles of Estrus Synchronization	11
2.4. Estrus Synchronization Protocols	13
<i>2.4.1. GnRH- PGF2α -GnRH-based protocol</i>	14
<i>2.4.2. GnRH and or CIDR- PGF2α -GnRH-based protocol</i>	14
<i>2.4.3. GnRH- PGF2α -based protocol</i>	15
<i>2.4.4. PGF2α -based protocol</i>	16
2.5. Estrus Synchronization in Ethiopia	18
3. MATERIAL AND METHODS	21
3.1. Study Areas	21
3.2. Study Animals	21
3.3. Study Design and Treatment Protocols	22
<i>3.3.1. Ultrasonography</i>	22
<i>3.3.2. Treatment protocol for postpartum dairy cows</i>	22
<i>3.3.3. Treatment protocol for dairy heifers</i>	23
<i>3.3.4. Estrus response rate of cows/heifers and record of estrus characteristics</i>	24
<i>3.3.5. Type of Insemination (AI on detected estrus versus TAI)</i>	25
<i>3.3.6. Determination of Conception rate</i>	26
3.4. Data Management and Analysis	26
4. RESULTS	27

4.1. Characteristics of estrus induced by treatment protocols	27
4.2. Behavioral characteristics of estrus	28
4.3. Estrus response and Conception rate to different treatments protocols.....	28
4.4. Effect of BCS and parity on conception rate	29
4.5. Conception rate to AI on estrus and timed insemination	31
5. DISCUSSION	32
6. CONCLUSION AND RECOMMENDATIONS	36
7. REFERENCES	37

ACKNOWLEDGEMENTS

Above all, I would like to render my utmost praise to the Almighty GOD, for giving me the opportunity for this postgraduate study and for giving me health, energy, and favorable situations to carry out the research work.

This work would have not been possible without the support and contribution of many people; therefore, I would like to thank my advisors Dr. Tilaye Demissie and Prof. Alemayehu Lemma for their constructive advice, provision of different materials, overall intellectual and practical guidance.

I would like to express the respect I have for them my beloved families for all their support, especially to my beloved wife W/ro Bizunesh Adimasu and to all my family members. I would also like to express my respect to all of them my best friends of MVSc and MSc Batch of 2010 E.C entry and to all of my friends.

Many thanks goes to my institute, Livestock and Fish resources development head office of Duna Worade giving me this chance and Assefa Bogale, Prime and S and S plc., dairy farms for their admission and provision of study animals.

This work was funded by a thematic research “*Optimizing dairy herd structure and performance through complete evaluation of endocrine parameters of crossbred cattle and application of selected assisted reproductive technologies*” that was funded by Addis Ababa University, Research and Technology Transfer. Hence I would like to thank Addis Ababa University, College of Veterinary Medicine and Agriculture and especially a research team led by Dr. Tilaye Demissie which have taught me a lot what a team work meant in general and manipulation of reproductive system of dairy cattle with application of dairy technology in particular.

LISTS OF TABLES

Table 1. List of common estrus detection aids.....	11
Table 2. Description of behavioral estrus observed during estrus.....	25
Table 3. Mean interval to estrus (in hours) across different factors	27
Table 4. Mean duration (in hours) of estrus across different factors.....	27
Table 5. Behavioral estrus characteristics.....	28
Table 6. Effects of treatment protocols on induction of estrus and conception rate.....	29
Table 7. Effects of BCS on estrus response and conception rate	30
Table 8. Effect of parity of cows on estrus response and conception rate.....	30
Table 9. Fixed time AI versus AI at detected estrus.....	31

LIST OF FIGURES

Figure 1. Schematic diagram representing 2-wave (a) and 3-wave (b) follicular growth in the Bovine estrous cycle.....	9
Figure 2. PGF2 α -based treatment protocol used in the study.....	22
Figure 3. GnRH-PGF2 α or GnRH-based treatment protocols used in the study	23
Figure 4. GnRH and/or CIDR-PG or GnRH- based treatment protocols used in the study...	24

ANNEXES

Annex 1. Investigation format for collecting data from selected individual cows/heifers for hormonal manipulations, detected AI or TAI.....	51
Annex 2. Different photos were taken during this study.....	53
Annex 3. Ethical approval sheet.....	61

ABBREVIATIONS

AI	Artificial Insemination
BCS	Body Condition Score
BoA	Bureau of Agriculture
CIDR	Controlled Intra Vaginal Drug Releasing
CL	Corpus Luteum
CSA	central statistical agency
d (s)	day(s)
DF	dominant follicle
ES	Estrous synchronization
FSH	Follicular Stimulating Hormone
FTAI	Fixed Time Artificial Insemination
GDP	Gross Domestic Product
GnRH	Gonadotropin Releasing Hormone
hr (s)	hour(s)
IDMS	Improving the development and Market Success
ILRI	International Livestock Research Institute
i.m	intramuscular injection
IPMS	Improving the Productivity and Market Success
L	Liter
LH	Luteinizing Hormone
LIVES	Livestock and Irrigation Value Chains for Ethiopian Smallholders
MGA	Melengestrol Acetate
mg	milligram
mL	milliliter
MoA	Ministry of Agriculture
mRNA	messenger ribonucleic acid
NMSA	National meteorology service agency
OSMAI	Estrus Synchronization mass Artificial Inseminations

ABBREVIATIONS (Continued)

Ovsynch	Ovulation synchronisation
PGF2 α	Prostaglandin F two Alpha
P4	progesterone
SEM	standard error of mean
SNNP	Southern Nations, Nationalities, and Peoples' Region
TAI	Timed Artificial Insemination
US	Ultrasound
USD	United State Dollar

ABSTRACT

The study was conducted from November 2018 to June 2019 to evaluate conception rate to different hormonal manipulation of the estrous cycle and insemination on estrus and without estrus at fixed time in crossbred dairy cattle. Before assigning cows to treatments ovaries were scanned by ultrasonography and postpartum cows (>60 days postpartum) were divided into corpus luteum positive (n= 15) and corpus luteum negative (n= 19). All cows with corpus luteum received single PGF2 α and detected for estrus and inseminated at standing estrus. Cows without corpus luteum received GnRH at starting day and PGF2 α at day seven, detected for estrus within 48 hours and while those in estrus inseminated (Selectsynch) those did not show estrus received second GnRH at day 9 and inseminated at 16-24 hours of the second GnRH (ovulation synchronization). Dairy heifers (≥ 2 years) were randomly assigned into two groups. One group (n=14) received CIDR and GnRH at starting day and PGF2 α at day seven with CIDR removal. The second group heifers (n =13) received GnRH-on day of start (without CIDR) and PGF2 α at day seven. All heifers were inseminated either at standing estrus (Selectsynch) or at fixed time (ovulation synchronization). The estrus response was 93.3% in cows treated with single PGF2 α , 68.4% for cows receiving GnRH-PGF2 α , 50% for heifer receiving CIDR-GnRH-PGF2 α and 53.8% for heifers receiving GnRH-PGF2 α . The estrus response for all groups was 67.2%. The conception rate was 66.6% to single PGF2 α administration and insemination at estrus, 30.7% for GnRH-PGF2 α protocol and insemination at estrus, 33.3% for GnRH-PGF2 α protocol and timed insemination for cows. Overall conception rate was 71.4% for heifers receiving CIDR-GnRH-PGF2 α protocol and 53.8% for GnRH-PGF2 α protocol. The difference in conception rate was not statistically significant ($P>0.05$). In heifers receiving CIDR-GnRH-PGF2 α and inseminated at estrus all conceived (100%), while only 42.8% heifers were conceived in CIDR-GnRH-PGF2 α and fixed time insemination and difference was not significant ($P>0.05$). Conception rate was 57.1% in heifers receiving GnRH-PGF2 α and inseminated at estrus while it was 50% in GnRH-PGF2 α protocol with inseminated at fixed time insemination. BCS and parity showed statistically significant difference ($P<0.05$) on estrus response and conception in all treatment protocols. It could be concluded that ultrasound scanning for presence of corpus luteum, single PGF2 α administration and insemination at standing estrus would give better conception rate in dairy cows than GnRH-PGF2 α protocol. In dairy heifers CIDR-GnRH-PGF2 α protocol improve conception rate than GnRH-PGF2 α without CIDR in general and in particular CIDR-GnRH-PGF2 α with insemination at standing estrus gives 100% conception rate.

Keywords: *Artificial insemination, Conception rate, Dairy cattle, Ethiopia, Hormones*

1. INTRODUCTION

Livestock production is an integral part of the agricultural activities in Ethiopia. The livestock sector contributes about 12-16% of the total national Gross Domestic Product (GDP), 30-35% of the agricultural GDP, 15% of export earnings, and 30% of agricultural employment in the country (Land O'Lakes, 2010). In Ethiopia exotic and crossbred cattle are the main contributors of milk to the growing dairy industry. Although the country holds the largest livestock population in Africa, production is too low. The domestic production of meat and milk remains very low; indeed, the importations of dairy products in Ethiopia (mostly for local consumption) and demand of milk are increasing each year to more than domestic production compared with the cattle population (Alemayehu and Getu, 2015). The low production may be mainly due to poor genetic performance, nutrition, management, infertility, and reproductive disorders (Mureda and Mekuriaw, 2007; Mekonnen *et al.*, 2010; Bitew and Prasad, 2011; Haileselassie *et al.*, 2011).

A major goal in dairy cattle production is to increase the number of calves produced each year. To accomplish this goal and maximize profits, producers must have an understanding of reproductive processes of the bovine. Reproductive efficiency in farm animals, especially dairy cattle, can be increased by decreasing the interval from parturition to conception. It is well known that failure of females to display estrus early in the breeding season is a primary cause of decreased reproductive performance in dairy cattle (Wiltbank, 2011). Furthermore, it is generally accepted that in order to produce a calf each year a cow should conceive and maintain pregnancy by Day 85 postpartum. Therefore it is essential to have at least one normal ovulation before this time (Wiltbank, 2011).

It has generally been accepted that for the majority of the dairy herds where artificial insemination is practiced, the limiting factor toward obtaining efficient reproductive performance is the failure to detect estrus in a timely and accurate manner. The most productive approach to improve dairy herd reproductive performance is by working to increase heat detection or AI submission rates.

The tools to control the timing of the onset of estrus is either by controlling the length of the estrous cycle and choices of approaches for controlling cycle length are, to regress the corpus luteum (CL) of the animal before the time of natural luteolysis, and thereby shorten the cycle or to administer exogenous progestins to delay the time of estrus following natural or induced luteolysis which may extend the length of the estrous cycle (Yizengaw, 2017). Variations on one of the two approaches to cycle control are the basis for commercially-available products which successfully synchronize estrus in the majority of cows or heifers within 5 to 7 day period. This yields high conception rates following heat detection and AI breeding that are similar to those following AI after a spontaneous estrus (Yizengaw, 2017).

Several attempts have been made to improve reproductive efficiency, including monitoring of ovarian activity by ultrasound scanner. Estrus Synchronization (ES) is one of the reproductive management tools that involve induction of estrus in group of females to breed relatively in around the same time. Its programs improve reproduction efficiency by reducing the length of breeding and calving seasons and increasing calf-weaning weights (Jancy *et al.*, 2009). Products such as Prostaglandin (PGF₂α), Melengesterone Acetate (MGA), Controlled Internal Drug Release (CIDR) and Gonadotropin releasing hormone (GnRH) are some of the product used in synchronization of estrus. An understanding of how these products influence the bovine estrous cycle and of how management decisions determined pregnancy success would have an effect on the success of any reproductive program (Perry *et al.*, 2004).

Infertility is the main influencing factor that adversely affects the production and productivity of local and crossbred cows and heifers in Ethiopia. Consequently, cow and calf production and productivity continues to be unsatisfactory. Calving interval is generally longer than 12 months in most cows, including crossbreds, kept by small holder farmers. Heifers are reported to have a higher age at first calving (Shiferaw *et al.*, 2003) and rarely calve every 12-13 months after the first calving.

Artificial insemination (AI) has been a vital tool for the genetic improvement of livestock for many years in Ethiopia. However, conception rate to AI is generally very low. Many reasons may contribute to this low conception rate to AI. In adequate estrus detection for AI,

insemination at an improper time and lack of sufficient knowledge to detect estrus are among the most important factors contributing. Thus, estrus synchronization protocols that include fixed timed AI (FTAI) to minimize reliance on estrus detection may increase use of AI for breeding dairy heifers (Peeler *et al.*, 2004).

A synchronization protocol called ovulation synchronization (Ovsynch) was developed to successfully synchronize ovulation thereby allowing for a fixed time artificial insemination (FTAI) in lactating dairy cows of *Bos Taurus* cattle (Pursley *et al.*, 1995). Unfortunately, dairy heifers respond poorly to Ovsynch and FTAI, exhibiting a conception rate per AI of 20 to 40% lower than heifers receiving AI to a standing estrus (Pursley *et al.*, 1997). The greater incidence of estrus before FTAI in dairy heifers (18%) compared to lactating dairy cows (6 to 9%) may likely accounts for the low pregnancy to FTAI of Ovsynch for dairy heifers (Rivera *et al.*, 2004). Decreased pregnancy rate of virgin heifers on Ovsynch protocols could also be due to the number of follicular waves (Pursley *et al.*, 1997) or inconsistent follicular wave emergence (Nebel and Jobst, 2000).

An ovulation synchronization protocol that utilized gradual release of progesterone by control internal drug releasing device (CIDR) has been developed and has been proved to be effective for inducing and synchronizing estrus in dairy heifers of *Bos taurus* cattle. The CIDR inserted placed in to the vagina with lubricated applicator, after disinfection of the vulva. The device has a flexible polyester tail that protrudes from the vulva and is easily removed by pulling the polyester tail. The CIDR treatment includes insertion of a CIDR device on day 0, injection of PGF2 on day 6 or day 7 and CIDR insert removal on day 7. Heifers are either observed in estrus 24 to 72 hours after CIDR insert removal, and they commonly are inseminated after detection of estrus or FTAI in those heifers not in estrus (Ahmed and Derar, 2015). This system should effectively synchronize estrus in a large proportion of cyclic females since treatment for 7 days with the CIDR prevents animals from being in the early stage of the cycle and not responsive to PGF2 induced regression of the CL (Lucy *et al.*, 2001).

Injection of GnRH at CIDR insertion in this protocol has been shown to increase pregnancy rates compared to administration of GnRH-PGF or PGF alone in *Bos taurus* cattle (Stevenson *et al.*, 2003). However, these protocols were repeatedly evaluated for animals of *Bos taurus* breed and there is a scarcity of data on *Bos taurus* cattle crossbred to *Bos indicus* cattle especially in the tropical environments. There are differences in ovarian morphology and function, as well as circulating hormones and metabolites between *Bos indicus* and *Bos taurus* (Stevenson *et al.*, 2003).

Protocols that effectively used for animals of *Bos taurus* may not equally be effective in *Bos taurus* cattle that have *Bos indicus* blood (Crossbred animals). We used these differences in reproductive physiology as a study gap and derived our objectives based on these differences.

The objectives of the present study were:

- ✚ To evaluate the conception rates in synchronization that utilized a combination of gonadotropin releasing hormone (GnRH) and prostaglandin F₂α (PGF₂α) in insemination at estrus detection or at fixed time artificial insemination (FTAI) in postpartum crossbred dairy cattle.
- ✚ To evaluate the effect of CIDR insertion on conception rate of crossbred dairy heifers synchronized by GnRH-PGF₂α based protocol and inseminated either on estrus manifestation or FTAI.

2. LITERATURE REVIEW

2.1. Current status of dairy farming in Ethiopia

In Ethiopia the dairy industry plays an enormous role in ensuring food security and creating rural employment in the highlands and lowland areas. In 2013 the annual milk production was estimated to be 3.8 billion liters from cattle and 165 million liters from camel. From this, the total production of cow milk the average daily milk production/cow of 1.86 liters/day. However, Milk yield per day per cow remains to be low for indigenous dairy cattle compared to crossbred in different part of the country. Cross breeding program had significantly improved the genetic level of an overwhelming majority of indigenuous dairy cattle. Aside from the genetics, the milk yield is constrained by shortage of feed availability, water and lack of improved herd management. Therefore, improvement on the genetic makeup of dairy cattle should be complemented with provision of improved forage crops, supplementation of concentrate, adequate veterinary services and improved herd management (Metekia and Nezif, 2017).

Livestock remains to be a major national resource and form an interconnected chain in the mixed crop-livestock production system (Gebrewold *et al.*, 2000). Cows contribute to about 95% of the total annual milk produced compared to other livestock species and the remaining 5 % comes from camels from the pastoralist areas (CSA, 2010). Around 99.19 percent are indigenous breeds while the hybrids and pure exotic breeds accounted 0.72 and 0.09 percent, respectively. With this wide array of functions, livestock can be considered as a vehicle for improving food security and better livelihood of the rural population. (Land O'Lakes Inc., 2010).The huge and diverse cattle population, varied and favorable agro-ecology for dairying, increasing demand for dairy products in urban and peri-urban areas, long standing culture of dairy products consumption, and favorable policies are indicators of the importance and potential of dairying in the country (Tegegne *et al.*, 2013).

Milk production system in Ethiopia: Different species of livestock have been reared in all of the production systems of Ethiopia by pastoralists, agro pastoralists, and crop/livestock farmers (Ahmed *et al.*, 2003). Based on climate, land holdings and integration with crop as criterion, dairy production systems are classified into three main systems: **(1) rural, (2) peri-urban and urban and (3) commercial dairy systems:** The rural dairy small-holder system produces the largest share of total milk produced, contributing 98% of the milk supply (Land O'Lakes Inc, 2010).

Rural dairy production system: Majority of livestock keepers rely on rural dairy system which is part of the subsistence farming system that contribute up to 98% of the total milk production of in Ethiopia and includes pastoralists, agro-pastoralists, and mixed crop–livestock producers (Ketema, 2000; Tsehay, 2002; Yoseph *et al.*, 2003; Zegeye, 2003; Dereje *et al.*, 2005).The system is not commercial based and most of the milk produced in this system is left for home consumption (Ahmed *et al.*, 2003).

Peri-urban and urban milk production system: Peri-urban milk production has been performed in areas where the population becomes high and the agricultural land is scarce due to urbanization around major cities like Addis Ababa and other regional towns (Tsehay, 2001).This production system is now becoming prominent in the highlands among mixed crop-livestock farmers (Gebrewold *et al.*, 2000). Dairy farmers and cooperatives involved in milk production in the peri-urban and urban areas are selling milk to consumer in the nearby town and city. Dairy Producers in this production system have a better understanding of dairy management, processing facilities, better genetics (50-62.5% crosses) with experience of receiving AI services (Land O'Lakes Inc., 2010).

Urban dairy farming is a highly specialized dairy farming owned by state or businessmen and is mainly concentrated in major cities of the country. Pure exotic and cross bred cows have been used in this production system comprising 40,000 pure exotic and crossbred cows in urban and peri urban areas of the country. In Addis Ababa alone, there are about 5200 dairy farms with some 58,500 cattle (almost 50 percent crossbred). Total annual milk production from 5200 dairy farms is estimated to be 44

million liters from which 83% is marketed, while the rest is used for household consumption (Azage, 2004). However, the number of smallholder and commercial dairy farms are limited to be emerging mainly in the urban and peri-urban areas of the capital (Felleke and Geda, 2001; Azage, 2003) and most regional towns and districts (Nigussie, 2006).

Commercial Milk Production: A majority of the pure exotic animals are limited to commercial or government farms; within the Greater Addis milk shed (there are 269 commercial dairy farms with 10 or more mature cows) (Land O'Lakes Inc., 2010). Dairy Producers would rely almost exclusively on AI for good semen and keep breeding records with paying for the more expensive imported genetics and breeding supplies (SNV, 2008). In around Addis Ababa there are an estimated 5,000 dairy producers with pure and cross-bred cows producing 34 million liters per year (Land O'Lakes Inc., 2010).

According to Kumar *et al.* (2014), the production system influenced the production potential of dairy cattle in the study area. There was a significant difference in terms of total milk yield and peak milk yield being produced in the three production system having the highest milk yield of 1403.44, 1464.65 and 1352.12l/lactation lengths with peak milk yield of 7.34, 6.92 and 6.54 liters in urban followed by mixed farming and Peri-urban respectively. However, the lactation lengths between farming system were not affected by production system which might be attributed to genetic factor of the animal (Metekia and Nezif, 2017).

2.2. Estrous Cycle in Bovine

The bovine is a non-seasonal polyestrous animal that is capable of conception year round. The estrous cycle in cattle is comprised of 21 d, with the first day (d 1) of the cycle being deemed estrus or heat. Estrus occurs when the female is physiologically and psychologically receptive to the male. The estrous cycle is characterized by repeated patterns of ovarian cell proliferation, differentiation, and transformation that aid in follicular development as well as

the formation and regression of the corpus luteum (CL). Timing of ovulation is directly correlated with estrus in vertebrates. In the bovine, ovulation occurs in the window of 24 to 32 h after the onset of estrus, which could last from 6 to 24 hr (Senger, 2003).

A cascade of events leads to the ovulation of the dominant follicle (DF). The estrous cycle consists of 2 distinct phases termed follicular and luteal, both of which occur within the 21-d cycle. The ability to complete the estrous cycle in this short time frame relies on the adult female's rapidly growing tissues including the follicle and CL, which can both undergo periods of dynamic growth and regression. The follicular phase, the shorter of the 2 phases, consists of the period from regression of the CL to ovulation at which time the dominant follicle is the primary structure. The luteal phase occurs from ovulation to regression of the CL and comprises 80% of the estrous cycle with the CL as the dominant structure (Senger, 2003).

During the luteal phase, there are 2 to 3 waves of growing follicles with each wave culminating at the formation of a large follicle and the eventual selection of the dominant follicle as seen in Figure 1. In a 2-wave cycle, each wave lasts approximately 10 to 11 d; however, in a 3-wave cycle, the second and third waves are much shorter (Stewart, 2004). With these 2 patterns, waves emerge on d 0 and 10 for a 2-wave cycle and d 0, 9, and 16 for a 3-wave cycle. Each wave produces several large follicles 4 to 5 mm in diameter followed by selection and growth of the dominant follicles. All large and dominant follicles will undergo atresia, except the largest dominant follicle in the final wave prior to ovulation (Lucy, 1992).

Each wave has a dominant follicle that will achieve the greatest diameter and will suppress the growth of subordinate follicles. This suppression is the result of increasing and decreasing estradiol and inhibin hormonal concentrations. The concentration of luteinizing hormone (LH), however, consistently increases, encouraging growth of the dominant follicle. Each of these dominant follicles undergo a series of events during their lifespan including 6 d of growth (growing phase), 6 d of no growth (static phase), and followed in the remaining time by regression (regressing phase) (Noseir, 2003). An increase in follicular size of the dominant follicle is directly related to increased estradiol concentrations and decreased progesterone

concentrations (Noseir, 2003). Estradiol concentrations peak at the end of the growing phase and begin to decline upon the beginning of the static phase (Bartlewski, 2015).

During this time, inhibin secretion is gradually increasing. Increased estradiol and inhibin concentrations suppress follicle stimulating hormone (FSH) release, reducing FSH secretions significantly when selection of the dominant follicle occurs. Granulosa cells of atretic follicles show little to no mRNA expression of the estrogen receptor (Bao, 2000). The decrease in estradiol during the static phase initiates the increasing release of FSH and a subsequent follicular wave (Kaneko, 1991). This cycle repeats for each wave that does not produce the preovulatory follicle.

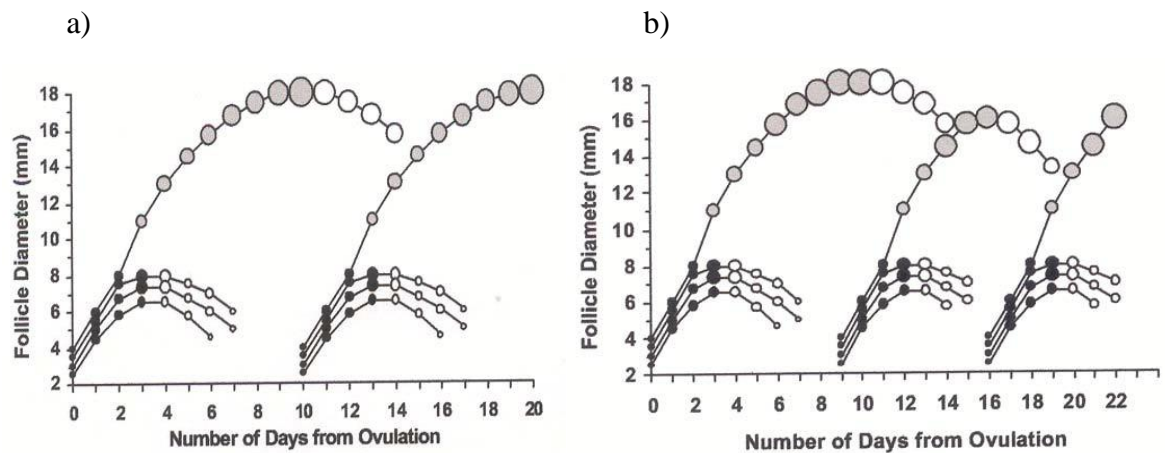


Figure 1. Schematic diagram representing 2-wave (a) and 3-wave (b) follicular growth in the Bovine estrous cycle: Source, Jillian (2005).

The number of follicular waves is immediately dependent upon the surge of GnRH and subsequent FSH and estradiol. Follicular wave number is also influenced by age, parity, dietary intake, and lactational status with the general guideline of primiparous and multiparous lactating cows having 2 waves and nulliparous heifers have 3 waves (Sartori, 2000). The compensation for increased wave numbers is accounted for with decreased wave time span (Kastelic, 1991). Growing follicles before the selection of the dominant follicle are depicted as black circles, the dominant follicles are the grey circles, and the open circles are atretic follicles undergoing death and regression (Fricke *et al.*, 2001).

In tandem with the follicular dynamics of the luteal phase is the maintenance of the CL and progesterone production. Throughout this time, the CL remains the dominating structure and progesterone synthesis maintains a pulsatile secretion of GnRH from the hypothalamus. Beginning on or around d 14 to 16, the uterus begins to release pulses of prostaglandin into its venous drainage into the ovaries. Within hours, the functional role of the CL has been removed, with the structural portion of the CL often taking more than 12 h to be fully removed. The uterus possesses the most influence on regression of the CL. In cows that have undergone a removal of the uterus (hysterectomy), the functional lifespan of the CL is prolonged in an occurrence of pseudopregnancy. By d 17, the CL is fully lysed, and both functional and structural components are removed in a process termed luteolysis that requires a number of external factors to complete the process. With the regression of the CL and the formation of the preovulatory follicle, the luteal phase has come to an end (Jillian, 2005).

Table 1. List of common estrus detection aids

Detection Aids	How it Works	Potential concerns
Tail chalk	Chalk is applied to tail head. When animal is mounted the color will be rubbed off and hair be ruffled	Removal by trees, water, fences, or licking by other animals
Heat mount detectors	Detectors are applied to tail head and turn a different color when mounted.	Partial activation or loss of detector requires interpretation, false activation; trees, fences, other animals
Heat watch	Transmitters are attached to tail head region. When transmitter is depressed a signal is sent to receiver	Expensive to replace lost sensors, data interpretation, appropriate facilities/terrain
Gomer Bulls	Vas-ectomized, epididym-ectomized, and or penile-deviated animals are used as teaser animals and will mount females in estrous	Feeding and maintenance expense, potential loss of desire to mate, and disease transmission by non-penile deviated animals
Chinball marking harness	Detector animal is fitted with harness leaving an ink mark on the back and neck of females that have been mounted	Maintenance of equipment, feeding and maintenance of animal, ill-defined markings
Androgenized Cows	Testosterone injections before and during the breeding season or androgen implant causes cow to mount other females in heat.	Cost and labor of administering drug, variable response to hormone

Source: (Smith *et al.*, 2011)

2.3. Principles of Estrus Synchronization

Most estrus synchronization systems employ a method for controlling follicular wave development, preventing premature ovulation in cyclic cows and promoting ovulation in anestrus cows accomplished through progesterone supplementation, regressing the corpus luteum in cyclic cows, synchronizing estrus and or ovulation at the end of treatment (Lucy *et al.*, 2004). Synchronizing estrous cycle of domestic cattle depends on control of the functional life span of the corpus luteum (Hansel and Convey, 1983).

There are two ways to facilitate control of the corpus luteum that result subsequently in estrus and ovulation. The first method involves long-term administration of a progestin with subsequent regression of the corpus luteum during the time the progestin is administered (Britt, 1987). Estrus and ovulation occur within 2 to 8 days after progestin withdrawal. The second method involves the administration of a luteolytic agent that shortened the normal life span of the CL. This is accompanied generally with estrus and ovulation within 48 to 120 h after injection (Murugavel, 2004). Therefore, Synchronization of estrus in cows is feasible by either prolonging or shortening of the luteal lifespan.

The success of an estrus synchronization program is largely based on understanding the estrous cycle, the biological actions of estrus synchronization products and the selection of heifers and cows that have a high likelihood of responding appropriately to the preceding products (Smith *et al.*, 2006). All synchronization programs require good management, cows having regular estrous cycles and in good body condition (Hopkins and Schrick, 2012).

Cows should be selected based on the following criteria; adequate time has elapsed from calving to the time of synchronization treatments; a minimum of 40 days postpartum at the beginning of treatment is suggested. Average or above average body condition score; at least 5 on a scale of 1 to 9, Minimal calving problems. Replacement heifers are developed to pre-breeding target weight that represent at least 65% of their projected mature weight and reproductive tract scores are assigned to heifers two weeks before a synchronization treatment begins; scores of 2 or higher on a scale of 1 to 5; to avoid problems during synchronization (Patterson *et al.*, 2000).

Heifers need to reach puberty prior to estrus synchronization to increase the likelihood of responding to a synchronization program. Furthermore, a 21% increase in fertility is experienced at a heifer's third estrus compared to her pubertal estrus (Byerley *et al.*, 1987). If labor is available or can be hired, protocols using heat detection are generally lower cost than fixed-timed AI. Treatments, semen and number of handlings would contribute to cash costs of synchronization (Brtf, 2006).

2.4. Estrus Synchronization Protocols

Effective synchronization of estrus in beef and dairy cattle has been a goal of the cattle industry since AI techniques were available. Prostaglandin F₂ α and its analogues are the most common treatment used to shorten the luteal phase and synchronize estrus. Progestogen have been used to extend the luteal phase, resulting in synchronous estrus after progestogen withdrawal. More recently, GnRH and estradiol alone or in combination with progesterone have been used in estrus synchronization programs. Effective estrus synchronization protocols are designed to synchronize follicular maturation with the onset of corpus luteum regression. In general, development of estrus synchronization protocols in cycling animals has involved the following three approaches: First, inhibit ovulation following spontaneous corpus luteum regression (long-term progestin treatment), Second, Induction of corpus luteum regression (PGF₂ α treatment) and third, combination of the two. Most of the protocols utilized today can be categorized under the third approach (Smith *et al.*, 2006).

The first approach requires long-term progestin treatment; 7 to 14 days and is effective at synchronizing estrus; however, the second approach results in good fertility; however, animals that are in the first 5 to 6 days of their cycle will not respond to the PGF₂ α injection, resulting in a reduced synchronization response. The third approach allows effective synchronization of estrus, regardless of stage of the cycle, without compromising fertility. This is particularly true when an injection of GnRH is administered at the beginning of progestin treatment to ovulate dominant follicle and synchronize a new follicular wave (Smith *et al.*, 2006). Injection of prostaglandin causes luteolysis and allows for the final growth and maturation of the dominant follicle followed by ovulation at a predictable time post injection (Murugavel *et al.*, 2003).

Most estrus synchronization protocols are mainly based on the use of the luteolytic agents, prostaglandins or their synthetic analogues. The use of prostaglandin as a drug for estrus synchronization 10–17 or as a therapeutic agent 18–25 has been extensively reviewed in both dairy and beef cows. Furthermore, for PGF₂ α treatment to achieve its luteolytic effects; animals must be in the diestrus stage of the estrus cycle i.e. in day 7 to day 17. However, Prostaglandin treatment in the early stage of estrous cycle i.e. the first 5 days was found to be ineffective in

causing a luteolytic response in cattle (Murugavel *et al.*, 2003).

2.4.1. GnRH- PGF2 α -GnRH-based protocol

In the United States, most dairy herds have an estrus detection efficiency of < 50% (Washburn *et al.*, 2002), resulting in substantial economic losses for dairy producers. Estrus synchronization protocols, such as Ovsynch, that allow for timed artificial insemination (TAI) have been developed to alleviate this problem and increase reproductive efficiency (Pursley *et al.*, 1997). Conception rates are greatest when Ovsynch is initiated on Days 5 to 12 of the estrous cycle, because of increased rates of ovulation following the first injection of GnRH of Ovsynch (Bello *et al.*, 2006).

The primary synchronization of ovulation protocol was given by Pursley *et al.* (1995) which consists of first injection of GnRH followed 7 days later with an injection of PGF2 α , followed in 48 hrs by a second injection of GnRH; TAI could be performed 0 to 24 h (optimally 16 to 18 h) later. Following this preliminary report, many protocols have been proposed and routinely applied in high production dairy cows (Wiltbank *et al.*, 2011). Although it allows for satisfactory pregnancy rates without heat detection, it does not necessarily eliminate the need for heat check. Treated animals should be observed closely for returns to estrus 18 to 24 days later. Additionally, up to 20 percent of treated cows will display standing estrus between days six and nine of this protocol (Geary *et al.*, 2000).

2.4.2. GnRH and or CIDR- PGF2 α -GnRH-based protocol

It is another option to GnRH-PGF2 α -GnRH-based protocol that availed more extensively in beef herds (Geary *et al.*, 2001). It eliminates one animal handling by breeding cows coinciding with the second GnRH injection. Most field trials indicated only a small reduction in conception rates in this protocol than previous one. As with previous one, pregnancy rates are maximized if early heats (\pm 24 hours of PGF2 α) are visually detected and bred using the a.m.-p.m. rule (Pursley *et al.*, 1998).

Accordingly, the most recently developed synchronisation treatments combine traditional methods to control cycle length and also manipulate follicular dynamics to select the ovulatory follicle and decrease variation in estrus onset (Beal, 1998). Gonadotrophin-releasing hormone may be given in combination with progesterone to synchronise follicular waves. The effect of a low or an intermediate dose of GnRH given to heifers (Mihm *et al.*, 1998) or a high dose given to post-partum cows (Ryan *et al.*, 1998) is dependent on follicular wave status at initiation of treatment. Ovulation will occur if GnRH is given at follicular dominance, there is, however, no effect if given at emergence of a follicular wave. The cohort of follicles continues to develop until dominance is attained approximately 3-4 days later.

Therefore, it is necessary to minimize the length of the progesterone treatment to ensure a dominant follicle of short duration is present at the end of treatment. Additionally it is necessary to include a luteolytic agent in the regimen in both cyclic and non-cyclical animals. Ryan *et al.* (1995) reported that estrus synchronisation resulting in high estrus detection rate (86%) and good pregnancy rates (58%) are achievable following an 8-day progesterone treatment and GnRH agonist (Buserelin) given at the start of treatment and PGF2 α on the day before progestogen withdrawal. However, research from our laboratory (Lane *et al.*, 2001) showed that GnRH given at the start of progesterone treatment of 8 days duration decreased submission rate, at 72 h, in beef heifers compared with estradiol benzoate given at the start of treatment (69% vs 95%). So although not as effective as estradiol benzoate to improve estrus synchrony in heifers, GnRH is currently the only available alternative to use at the start of a synchrony regimen to manipulate follicular growth.

2.4.3. GnRH- PGF2 α -based protocol

This is a breeding option for those herds with good heat detection programs and that prefer to breed cows based to standing estrus. Cows are either bred to detected estrus for three to five days after PGF2 α (Option 1 (Geary *et al.*, 2000) or bred to estrus for 72 hours after PGF2 α with no responders' time bred at 72 hours with a concurrent injection of GnRH (Option 2 (De Jarnette *et al.*, 2001). This approach allows most cows (50 to 70 %) to be bred at standing estrus and gives

all cows an opportunity to conceive with the clean-up AI at 72 hours. This approach saves additional hormone costs because only those cows that fail to show estrus receive the second GnRH injection. It facilitates more efficient use of expensive or genetically valuable semen by targeting its use in cows at estrus, whereas less expensive semen can be reserved for the timed AI services (Geary *et al.*, 2000).

The administration of GnRH or a derivative induces a gonadotrophin surge (Mihm *et al.*, 1998), alters the pattern of follicular growth and may induce ovulation of a dominant follicle. The effect of GnRH is dependent on follicular status at the time of administration. Research conducted by Ryan *et al.* (1998) showed that 250 g GnRH resulted in ovulation, in 20 of 20 cows, when given at dominance of a follicular wave, this was followed by emergence of a new wave of ovarian follicular growth 1.6 ± 0.3 days later and dominance of the subsequent wave was attained in 5 ± 0.3 days. However, there was no effect of GnRH on follicular dynamics when given at emergence of a follicular wave. The existing cohort of follicles continued to develop unaffected in 17 of 17 cows, and dominance occurred 3.6 ± 0.5 day later. Thus, GnRH may cause ovulation or no effect on follicle development depending on the animals' stage of follicle development at treatment. The interval to formation of the next dominant follicle was different in cows given GnRH at emergence compared with those given GnRH at dominance of a follicular wave.

Despite this difference, if GnRH administration were followed with PGF2 α injection, 7 days later a dominant follicle should be present irrespective of follicle wave stage at time of initial GnRH treatment. Consequently, it has been reported that the use of a GnRH agonist followed by PGF2 α injection 7 days later effectively synchronises estrus. However, the problem is that in non-cyclic anestrus animals, when GnRH fails to induce ovulation (i.e., when administered during non-dominance phases) there is no CL present to respond to the PGF2 α injection 7 days later. The estrus response to this treatment is good and fixed time insemination may be used if a further injection of GnRH is given approximately 48 h after PGF2 α administration while insemination is carried out 15 h after the last GnRH injection (Schmitt *et al.*, 1996).

2.4.4. PGF2 α -based protocol

Prostaglandin is a luteolytic hormone that regulates the life-span of the CL. A major limitation of using PGF2 α is that animals will only respond if they have a functional CL present at the time of treatment and is therefore not suitable for use in pre-pubertal heifers and cows in the early postpartum period (Kinder *et al.*, 1996). If PGF2 α is administered from day 6 of the estrus cycle until the time of natural luteolysis it will cause immediate regression of the CL, decreased progesterone secretion, and transition to the follicular phase, resulting in increased LH pulse frequency, allowing ovulation to occur. To illustrate the role of PGF2 α , treatment of diestrus cows with a single dose of PGF2 α significantly improved conception rate to first insemination compared with untreated controls (Macmillan and Day, 1982).

Prostaglandin does not directly affect follicular wave dynamics and the timing of estrus onset is dependent on follicular status at time of PGF2 α administration. If administered at dominance of a follicular wave, the onset of estrus will be reduced 2-3 days; (Savio *et al.*, 1990). If PGF2 α is given before follicular dominance is attained the interval from administration to onset of estrus will be lengthened 4–5 days (Macmillan and Henderson, 1984) to allow development of an ovulatory follicle. In a random group, animals will be at different stages of a follicle wave and thus, variation in the interval from administration to onset of estrus will occur. The estrus response in heifers is improved if PGF2 α administration is strategically timed to coincide with the presence of a dominant follicle, for example using a regime of two PGF2 α injections given 11 days apart, when the animals tend to be at dominance of a follicular wave at the time of the second injection. Fixed time insemination resulting in good pregnancy rates may be used following this regimen in cows/ heifers (Roche *et al.*, 2000).

The effectiveness of double injections of PGF2 α in lactating cows to synchronise estrus is questionable. The estrus response in lactating dairy cows following two injections of PGF2 α 14 days apart was poor with only 55% of cows detected in estrus, although the conception rate in the cows that were inseminated was high (52%; Stevenson *et al.*, 1999). In this particular study, cows that were not detected in estrus were inseminated at 80 h after the second PGF2 α injection. The conception rate to this fixed time insemination was very low at 15%. Therefore, this treatment

regimen should only be considered in a herd in which a high proportion of the cows have been shown to be cyclic and where estrus detection rate is good. Some studies have investigated the use of a double insemination 24 h apart to improve conception rates. Tenhagen *et al.* (2000) reported that pregnancy rates were similar following insemination at a detected estrus (30%) compared with two fixed time inseminations at 60 and 90 h (33%) after synchronisation with two PGF2 α injections 14 days apart.

Furthermore, the interval to first service and number of days open were reduced by the double fixed time inseminations. The authors concluded that the cost of the extra inseminations was justified economically. In a commercial setting, double inseminations could perhaps be considered where the use of semen of reasonable cost is employed. To improve the response in anestrus cows' progesterone may be combined with PGF2 α in a double prostaglandin programme. Supplementation with progesterone (administered for 5 days before the second PGF2 α) in a double PGF2 α synchronisation programme increased both submission and conception rates (Xu *et al.*, 1997).

Research has shown that increased proportions of high-yielding dairy cows have prolonged post-partum anestrus periods (Harrison *et al.*, 1990) and more recently, Veerkamp *et al.* (2000) have described an unfavourable correlation between interval to first luteal activity post-partum and high milk yields. Similarly poor body condition in post-partum beef cows prolongs the anestrus period due to lack of ovulation of a dominant follicle (Stagg *et al.*, 1995). These cows do not respond to PGF2 α and require an alternative approach to estrus synchronisation, due to the absence of a CL on the ovaries. The estrus response may also be improved if prostaglandin is administered following diagnosis of an existing CL.

2.5. Estrus Synchronization in Ethiopia

Synchronization is regulating follicular development; provide multiple stimuli designed to induce females to cycle, and treatments that synchronize ovulation for the purposes of timed AI. Despite the wide application and success of AI and estrus synchronization throughout the developed world and the success rate in African and other developing countries is still low owing to a number of technical, system related, financial and managerial problems (Desalegn *et al.*, 2009).

The history of artificial insemination of cattle in Ethiopia goes back to 1938 by the veterinary institute in Asmara then part of Ethiopia; still Ethiopia manages largest livestock population in Africa, but the productivity of animals is very low due to many reasons. This large potential for dairy production development due to the country's favorable climate which supports use of improved, high yielding animal breeds and offers a relatively disease-free environment for livestock development. Given the considerable potential for smallholder income and employment generation from high-value dairy products, development of the dairy sector can contribute significantly to food security and nutrition of the country. The Land O'Lakes (2010) report indicated that most of the milk (85%) produced by dairy households is consumed by the household.

Ethiopia has over 50 million indigenous cattle and about 10 million are breeder cows, with annual calving rate of 45%. The number of improved dairy type animals is insignificant (Desalegn *et al.*, 2009). Due to the high demand-supply variance, annual import of dairy products is over USD 10 million. The current human population of 80 million will double by 2030; increasing the demand for dairy products. However, there is huge potential for dairy development due to the large human and livestock population and suitable agro-ecologies. One of the major problems hindering smallholder farmers from participating in milk production and marketing is lack of access to and high price of improved dairy animals. Hormonal estrus synchronization (Desalegn *et al.*, 2009) under smallholder context could be used, among others, to produce large number and dairy animals in a short period of time (kick start), to match calving with feed availability and market demand for dairy products and to improve the effectiveness and efficiency of AI service (Desalegn *et al.*, 2009).

Estrus synchronization was conducted first in field trial by IPMS project in Tigray and SNNP regions under on-farm condition to improve dairy genetics in smallholder farmers. Then after, the technology was adopted and scaled up by the MoA and regional BoAs in collaboration with international development partners; IDMS and LIVES projects of ILRI and the national research system. Finally, LIVES project initiated action research activities to appraise performance of the technology at a larger scale and introduce state of the art technologies to improve the performance of OSMAI in Ethiopia through LIVES-sponsored projects. Evaluation of OSMAI

was assessed in four (Oromia, Amhara, Tigray and SNNP) regions of Ethiopia by the researcher (Gizaw *et al.*, 2016).

3. MATERIAL AND METHODS

3.1. Study Areas

This study was conducted in Bishoftu and Modjo towns of Eastern Shoaw zone of Oromia regional state in selected dairy farms from November 2018 to June 2019. These study areas were selected based on the abundance of crossbred dairy cattle and willingness of the owners. The farms are privately owned farms producing milk. Bishoftu is located at 47 km Southeast of Addis Ababa at latitude and longitude of 8°45'N 38°59'E respectively and elevation of 1885 meters above sea level. The mean annual rainfall of the town is 866 mm with a bimodal pattern. The mean annual minimum and maximum temperatures are 14⁰C and 26⁰C, respectively and relative humidity was 61.3% (NMSA, 2003). Modjo town is located at 66 Km South-east of Addis Ababa and lies at latitude of 8°35'N and longitude 39°7'E. The altitude is at 1790 meters above sea level. The area gain rainfall twice a year those known as long and short rainy season. The main rainy season extends from June to September. The average annual rainfall, temperature, and mean relative humidity are: 776mm, 19.4 °C and 59.9% respectively (CSA, 2005).

3.2. Study Animals

A total of 61 crossbred dairy cattle (34 postpartum cows and 27 heifers) were selected from 4 dairy farms and included in this study. All cows and heifers were apparently normal and had no previous history of periparturient diseases or disorders. Routine deworming against worms was in practice. All animals were subject to clinical and gynecological evaluations of the reproductive tracts to check for any abnormalities and to determine the ovarian status of individual animals using rectal palpation and trans-rectal ultrasonography. Only apparently healthy animals without any reproductive disorder were selected as a candidate. In all farms, animals were fed teff straw and grass hay as a basal diet and supplemented concentrate feed (mixed from wheat bran, wheat middling, corn, Noug cakes, and mineral salts). Feeding was based on level of production, while water was given *ad libitum*.

3.3. Study Design and Treatment Protocols

Ethical considerations in this study were taken seriously. All activities were carried out in accordance with the ethical guideline of the Addis Ababa University College of Veterinary Medicine and Agriculture (Annex 3) after receiving the ethical approval.

3.3.1. Ultrasonography

Before the start of the experiment, the reproductive tracts of all animals were evaluated using a real-time B mode ultrasound with 7.5 MHz linear array probe (Mindray, China). The ovaries were examined and the cows were grouped into two based on presence or absence of corpus luteum (CL). But, the heifers were grouped in to two treatment groups randomly.

3.3.2. Treatment protocol for postpartum dairy cows

PGF2 α -based treatment protocol: All cows with corpus luteum (n= 15) were treated with 2ml PGF2 α (Synchromate, BREMER PHARMA GMBH 34414 Warburg, Germany) intramuscularly as single dose. All cows were meticulously monitored for any sign of estrus for 2-5 days. In cows that manifested estrus the onset from PGF2 α injection, duration and intensity of estrus were recorded. Signs related estrus behavior (restlessness, mucus vaginal discharge, sniffing, standing to be mounted, and mounting others etc...) were recorded. All cows were inseminated on standing estrus (Figure 2).

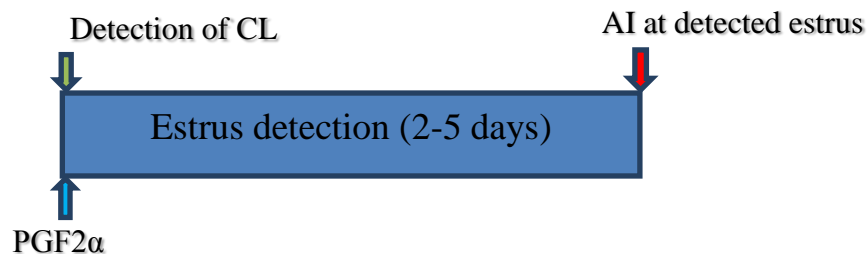


Figure 2. PGF2 α -based treatment protocol used in the study.

GnRH-PGF2 α based treatment protocol: All cows without CL (n= 19) were treated with 100 μ g GnRH (Gonadorelin acetate, Vetoquinol, Canada) on day zero (day of start) and by PGF2 α on the day 7 of GnRH injection. Cows were observed for estrus manifestation and AI was given on standing estrus in those that were in estrus. In those that were not in estrus second GnRH was given intramuscularly on the day 9 and TAI was conducted on day 10 (within 16 to 24hrs of the second GnRH) (Persely *et al.*, 1995) (Figure 3).

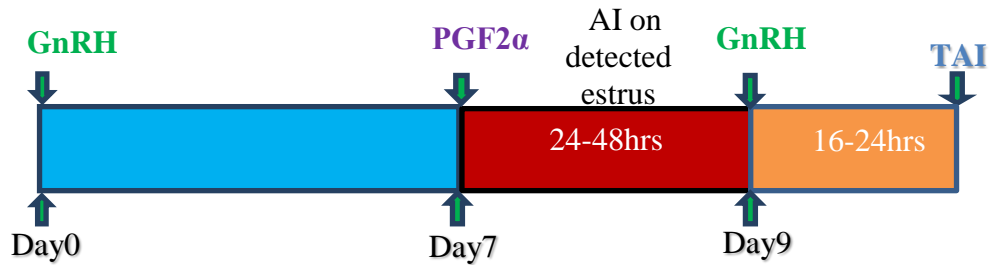


Figure 3. GnRH-PGF2 α or GnRH-based treatment protocols used in the study.

3.3.3. Treatment protocol for dairy heifers

The heifers were divided in to two groups in randomized block design based body condition scores and were assigned to two treatment protocols as follow.

Treatment One: CIDR - GnRH- PGF2 α - GnRH group: On day 0 (day of start) all heifers in this treatment (n= 14 heifers) received intravaginal CIDR insertion (1.38gm progesterone, EAZI BREED™, CIDR 1380, New Zealand) and intramuscular injection of 100 μ g GnRH (Gonadorelin acetate, Vetoquinol, Canada). On day 7 all heifers received intramuscular injection of 500 μ g cloprostenol sodium (Synchromate, GMBH, GERMANY) and the CIDR insert was removed. Then the heifers were followed for estrus for 48hrs three times a day. Heifers in estrus were inseminated on standing estrus (Select Synch) (Bhoraniya *et al.*, 2012). All heifers that did not show estrus received a second dose of GnRH (100 μ g i.m) on day 9 and Fixed-time AI (FTAI) were performed on Day 10 (16–24 hr after the second GnRH) (Modified Ovsynch) (EI-Zarkouny *et al.*, 2004; Stevenson, 2005; McDoughall, 2010).

Treatment Two: Treatment without CIDR: Heifers (n=13) received 100 µg i.m GnRH (Gonadorelin acetate, Vetoquinol, Canada) on Day 0 (day of start). On day 7 heifers were treated with a regular IM dose of 500 µg cloprostenol sodium (PGF2α) (Synchromate, GMBH, GERMANY). Then the heifers were followed for estrus for 48hrs three times a day. Heifers in heat were inseminated on standing heat (Select Synch) (Bhoraniya *et al.*, 2012). All heifers that did not show heat received a second dose of GnRH (100 µg i.m) on day 9 and Fixed-time AI (FTAI) were performed on Day 10 (16–24 hr after the second GnRH) (Modified Ovsynch) (EI-Zarkouny *et al.*, 2004; Stevenson, 2005; McDougall, 2010).

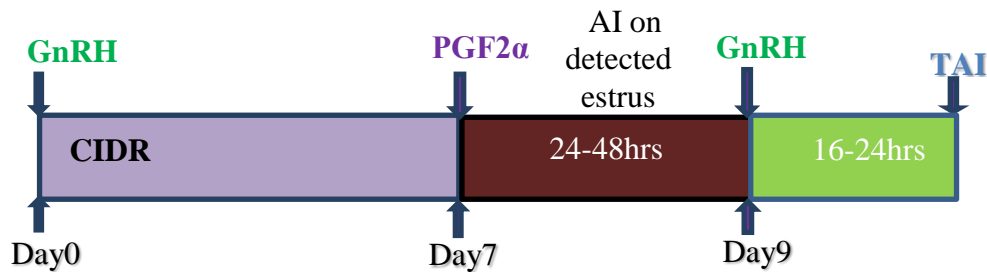


Figure 4. GnRH and/or CIDR-PG or GnRH- based treatment protocols used in the study.

3.3.4. Estrus response rate of cows/heifers and record of estrus characteristics

The time interval from the PGF2α administration to the onset of estrus, for the animals was recorded for animals that manifested estrus at 24,48,72,96 and 96 hours.

Visual observation was carried out three times a day (7 am, 1 pm and 5 pm) in each treatment group until the last animal stopped exhibiting any sign. All observations were carried out by one observer and sessions lasted about one hour. The observer recorded the onset and duration of occurrence of behavioral signs; quiet acceptance (standing) to be mounted, mounting others/disoriented mounts, chin resting, supporting chin of others, ruffled hair or excoriation on tail head or rump, vulva mucosa red or pink, vaginal mucus discharge, urination, sniffing the ano-genital, restlessness/ head-Butting, rejection to mount, soliciting with others, and bellowing. Detailed descriptions of each of the signs are depicted in (Table 2).

Table 2. Description of behavioral estrus signs observed during estrus

Behavior	Definition
Bellowing	A cow expresses excessive mobility
Chin resting or supporting chin of others	The actor (standing) puts or rests her head on the back or rump of the receptor (standing). The receptor stays in the same place for at least 3 sec (supporting chin of others).
Urination	A cow expresses excessive mobility
Quiet acceptance/standing to be mounted	The recipient stands immobile while another cow mounts.
Rejection to mount	One cow attempts to mount another without the recipient standing.
Ruffled hair on tail head or rump	Due to mounting activity the tail head or rump is ruffled.
Sniffing the ano-genital	One cow investigates the front or back half of another with her muzzle.
Soliciting with others	Cows activity stays together, the receptor walk away the actor immediately follows the receptor.
Standing to mounts/disoriented mounts	A cow mounts another from the front, back, or side.
Vaginal mucus discharge	Vaginal mucus is protrude out and hanging on vulva
Vulva mucosa red or pink	Vaginal mucosa becomes red or pink.
Walking in circles/restlessness/butting head to head	A cow follows another in a circular pattern and the cow sticks another with her head.

3.3.5. Type of Insemination (AI on detected estrus versus TAI)

To know more precisely the estrus onset, animals were monitored starting 24 hours after PGF₂ α administration 3 times a day (in the morning, at noon and late in the evening). Animals were artificially inseminated when they were detected in estrus after PGF₂ α injection.

In those animals which did not manifest estrus were inseminated at fixed time AI (at 16 to 24 hr) on day 10.

3.3.6. Determination of Conception rate

All animals that exhibited standing estrus were inseminated with Holstein semen purchased from the National Artificial Insemination Center, Kality, Ethiopia. Quality of semen was evaluated before insemination at the Reproduction laboratory of the College of Veterinary Medicine and Agriculture, Addis Ababa University.

Conception was evaluated by ultrasonography on 30 to 42 day post AI. Conception rates were calculated by dividing the number of cows that were diagnosed conceived to those inseminated in protocol.

3.4. Data Management and Analysis

All collected data were managed in Microsoft excel sheet and analyzed using R-studio version 12.0. Data were summarized using descriptive statistics (mean \pm standard error of mean and percentage) and frequency distribution. Effects of the BCS, parity and different treatment protocols, insemination on detected heat versus at FTAI on conception rate were analyzed using Chi-square. Data on the mean interval to onset of estrus manifestations and duration of estrus were analyzed using student t-test and total intensity of each estrus was calculated by summing up scores assigned to each value. The level of significance was held at $P \leq 0.05$ and 95% confidence interval.

4. RESULTS

4.1. Characteristics of estrus induced by treatment protocols

The overall mean (\pm SE) interval to estrus and duration of estrus were 72.38 ± 2.1 hr and 22.4 ± 2.1 hr, respectively. Interval to estrus was highly influenced ($P < 0.05$) by the treatment protocols with cattle received GnRH-PGF2 α protocol came to estrus at longer time than others. Details were indicated in (table 3 and 4).

Table 3. Mean interval to estrus (in hours) across different factors.

Factor	Category	N $^{\circ}$. responded	Mean \pm SEM(hrs)	<i>P</i> -value
Treatment	(a)	20	76.86 ± 5.86	0.036
	(b)	14	67.20 ± 3.39	
	(c)	7	69.20 ± 2.31	
BCS	2.5-3.0	27	72.00 ± 3.90	0.621
	3.5-4.0	14	68.82 ± 5.07	
Parity	0	14	62.80 ± 3.93	0.014
	1-3	15	64.80 ± 3.93	
	4-5	12	68.40 ± 3.60	

Where, (a) =GnRH-PGF2 α based protocol, (b) = PGF2 α based protocol and (c) = GnRH+CIDR-PGF2 α based protocol.

Table 4. Mean duration (in hours) of estrus across different factors.

Factor	Category	N $^{\circ}$. responded	Mean \pm SEM(hrs)	<i>P</i> -value
Treatment	(a)	20	22.29 ± 1.16	0.552
	(b)	14	23.04 ± 4.21	
	(c)	7	20.03 ± 2.11	
BCS	2.5-3.0	27	24.86 ± 3.65	0.881
	3.5-4.0	14	17.45 ± 1.89	
Parity	0	14	20.21 ± 2.31	0.822
	1-3	15	22.29 ± 1.16	
	4-5	12	24.20 ± 3.14	

Where, (a) =GnRH-PGF2 α based protocol, (b) = PGF2 α based protocol and (c) = GnRH+CIDR-PGF2 α based protocol.

4.2. Behavioral characteristics of estrus

Estrus characteristics were evaluated for each animals. Details of behavioral estrus characteristics were indicated in table below (table 5).

Table 5. Behavioral estrus characteristics.

Estrus behavior	Number examined	Number manifested behavior	Percent (%)
Redness of vaginal mucosa	61	31	50.8 %
Standing to be mounted	61	33	54.0%
Chin resting	61	27	44.2%
Support chin of others	61	27	44.2%
Mounting others	61	32	52.4%
Mucus vaginal discharge	61	36	59.0%
Restlessness	61	38	62.2%
Soliciting with others	61	21	34.4%
Frequent urination	61	13	21.3%
Sniffing	61	35	57.3%
Bellowing	61	14	22.9%
Ruffled hair	61	19	31.1%

4.3. Estrus response and Conception rate to different treatments protocols

Effects of hormones on post-partum cows and heifers were shown in Table 6. It showed that when PGF₂ α -based hormonal treatment was given for induction of estrus, higher (93.3%) proportion of cows showed estrus compared to GnRH-based treatment counterpart (68.4%). In addition, 67.2% (41/61) cows and heifers manifested detected estrus and 54.0% (33/61) cows and heifers conceived after receiving hormonal treatment at whole. Details were indicated in (table 6).

Table 6. Effects of treatment protocols on induction of estrus and conception rate

Category	Treatment protocols	N \bar{O} . treated	N \bar{O} . showed estrus	% showed estrus	TAI	N \bar{O} . conceived			% Conceived	χ^2	P-value
						ER	TAI	total			
Cows	a	19	13	68.4	6	4	2	6	31.5	0.133	0.715
	b	15	14	93.3	-	10	-	10	66.6		
Heifers	c	13	7	53.8	6	4	3	7	53.8	1.814	0.177
	d	14	7	50	7	7	3	10	71.4		
Total	all	61	41	67.2	19	25	8	33	54.0		

Where, a = GnRH-PGF2 α protocol (cows), b = PGF2 α protocol (cows), c= GnRH-PGF2 α protocol (heifers), d=CIDR+GnRH-PGF2 α protocol (heifers) protocol, ER= estrus response, TAI = Timed AI

4.4. Effect of BCS and parity on conception rate

Effect of BCS of cow on the different treatments are shown in Table 7. BCS is widely used for the evaluation of nutritional status of animals, which influences the fertility indexes. It was found that higher (100%) proportion of cows and heifers (83.3%) showed estrus after receiving treatment with PGF2 α when their BCS ranged 2.75-3.0 and 3.5-4 respectively. After AI, conception rate was higher (66.6%) in cows and (77.7%) in heifers with BCS ranged 2.75-3.0 and 3.5-4 treated with PGF2 α and CIDR+GnRH-PGF2 α based protocols respectively. In contrast, (33.3%) cows and (28.5%) heifers with BCS 3.5-4.0 and 2.75-3 showed estrus behavior after treatment in GnRH-PGF2 α based protocol, as well as conception rate was lower (33.3%) and (42.8%) in this group respectively. The difference in BCS among the dairy cattle received three hormonal treatment protocols was statistically significant ($P < 0.05$) within treatment groups. There is paucity of research to compare our findings in this regard.

Table 7. Effects of BCS on estrus response and conception rate.

Category	Treatment protocols	BCS (range)	N ₀ . treated	N ₀ . in estrus	% in estrus	N ₀ . conceived			% conceived	<i>P</i> -value
						ER	TAI	total		
Cows	a	2.75-3	13	11	84.6	2	2	4	38.4	0.0002
		3.5-4	6	2	33.3	2	-	2	33.3	
	b	2.75-3	12	12	100	8	-	8	66.6	
		3.5-4	3	2	66.6	2	-	2	66.6	
Heifers	c	2.75-3	7	2	28.5	2	1	3	42.8	0.017
		3.5-4	6	5	83.3	2	2	4	66.6	
	d	2.75-3	5	2	40	3	-	3	60	
		3.5-4	9	6	66.6	4	3	7	77.7	

Where, a= GnRH-PGF2 α protocol, b= PGF2 α protocol, c= GnRH-PGF2 α protocol (heifers), d=CIDR+GnRH-PGF2 α (heifers), ER= estrus response, TAI = Timed AI

The study determined the effect of parity of animals on induction of cyclicity and conception in postpartum crossbred cows and results were showed in Table 8. It showed that the highest proportion of cows with parity range 1-3 showed estrus (100%) and conception rate (75.0%) after receiving treatment with PGF2 α . Induction of estrus (66.6%) and conception rate (25%) were lower in cows with parity range 1-3 and 4-5 GnRH treatment group respectively. The effect of parity between the two cows groups were statistically significant ($P < 0.05$).

Table 8. Effect of parity of cows on estrus and conception rate

Treatment protocols	Parity	N ₀ . treated	N ₀ . showed estrus	% in estrus	N ₀ .conceived			% concei ved	<i>P</i> -value
					ER	TAI	total		
a	1-3	15	10	66.6	3	2	5	33.3	5.9e ⁻⁰⁵
	4-5	4	3	75.0	1	-	1	25.0	
b	1-3	12	12	100	9	-	9	75.0	
	4-5	3	2	66.6	1	-	1	33.3	

Where, a= GnRH-PGF2 α or GnRH protocol, b= PGF2 α protocol in cows group

4.5. Conception rate to AI on estrus and timed insemination

Comparing the conception rate of 41 cows and heifers treated with the hormonal injections and inseminated after estrus detection and 19 cows and heifer fixed time inseminated after injection of the hormones, 61.0% (n=25) and 42.1% (n=8) were conceived respectively. AI at detected estrus was found higher proportion (61.0%) of conception rate than fixed time AI (40.0%) after PGF2 α injection. Even though there was no statistical significance ($P>0.05$) between the detected estrus and fixed time AI, but the conception rate was higher in detected estrus AI.

Table 9. Fixed time AI versus AI at detected estrus.

AI	N $^{\circ}$. of cattle inseminated	N $^{\circ}$. of cattle conceived	% cattle conceived	<i>P</i> -value
On detected estrus	41	25	61.0	0.522
TAI	19	8	42.1	

5. DISCUSSION

In this study the average or mean interval from PGF2 α to estrus was shorter (67.20 ± 3.39) in treatment protocol that utilized single PGF2 α only and longer (76.86 ± 5.86) for treatment protocol that utilized GnRH-PGF2 α combination. This interval to estrus was longer than (Meles, 2007) who reported interval to estrus of 34.3 ± 1.3 hrs local cattle and 32 ± 1.2 hrs for crossbred cattle. The average duration of estrus was among the three synchronization protocols (22.4 ± 2.1 hr) in this study was in agreement with that of Chaudhari *et al.* (2012) reported average duration of 25.41 ± 0.94 , 21.95 ± 0.20 and 22.68 ± 1.46 using CIDR, with or without PMSG, and finally PGF2 α . Intervals to estrus following treatment with PGF2 α are dependent on the stage of follicle development at the time of treatment; cows with mature follicles at the time of luteolysis enter estrus sooner to estrus than cows with immature follicles.

Similarly the stage of the estrous cycle (Martinez *et al.*, 1999; Vasconcelos *et al.*, 1999; Moreira *et al.*, 2000) and cyclic status (Bisinotto *et al.*, 2010) at the time that GnRH is administered has been shown to affect estrus intervals and ovulation time (Wiltbank *et al.*, 2011). In this study cattle with parity zero (heifers) to parity three showed shorter average interval to estrus (62.80 ± 3.93) than cattle with greater than 4th parity (68.40 ± 3.60). This may be due to amount of circulating hormones in older cows (Roberts, 1986). Further, younger (heifer) or (parity 0) cattle had shorter length of estrus duration (20.21 ± 2.31) than the older cattle (24.20 ± 3.14).

Cows and heifers treated by single PGF2 α only showed higher estrus response (93.3%) compared to cattle that received GnRH (68.4%) and CIDR (50%) based treatment protocols. Fifty percent of heifers that received CIDR-GnRH- PGF2 α manifested estrus. Details on over estrus response to treatment protocols and conception rates were indicated in table 6. As indicated several studies the estrus response in cows/heifers to GnRH treatment at initiation of an Ovsynch program ranged from 54.2 to 58.3% (pursley *et al.*, 1995; Moriera *et al.*, 2000). A

synchronous emergence of a new follicular wave is most likely to occur if the dominant follicle ovulates following administration of GnRH (Moriera *et al.*, 2000).

The various behavioral signs recorded in the current study were similar in type and sequence to those reported by Bekana *et al.* (2005) and (Meles, 2007). The frequent signs observed in the present study were restlessness, sniffing, standing heat, mounting other cows/heifers, mucus vaginal discharge and redness of the vaginal mucosa. Various factors are known to affect estrus manifestation in dairy animals (Mukasa-Mugerwa *et al.*, 1989; Lamothe Zavalete *et al.*, 1991; Valle *et al.*, 1994; Bekana *et al.*, 2005).

Although 'standing to be mounted by others' behavior is considered as reliable behavioral expression during estrus, it can occur at as low as 50-53% cases in Holstein cows (Lyimo *et al.*, 2000) which was in agreement the 54% in present study. In other study (Layek *et al.*, 2011) observed standing to be mounted by other cows followed by mounting to other cows as 100%. In some other studies sniffing/licking of vulva, chin resting and restlessness in majority of estrus reported from 94.1% to 100% estrus in crossbred cows (Sood and Nanda, 2006). It is reported that the endocrine profile during the peri-estrual period of an individual animal has also been reported to affect the estrus behavior expression (Roelofs *et al.*, 2005). It has been reported that mounting on herd mates' behavior could be used as a good predictor of ovulation in Holstein Friesian cows (Roelofs *et al.*, 2005).

In the present study BCS did not show statistically significance ($P>0.05$) effect on both average interval to estrus and duration of estrus. According to Centurin-Castro *et al.* (2013), body condition score in synchronized Cows did not affect estrus characteristics. The current result is also in line with previous report of (Lamb *et al.*, 2001) who concluded that body condition score of cows have an effect on the estrus response and under or over conditioning of cows may lead to both an ovulatory state which contributes to reduced rates of success in synchronization. Hillegass *et al.* (2008) has also reported that increasing BCS during the synchronization protocol enhanced the proportion of high-producing dairy cows to display estrus after the estrus synchronization protocol which goes in line with the current finding.

Overall, CIDR insertion markedly improve conception rate (71.4%) than non-CIDR protocol (53.8%) in heifers although the difference was not statistically significant ($P>0.05$). However, CIDR insertion and insemination on estrus detection markedly influence conception rate (50%) than CIDR insertion with timed insemination (21%), details in table 6. This finding was in agreement with of Lucy *et al.*, (2001) who reported, the synchronization rate measured in a 3-day period of detecting estrus confer higher ($P<0.05$) conception rate in dairy heifers that received a combination of CIDR insert and PGF2 α (84%) compared with heifers receiving only PGF2 α (57%). CIDR-GnRH-PG and insemination at detected estrus was effective in heifers. This may warrant thinking on the effectiveness of visual observation of estrus with CIDR-GnRH-PG than TAI.

Similarly, many authors including Bo *et al.* (2002); Baruselli *et al.* (2004); El-Zarkouny *et al.* (2004) ; Stevenson *et al.* (2006) ; Meneghetti *et al.* (2009); Santos *et al.* (2009); Vasconcelos *et al.* (2009); Teixeira (2010) reported improved conception rates, on one farm, when a CIDR inserted for short treatment period, result in fertility that lower after TAI than AI upon estrus detection but with the difference close to each other. What was so ever on AI type (on detect estrus or TAI), the CIDR-GnRH-PG with CIDR removed after 7 days improve conception rates in crossbred dairy heifers than GnRH-PG without CIDR.

In postpartum cows the overall conception rate following synchronization by single PGF2 α and insemination on detected estrus was higher (66.6%) than synchronization by GnRH-PGF2 α and insemination on detected estrus (31.5%). However, the difference was not statistically significant ($P>0.05$). When conception rate was compared by the type of insemination (on estrus versus TAI) and the treatment protocol (single PGF2 α versus GnRH-PGF2 α), conception rate was 71.4 % (10 /14 cows in estrus) to insemination on detected estrus for single PGF2 α . For GnRH-PGF2 α protocol conception rate was 30.7% (4/13) to insemination on detected estrus but was 33.3% (2/6) for TAI ($P>0.005$).

The conception rate to insemination on detected estrus was better than TAI in all treatment protocol except GnRH-PGF2 α protocol with TAI. Several studies have established that cows that exhibit estrus before AI achieve higher conception rates than those that fail to exhibit

estrus (Perry and Smith, 2015). This might be due to variation in follicular size at start of treatment or circulating concentrations of hormones especially of E2 as many studies indicates as diameter of the ovulatory follicle increases (Vasconcelos *et al.*, 2001; Atkins *et al.*, 2010) during the interval from PGF2a administration to serum concentrations of E2 increased as well. Perry *et al.* (2005) reported that when GnRH was used to induce ovulation of follicles less than or equal to 11 mm in diameter, lower circulating concentrations of E2 on the day of insemination were observed, which then correlated to decreased conception rates compared with cows with larger size follicles.

The higher conception rate was observed in cows conceived (66.6%) after receiving treatment with PGF2 α -based treatment and heifers conceived (71.4%) in CIDR based-treatment than cows conceived (37.5%) and heifers conceived (53.8%) in GnRH-based treatment in the cows and heifers group respectively. The difference in estrus response as well as in conception rate within cows/heifers group in the dairy cattle received hormonal treatment protocols was not statistically significant ($P>0.05$).

Regarding to parity, conception rate was higher in primiparous than multiparous dairy cows. The difference was statistically significant ($P<0.05$). Similarly, Tenhagen *et al.* (2003); Quintel *et al.* (2004); Paul *et al.* (2011); Grimand *et al.* (2013); Potdar *et al.* (2016) all reported that conception rate was higher in primiparous cows than in multiparous cows. However, Diskin (2002); wathes *et al.* (2001); William *et al.* (2002) reported that conception rate was higher in multiparous dairy cows. Higher conception in primiparous might be due to greater chance of multiparous cows for metabolic disorder or disease than primiparous cows. Additional reasons for better fertility in primiparous cows may include a reduced risk of metabolic disorders in early lactation and reduced reproductive problems (Gröhn and Rajala-Schultz, 2001).

6. CONCLUSION AND RECOMMENDATIONS

In general the conception rate was higher on insemination at standing estrus in all protocols in this study. Ultrasonographic scanning of the presence of corpus luteum with single PGF₂ α and insemination at standing estrus gives better conception rates in postpartum dairy cows. Similarly, a combination of CIDR-GnRH-PGF₂ α and insemination at standing estrus improves conception in dairy heifers than GnRH-PGF₂ α combination without CIDR. Based on this study the following recommendations were set as follows;

- ✚ The PGF₂ α -based synchronization protocol should be implemented with great care on appropriate animal selection to get high estrus response rate and conception rate in postpartum dairy cows.
- ✚ CIDR-GnRH-PGF₂ α with insemination at standing estrus can be used in crossbred dairy heifers to have higher conception rate.
- ✚ Because the conception rate was influenced by BCS and parity, for synchronization animals, optimal BCS and parity are primarily considered.

7. REFERENCES

- Ahmadzadeh A., Carnahan K., and Autran C. (2011). Understanding puberty and postpartum anestrus. Proceedings, Applied Reproductive Strategies in Beef Cattle.
- Ahmed Sh. Mohamed B. P. Hegede and Bekele T., (2003). Traditional processing of camel meat and milk, and marketing of camels, milk and hides in After Zone of Somali National Regional State, Ethiopia. In: Proceeding 10th Annual conference of the Ethiopian Society of Animal production (ESAP) held in Addis Ababa, Ethiopia, August 22-24, 2002. ESAP, Addis Ababa. Pp 201-209.
- Ahmed N. and Derar J.M. (2015). Pattern of induced estrus and conception rate following Ovsynch and Ovsynch based gonadotrophin-releasing hormone treatments initiated on day 6 of estrous cycle in repeat breeding crossbred cows. *Vet. World.* **9**(4): 342-345.
- Alemayehu K. and Getu A. (2015). The Status of Estrus Synchronization and Conception Rate in Ethiopian Cattle.
- Atkins J.A., Smith M.F., Wells K.J., Geary T.W. (2010). Factors affecting preovulatory follicle diameter and ovulation rate after gonadotropin-releasing hormone in postpartum beef cows. Part I: cycling cows. *Journal of Animal Science*; **88**: 2300-10.
- Azage T. (2003). Urban livestock production and gender in Addis Ababa, *Urban Agriculture Magazine*; **12**:30-31.
- Azage T. (2004). Financing market-oriented dairy development: The case of Ada'a-Liben district Dairy Association, Ethiopia. *Urban Agricultural Magazine* No. 9. Koninklijke, Netherlands. Pp. 45.
- Bao B., Kumar N., Karp R.M., Garverick H.A. and Sundaram K. (2000). Estrogen receptor- β expression in relation to the expression of luteinizing hormone receptor and cytochrome P450 enzymes in rat ovarian follicles. *Biology of reproduction*; **63**:1747-1755.
- Bartlewski A. (2015). LH peak and ovulation after two different estrus synchronization treatments in buffalo cows in the daylight-lengthening period. *Theriogenology*; **84**(2): 286-293.

- Baruselli P.S., Berber R.A., Madureira E.H., Carvalho N.T. (2003). Half dose of Prostaglandin F2 is effective to induce luteolysis in the synchronization of ovulation protocol for fixed time artificial insemination in buffalo (*Bubalus bubalis*). *Brazilian Journal of Veterinary Research and Animal Science*; **40**: 397-402.
- Beal W.E. (1998). Current estrus synchronization and artificial insemination programs for cattle. *Journal of Animal Science*; **76** (Suppl. 3): 30-38.
- Beef Reproduction Task Force (BRTF). (2006). Applied Rep. Strategies in Beef cattle. Conference Proceedings. Breed. *International Journal of Agriculture and Veterinary Sciences*; **1**: 19-26.
- Bekana, M., Gizachew, A. and Regassa, F. (2005). Reproduction performances of Fogera heifers treated with prostaglandin F2 α for synchronizations of estrus. *Trop Animal Health and production*; **37**: 373-379.
- Bello N.M., Steibel J.P., Pursley J.R. (2006). Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of Ovsynch in lactating dairy cows. *J Dairy Science*; **89**: 3413-24.
- Bisinotto H., Guilbault L.A., Dufour J.J. (2010). Synchronization of ovarian follicular waves with a gonadotropin-releasing hormone agonist to increase the precision of estrus in cattle: a review. *Journal of Animal Science*; **73**:3141-51.
- Bitew M. and Prasad S. (2011). Study on major reproductive health problems in indigenous and crossbred cows in and around bedelle, south west Ethiopia. *Animal and veterinary Advances*; **10**(6):723-727.
- Bo G.A., Baruselli P.S. and Martinez M. F. (2003). Pattern and manipulation of follicular development in *B. indicus* cattle. *Animal Reproduction Science*; **78**: 307-326.
- Britt J.H. (1987). Induction and synchronization of ovulation. In: ESE Hafez (eds.), *Reproduction in Farm Animals*. 5th Ed., Lea and Febiger, Philadelphia, PA; Pp 507-516.
- Byerley D.J., Staigmiller, R.B., Beradinelli J.G., and Short R.E. (1987). Pregnancy rates of beef heifers bred on puberal or third estrus. *Journal of Animal. Science*; **65**:645-650.

- Center for Disease Control (CDC) (2005). Food borne illness, January 10: 1-13.
- Central Statistical Agency (CSA), (2005): Agricultural Sample Survey 2004/05. Central Statistical Authority No.2. Report on Livestock and livestock characteristics. Statistical Bulletin, Addis Ababa, Ethiopia, Pp. 331.
- Centurin-Castro F., Orihuela Porcayo J., AkLpez R. J., Magaa Monforte J. G., Montes Prez R. C., Segura Correa J. C. (2013). Effect of body condition score on estrus and ovarian function characteristics of synchronized beef-master cows. *Tropical and Subtropical Agro ecosystems* **16**: 2.
- Chaudhari J., Hepworth G., Parker K.I., Wright P.J., Macmillan K.L. (2012). Effect of treatment with progesterone and oestradiol when starting treatment with an intravaginal progesterone releasing insert on ovarian follicular development and hormonal concentrations in Holstein cows. *Animal Reproductive Science*; **76**:177-93.
- CSA (Central Statistical Agency) (2005). Federal Democratic Republic of Ethiopia Agricultural sample survey. Livestock and livestock characteristics bulletin, Volume II. Addis Ababa, Ethiopia.
- CSA (2010). Agricultural sample survey. Report on crop and livestock product utilization. The Federal Democratic republic of Ethiopia, Central Statistical Agency (CSA). Private Peasant Holdings. Statistical Bulletin 468, Addis Ababa, Ethiopia, 2010.
- De Jarnette J.M., Day M.L., House R.B., Wallace R.A. and Marshall C.E. (2001). Effect of GnRH pretreatment on reproductive performance of postpartum suckled beef cows following synchronisation of estrus using GnRH and PGF₂ α . *Journal of Animal science*; **79**: 1675-1682.
- Desalegn G., Merga B., Azage T. and Kelay B. (2009). Status of artificial insemination service in Ethiopia. In: The 17th Annual Conference of the Ethiopian Society of Animal Production (ESAP), Sept 24-26, 2009 Addis Ababa, Ethiopia, 87-104.
- Diskin M.G., Austin E.G., Roche J.F. (2002). Exogenous hormonal manipulation of ovarian activity in cattle. *Domest Animal Endocrinology*; **23**: 211-228.
- Dudhatra G. B., Mody S. K., Patel H. B., Modi C. M., Chukewar A. B., Kumar A. and Awale M. M. (2012). Prostaglandins and its Analogues: An approach for Treatment of Anoestrus and to enhance Breeding Efficiency. *Vet. World*; **5**(6): 378-384.

- Edward E. C. (1988). Postpartum interval to estrus and patterns of luteinizing hormone (LH) concentrations in first-calf suckled beef cows exposed to mature bulls. A thesis submitted for Master in Animal Science.
- El-Zarkouny S.Z. and Stevenson J.S. (2004). Resynchronizing estrus with progesterone or progesterone plus estrogen in cows of unknown pregnancy status. *Journal of Dairy Science*; **87**: 3306-3321.
- El-Zarkouny S.Z., Cartmill J.A., Hensley B.A., Stevenson J.S. (2004). Pregnancy in dairy cows after synchronized ovulation regimens with or without presynchronisation and progesterone. *Journal of Dairy Science*; **87**: 1024-1037.
- Felleke G. and Geda G. (2001). The Ethiopian dairy development policy: a draft policy document. Ministry of agriculture (MoA), Addis Ababa, Ethiopia.
- Fricke P.M. (2001). Ovsynch, pre-synch, the kitchen-synch: What's up with Synchronization Protocols? University of Wisconsin – Extension Brochure.
- Geary T.W., Downing E.R., Bruemmer J.E., and Whittier J.C. (2000). Ovarian and estrous response of suckled beef cows to the Select Sych estrous synchronization protocol. *Prof. Animal Science*; **16**: 1-5.
- Geary T.W., Downing E.R., Bruemmer J.E., and Whittier J.C. (2001). Ovarian and estrous response of suckled beef cows to the Select Sych estrous synchronization protocol. *Prof. Animal Science*; **16**: 1-5.
- GebreWold A., Alemayehu M., Demeke S., Bediye S and Tadesse A. (2000). Status of dairy development. Smallholder Dairy Development Project (SDDP) dairy research in Ethiopia. In: The role of village dairy cooperatives in dairy development. SDDP smallholder Dairy Development Project) Proceedings, MOA (Ministry of Agriculture), Addis Ababa, Ethiopia.
- Gizaw S., Tesfaye Y., Mekuriaw Z., Tadesse M., Hoekstra D., Gebremedhin B. and Tegegne A. (2016). Oestrus synchronization for accelerated delivery of improved dairy genetics in Ethiopia: Results from action research and development interventions. LIVES Working Paper 12. Nairobi, Kenya: International Livestock Research Institute (ILRI).S. and Orihuela A., (2007): The detection of estrus in cattle raised under tropical conditions: What we know and what we need to know. *Hormones and Behavior*; **52**: 32.

- Grimand B., Marquant-Leguienne B., Remy D., Richard C., Nuttinck F., Humblot P., Ponte and quality in dairy cows (2013). Relationships with parity and subsequent Fertility. *Reproduction in Domestic animals*; **48**:183-194.
- Gröhn Y.T., and Rajala-Schultz P.J. (2001). Epidemiology of Reproductive performance in dairy cows. *Animal Reproductive Science*; **6**: 605-614.
- Haileselassie M., Kalayou S., Kyule M., Asfaha M. and Belihu K. (2011). Effect of brucella infection on reproduction conditions of female breeding cattle and its public health significant in western Tigrai, Northern Ethiopia. *Veterinary Medicine International*; **2**: 1-7.
- Hansel W., and Convey E.M. (1983). Physiology of the estrous cycle. *Journal of Animal Science*; **57**(Suppl.2):404-424.
- Hillegass J., Lima F. S., S Filho M. F. and Santos J. E. (2008). Effect of time of artificial insemination and supplemental estradiol on reproduction of lactating dairy cows. *Journal of Dairy Science*; **91**(11): 4226-4237.
- Hopkins M.F. & Schrick N.F. (2012). Estrous Synchronization. Extension Dep. of An. Sci. Uni. of Tennessee. <https://beefandlamb.ahdb.org.uk>.
- Hussain A.M. and Daniel RCW. (1991). Bovine normal and abnormal reproductive and endocrine functions during the postpartum period: A review. *Reproduction of Domestic Animal*; **26**: 101-11.
- Ill-Hwa K., Gook-Hyun S., Dong-Soo S. (2003). A progesterone-based times AI protocol more effectively prevents premature estrus and incomplete luteal regression than Ovsynch protocol in lactating Holstein cows. *Theriogenology*; **60**: 809-817.
- IPMS (Improving Productivity and Market success of Ethiopian Farmers) (2011). 14th progress report. Addis Ababa, Ethiopia. Pp. 11-12.
- Jancy J., Anand L. Singh O. (2009). A Comparative Study on Evaluation of Three Synchronization Protocols at Field Level in Bovines. *Indian journal of dairy science*; **62**(3): 218-221.

- Jillian L. F. (2005). Evaluating the effectiveness of using the controlled internal drug Release (CIDR) insert for synchronization of estrus and postinsemination Progesterone therapy to improve reproductive Performance of dairy cattle. A Thesis Submitted to the Graduate Faculty of The University of Georgia.
- Juyena N. S., Islam M. R., Bhuiyan M. M., Rahman M. M. and Ferdousy R. N. (2013). Treatment outcomes in postpartum anoestrus cows guided by transrectal ultrasonography. *Progressive Agriculture*; **24**(1 and 2): 93-100.
- Kaneko H. T., Terada K., Taya G., Watanabe S., Sasamoto Y., Hasegawa.L and Igarashi M. (1991). Ovarian follicular dynamics and concentrations of oestradiol-17 beta, progesterone, luteinizing hormone and follicle stimulating hormone during the preovulatory phase of the estrous cycle in the cow. *Reproductive Fertility Development*; **3**: 529-535.
- Kastelic J. P. and Ginther O. J. (1991). Factors influencing the origin of ovulatory follicle in heifers with induced luteolysis. *Animal Reproduction Science*; **26**: 13-24.
- Khair A. (2005). Hormonal interventions to increase fertility in anestrous and repeat breeder cows. M.S. Thesis. Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh.
- Lamb G. C., Stevenson J. S., Kesler D. J., Garverick H. A., Brown D. R. and Salfen B. E. (2001). Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2 α for ovulation control in postpartum suckled beef cows. *Journal of Animal science*; **79**(9): 2253-2259.
- Lamming G. I., Wathes D. C. and Peters A. R. (1981). Endocrine patterns of the postpartum cow. *Journal of Reproductive Fertility*; **30** (Suppl.I):155
- Lamothe-Zavaleta C., Fredriksson G. and Kindahl H. (1991). Reproductive performance of Zebu cattle in Mexico I. Sexual behavior and seasonal influence on estrous cyclisty, *Theriogenology*; **36**: 887-896.
- Land O'Lakes (2010). The Next Stage in Dairy Development for Ethiopia. *Dairy Value Chains, End Markets and Food Security*, Addis Ababa, Ethiopia. Pp. 13-30.
- Lane E.A., Austin E.J., Roche J.F., Crowe M.A. (2001). The effect of estradiol benzoate or a synthetic gonadotrophin-releasing hormone used at the start of a progesterone treatment on estrous response in cattle. *Theriogenology*; **56**: 79-90.

- Layek S.S. Mohanty T.K., Kumaresan A., Behera K. and Chand S. (2011). Behavioural signs of estrus and their relationship to time of ovulation in Zebu (Sahiwal) cattle. *Animal Reproductive Science*; **129**(3): 140-145.
- Lucy M. C., Stevenson J. S. and Call E. P. (1986). Controlling first service and calving interval by PGF2 alpha, gonadotropin-releasing hormone, and timed insemination. *Journal of Dairy Science*; **69**: 2186-2194.
- Lucy W. M. (1992). Physiology of the bovine estrous cycle. *Journal of Animal science*; **57**: 404.
- Lucy M. C., Billing H. J., Butler W. R., Ehnis L. R., Fields M. J., Kesler D. J., Kinder J. E., Mattos R.C., Short R. E., Thatcher W. W., Wettemann R. P., Yelich J. V. and Hafs H. D.(2001). Efficacy of an intravaginal progesterone insert and an injection of PGF2 for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, peripubertal beef heifers, and dairy heifers. *Journal of Animal. Science*; **79**: 982-995.
- Lucy M. C., McDougall S., Nation D. P. (2004). The use of hormonal treatments to improve the reproductive performance of lactating dairy cows in feedlot or pasture-based management systems. *Animal reproduction science*; **82**: 495-512.
- Lyimo Z.C., Nielen M., Ouweltjes W., Kruip T.A., and van Eerdenburg F.J. (2000). Relationship among estradiol, cortisol and intensity of estrous behavior in dairy cattle. *Theriogenology*, **53**: 1783-1795.
- Macmillan K.L., Day A.M. (1982). Prostaglandin F2 α is fertility drug in dairy cattle? *Theriogenology*; **18**: 245-253.
- Macmillan K.L., Henderson H.V. (1984). Analyses of the variation in the interval from an injection of prostaglandin F2 to oestrus as a method of studying patterns of follicle development during diestrus in dairy cows. *Anim. Reprod. Science*; **6**: 245-254.
- Madhuri G., Rajashr M. and Surabhi K. (2017). Post-partum anoestrus in dairy cows: a review. *International Journal of Science, Environment and Technology*; **6**(2):1447-1452.
- McDougall S., Compton C.W.R., Hanlon D.W., Davidson P.J., Sullivan D.J., Gore A.H. (2010). Reproductive performance in anestrous dairy cows following treatment with two protocols and two doses of progesterone. *Theriogenology*; **63**:1529-48.

- Mekonnen T., Bekana M. and Abayneh T. (2010). Reproductive performance and efficiency of artificial insemination smallholder dairy cows/heifers in and around Arsi-Negelle, Ethiopia. *Livestock research for rural development*; **22**(3):1-9.
- Meneghetti M., Sá Filho O., Peres R., Lamb G., Vasconcelos J. L. M. (2009). Fixed time artificial insemination with estradiol and progesterone for *Bos indicus* cows I: basis for development of protocols. *Theriogenology*; **72**(2): 179-189.
- Metekia T. and Nezif A. (2017). Review on the status of dairy cattle production in Ethiopia. *Journal of Gene c and Environmental Resources Conserve*; **5** (2):84-95.
- Mihm M., Delatang F., Roche J.F. (1998). The gonadotrophin and ovarian response to an intermediate or low dose of gonadoreline in beef heifers. *J. Reprod. Fertil. Abstr. Ser.* **21**: 32.
- Moriera F. and Hansen P. J. (2003). Pregnancy Diagnosis in the cow. Dept. of animal Sciences, university of Florida.
- Mukasa -Mugerwa E. (1989). A review of the Reproductive Performance of Female *Bos indicus* (Zebu) Cattle. Monograph No.6 ILCA, Addis Ababa, Ethiopia. Pp. 59-73.
- Mureda E. and Mekuriaw Z. (2007). Reproductive performance of crossbred dairy cows in Eastern lowlands of Ethiopia. *Livestock research for rural development*; **19**(161):3.
- Murugavel K., Yaíniz J. L., Santolaria P., Lpez-Bjar M. and Lpez-Gatius F. (2003). Prostaglandin based estrus synchronization in postpartum dairy cows: an update. *Journal of applied research in veterinary medicine*; **1**: 51-65.
- Murugavel K. (2004). Reproductive Performance of Dairy Cows Following Different Estrous Synchronization Protocols.
- Murugavel K., Yaniz J. L., Santolaria P., Lopez-Bejar M. and López-Gatius F. (2010). Prostaglandin Based Estrus Synchronization in Postpartum Dairy Cows: An Update. Schering Plough Animal Health, Spain. Pp. 1-24.
- Nebel R. L., Dransfield M. G., Jobst S. M., Bame J. H. (2000). Automated electronic Systems for the Detection of Oestrus and Timing of AI in Cattle. In: Animal Reproduction. II research and Practice. Proceedings of the 14th International Congress. Forsberg, N. **60**(61):713-723.

- Nigussie G. (2006). Characterization and evaluation of urban dairy production system in Mekelle city, Tigray region, Ethiopia, (unpublished MSc thesis, Hawassa University, Ethiopia).
- Niraj K., Kbrom T., Desalew T. and Awot T. (2014). Productive Performance of Crossbred Dairy Cows and constraints faced by dairy farmers in Mekelle, Ethiopia. *IOSR Journal of Agriculture and Veterinary Science (IOSRJAVS)* e-ISSN: 2319-2380, p-ISSN: 2319- 2372. Volume 7, Issue 1 Ver. V (Feb. 2014), PP 62-66 www.iosrjournals.org.
- NMSA (2003). National meteorology service agency report. Addis Ababa, Ethiopia.
- Noseir W. M. (2003). Ovarian follicular activity and hormonal profile during the estrous cycle in cows: the development of 2 versus 3 waves. *Reproductive Biology and Endocrinology*; **1**:50.
- Pacala N., Bencsik I., Dronca D., Acatincai S., Petroman I., Cornelia P., Ada C., Caraba, I., Alexandra B. and Papp S. (2009). Researches regarding the effect of PGF 2α administration interval on cow's estrus synchronization. *Banat's University of Agricultural Science and Veterinary Medicine, Timisoara*; **54**:258-260.
- Patterson D.J., Wood S.L., and Randle R.F. (2000). Procedures that support reproductive management of replacement beef heifers. *Proc. Animal Science*; Available at: <http://www.asas.org/jas/symposia/proceedings/0902.pdf>. Accessed July 13, 2017.
- Paul A.k., Alam M.G., Shamsuddin M. (2011). Factors that limit first service pregnancy rate in cows at char management of Bangladesh. *Livestock Research for Rural Develop.*23, Article#57. Retrieved, March, 24, 2018, from, <http://www.Irrd.org/Irrd23/3/paul23057.htm>.
- Peeler S., Compton C.W.R., Hanlon D.W., Davidson P.J., Sullivan D.J., Gore A.H. (2004). Reproductive performance in anestrous dairy cows following treatment with two protocols and two doses of progesterone. *Theriogenology*; **63**:1529-1548.
- Perry G. A., Smith M. F. Geary T. W. (2004). Ability of intravaginal progesterone inserts and melengestrol acetate to induce estrous cycles in postpartum beef cows. *Journal of animal science*; **82**: 695-704.
- Perry G.A., Smith M.F., Lucy M.C., Green J.A., Parks T.E., MacNeil M.D., Roberts J.A., Geary T.W.(2005). Relationship between follicular size at insemination and pregnancy success. *Proc Natl Acad Science USA*; **102**:5268-73.

- Perry G.A., Smith M.F. (2015). Management factors that impact the efficiency of applied reproductive strategies. Proceedings, Applied Reproductive Strategies in Beef Cattle, Davis, CA, Pp. 208-32.
- Peter A. T., Vos P. L. and Ambrose D.J. (2009). Postpartum anoestrus in dairy cattle. *Theriogenology*; **71**: 1333-1342.
- Potdar V.V., Bhawe K., Gaundare Y. (2016). Factors Influencing conception rate in Local and crossbred cows. *IOSR Journal of Agriculture and Veterinary Science*; **9**(10): 51-54.
- Pursley J. R., Mee M. O. and Wiltbank M. C. (1995). Synchronisation of ovulation in dairy cows using PGF2a and GnRH. *Theriogenology*; **44**: 915.
- Pursley J. R., Wiltbank M. C., Stevenson J. S., Ottobre J. S., Garverick H. A. and Anderson L. L. (1997). Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. *Journal of dairy Science*; **80**:295-300.
- Pursley J.R., Silcox R.W., and Wiltbank M.C. (1998). Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. *Journal of Dairy Science*; **81**: 2139-2144.
- Quintela L.A., Pena A.I., Taboada M.J., Alonso G., Varela portas B., Diaz C., Barrio M., Garcia M.E., Becerra J.J. and Heradon P.G. (2004). Risk Factors for Low Pregnancy Rate in dairy Cattle. Retrospective Study in the North West of Spain. *Archivos de Zootecnia*, **53**: 69-76
- Rivera H., Lopez H., Fricke P.M. (2005). Use of intravaginal progesterone-releasing inserts in a synchronization protocol before timed AI and first synchronizing return to estrus in Holstein heifers. *Journal of Dairy Science*; **88**: 957-968.
- Roberts S. L. (1986). Veterinary obstetrics and genital disease 2nd Ed. Lithographed by Edwards Brothers Inc. Ann. Arbor. Michigan. Pp. 500-506.
- Roelofs J.B., van Eerdenburg F.J., Soede N.M., Kemp B. (2005). Various behavioral signs of estrus and their relationship with time of ovulation in dairy cattle. *Theriogenology*; **63**:1366-1377.

- Ryan D.P., Snijders S., Yaakub H., O' Farrell K.J. (1995). An evaluation of estrus synchronization programs in reproductive management of dairy herds. *Journal of Animal Science*; **73**: 3687-3695.
- Santos J.E.P., Bartolome J.A., Cerri R.L.A., Juchem S.O., Hernandez O., Trigg T. (2009). Effect of a deslorelin implant in a timed artificial insemination protocol on follicle development, luteal function and reproductive performance of lactating dairy cows. *Theriogenology*; **61**:421-435.
- Sartori R., Haughian G. J. M., Rosa R. D., Shaver J. and Wiltbank M. C. (2000). Differences between lactating cows and nulliparous heifers in follicular dynamics, luteal growth, and serum steroid concentrations. *Journal of dairy science*; **83** (Suppl. 1): 212.
- Savio J.D., Boland M.P., Hynes N., Mattiacci M.R., Roche, J.F. (1990). Will the first dominant follicle of the estrous cycle of heifers ovulate following luteolysis on day 7? *Theriogenology*; **33**: 677-685.
- Schmitt E.J., Drost M., Diaz T., Roomes C., Thatcher W.W. (1996). Effect of a gonadotrophin releasing hormone agonist on follicle recruitment and pregnancy rate in cattle. *Journal of Animal Science*; **74**: 154-161.
- Senger P.L. (2003). Pathways to pregnancy and parturition. 2nd ed. Cadmus Professional Communications-Science Press, Ephrata, PA.
- Shiferaw Y., Tenhagen B. A., Bekana M. and Kassa T. (2003). Reproductive performance of crossbred cows in different production systems in the central highlands of Ethiopia. *Tropical Animal Health and production*; **35**:551-561.
- Smith M. F., Perry G. A., Atkins J. A., Jinks E. M., Pohler K. G. Patterson D. J. (2006). Physiological principles underlying synchronization of estrus.
- SNV (2008). (The Netherlands Development Organization), 2008. Study on Dairy Investment opportunities in Ethiopia, July 2008, Addis Ababa. Pp. 59.
- Sood P. and Nanda A.S. (2006). Effect of lameness on estrous behavior in crossbred cows. *Theriogenology*; **66**(5): 1375-1380.
- Stagg K., Diskin M.G., Sreenan J.M., Roche J.F. (1995). Follicular development in long-term anoestrous suckler beef cows fed two levels of energy postpartum. *Animal Reproductive Science*; **38**: 49-61.

- Stevenson J.S., Kobayashi Y., Thompson K.E. (1999). Reproductive performance of dairy cows in various programmed breeding systems including Ovsynch and combinations of gonadotropin releasing hormone and prostaglandin F2. *Journal of Dairy Science*; **82**: 506-515.
- Stevenson J. S., Thompson K. E., Forbes W. L., Lamb G. C., Grieger D. M. and Corah L. R. (2000). Synchronising estrus and (or) ovulation in beef cows after combinations of GnRH, norgestomet and prostaglandin F with or without timed insemination. *Journal of Animal Science*; **78**: 1747-1758.
- Stevenson J.S., Pursley J.R., Garverick H.A., Fricke P.M., Kesler D.J., Ottobre J.S., Wiltbank, M.C. (2006). Treatment of cycling and non-cycling lactating dairy cows with progesterone during Ovsynch. *Journal of Dairy Science*; **89**: 2567-2578.
- Stewart S., Rapnicki P. and Fricke P. (2004). Dairy Reproductive Synchronization Notes. Proceedings of Minnesota Dairy Herd Health Conference, St. Paul, MN.
- Tegegne A., Estifanos A., Tera, A. and Hoekstra D. (2012). Technological options and approaches to improve smallholder access to desirable animal genetic material for dairy development: IPMS experience with hormonal oestrus synchronization and mass insemination in Ethiopia. *Tropentag*. Pp. 1-4.
- Tegegne A., Gebremedhin B., Hoekstra D., Belay B. and Mekasha Y. (2013). Smallholder dairy production and marketing systems in Ethiopia: IPMS experiences and opportunities for market-oriented development. IPMS (Improving Productivity and Market Success) of Ethiopian Farmers Project Working Paper 31. Nairobi: ILRI. Reference.
- Teixeira S.Z., Cartmill J.A., Hensley B.A., Stevenson J.S. (2010). Pregnancy in dairy cows after synchronized ovulation regimens with or without presynchronization and progesterone. *Journal of Dairy Science*; **87**:1024-1037.
- Tenhagen B.A., Drillich M., Heuweiser W. (2000). Synchronization of lactating dairy cows with prostaglandin F2: insemination on observed oestrus versus timed artificial insemination. *Journal of Veterinary Medicine*; **47**: 577-584.
- Tenhagen B.A., Drillich M., Heuwieser W. (2003). Comparison of timed AI after synchronized ovulation to AI at estrus: reproductive and economic considerations. *Journal of Dairy Science*; **87**: 85-94.

- Tenhagen B.A., Drillich M., Heuwieser W. (2004). Comparison of timed AI after synchronized ovulation to AI at estrus: reproductive and economic considerations. *Journal of Dairy Science*; **87**: 85-94.
- Tenhagen B.A., Kuchenbuch S., Heuwieser W. (2005). Timing of ovulation and fertility of heifer after synchronization of estrus with GnRH and PGF2 α . *Reproduction of Domestic Animal*; **40**: 62-67.
- Tessema Z. K., Mihret J. and Solomon M. (2010). Effect of defoliation frequency and cutting height on growth, dry matter yield and nutritive value of Napier grass (*Pennisetumpurpureum* (L.) Schumach). *Grass and Forage Science*; **65**(4): 421-430.
- Tsehay R. (2002). Small-scale milk marketing and processing in Ethiopia. In: Smallholder dairy production and market opportunity and constraints. Proceeding of a South–South workshop held at NDDDB, Anand, India, and 13–16 March 2001. NDDDB (National Dairy Development Board), Anand, India, and ILRI (International Livestock Research Institute), Nairobi, Kenya. Pp. 352-367.
- Valle E. D., Encroach R.O., Schenk J. P. and Curvo J. V. (1994). Duration of estrus and time of ovulation in Nelore Cows. *Revista da Socioedade Brasilenia da zootecnia* **23**: 852-858.
- Vasconcelos J.L., Silcox R.W., Rosa G.J., Pursley J.R., Wiltbank M.C. (1999). Synchronisation rate, size of ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. *Theriogenology*; **52**: 1067-1078.
- Vasconcelos J.L., Sartori R., Oliveira H.N., Guenther J.G., Wiltbank M.C. (2001). Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. *Theriogenology*; **56**: 307-14.
- Vasconcelos J. L. M., Sá Filho O. G., Perez G. C., and Silva A. T. N. (2009). Intravaginal progesterone device and/or temporary weaning on reproductive performance of anestrous crossbred Angus \times Nelore cows. *Animal Reproduction Science*; **111**(2): 302-311.
- Veerkamp R.F., Oldenbroek J.K., Van der Gaast H.J., Van der Werf J.H. (2000). Genetic correlation between days until start of luteal activity and milk yield, energy balance and live weights. *Journal of Dairy Science*; **83**: 577-583.

- Washburn S.P., Silvia W.J., Brown C.H., McDaniel B.T., McAllister A.J. (2002) Trends in reproductive performance in southeastern Holstein and Jersey DHI herds. *Journal of Dairy Science*; **85**:244-51.
- Wathes D.C., Beever D.E., Cheng Z., Pushpakumara P.G., Taylor V.J. (2001). Life-time organization and management of Reproduction in dairy cow. In: British Society for animal Science. Occasional publication No 28, Pp. 59-69.
- Williams S.W., Stanko R.L., Amstalden M. and Williams G.L., (2002). Comparison of three approaches for synchronization of ovulation for timed artificial insemination in Bos indicus-influenced cattle managed on the Texas gulf coast. *Journal of Animal science*; **80**: 1173-1178.
- Wiltbank M. C., Weigel K. A. and Caraviello D. Z. (2007). Recent studies on nutritional factors affecting reproductive efficiency in US dairy herds.
- Wiltbank M.C., Sartori R., Herlihy M.M., Vasconcelos J.L.M., Nascimento A.B., Souza A.H., Ayres H., Cunha A.P., Keskin A., Guenther J.N., Gumen A. (2011). Managing the dominant follicle in lactating dairy cows. *Theriogenology*; **76**: 1568-1582.
- Xu Z.Z., Burton L.J., Macmillan K.L. (1997). Reproductive performance of lactating dairy cows following estrus synchronization regimens with PGF_{2a} and progesterone. *Theriogenology*; **47**: 687–701.
- Yizengaw L. 2017. Review on estrus synchronization and its application in cattle. *Int J Adv Res Biol Science*; **4**(4): 67-76.
- Yoseph M., Azage T., Alemu Y. (2003). Evaluation of the general farm characteristics and dairy herd structure in urban and peri-urban dairy production systems in Addis Ababa Milk Shed. In: Yilma Jobre and Getachew Gebru (editors), Proceedings of the 10th annual conference of the Ethiopian society of animal production, Addis Ababa, Ethiopia, August 21-23, 2003: 139-144.
- Zegeye Y. (2003). Challenges and opportunities of livestock marketing in Ethiopia. In: Proceeding of the 10th Annual Conference of Ethiopian Society of Animal Production (ESAP) 22-24 August 2002. ESAP, Addis Ababa, Ethiopia. Pp .47-54.

ANNEXS

Annex 1. Investigation format for collecting data from selected individual cows for hormonal manipulations and TAI

Part one: Cow's/heifer's characteristics

1. Cows /heifer I.D. _____
2. Age of the cow/heifer _____
3. Body condition Score (≥ 2.75) _____
4. Types of feed the cows/heifers are fed: Hay _____ straw _____ concentrate _____ industrial by product (wheat bran) Green feed Mixed concentrate, hay, straw, green feed and Stover others _____
5. How many times do the animals get feed per day? _____
6. Do the animals get water regularly? Yes No: If yes, how many times a day?

7. Number of births (Parity) _____

Part two: Reproductive performance

8. Age of 1st service _____
9. Number of services per conception _____
10. Gestation period _____
11. Dry period (dry off period) _____
12. Age of 1st calving _____
13. Date of last calving _____
14. Calving interval _____

Part three: production performance

15. Milk yield per day (liter) _____
16. Milk yield per lactation (liter) _____
17. Lactation length (month) _____

Part four: Fixed Time of Insemination

18. When the animal will be inseminated? Morning _____ Afternoon _____ Evening _____
19. Date of last insemination? _____
20. Does the animal come back to estrus again? Yes _____ No _____, if yes, how many times does

it come back to estrus after insemination? _____

21. When does it come back to estrus for the last time? _____

22. How many times the animal will be inseminated when it will be came to estrus? Once__
twice____ three times____ more than three times_____

Part five: Husbandry Practices

23. Do the animals encountered any reproductive diseases or problems in the past? Yes__
No__

24. If yes, what reproductive diseases or problems were observed?

25. Housing condition of the farm? Poor____Moderate____ Good_____

26. Hygienic condition (Shelter, feed and water) Poor____ Moderate____ Good_____

27. Do you use veterinary services? No____ Yes_____

28. What kind of genetic potential improvement practices do they use? Selection with in local
breeds____ up grading through crosses breeding with exotic breeds____ Others_____

29. Do you have any marketing problems in your farm? No____ Yes _____ If yes, why?

30. Do you any extension service relating to dairy production? No____Yes____ If no,
why? _____

31. What are the existing opportunities for the development of dairy production of the farm?

32. What are the most important constraints for the development of dairy production in the
farm? _____

Annex 2. Different photos were taken during this study



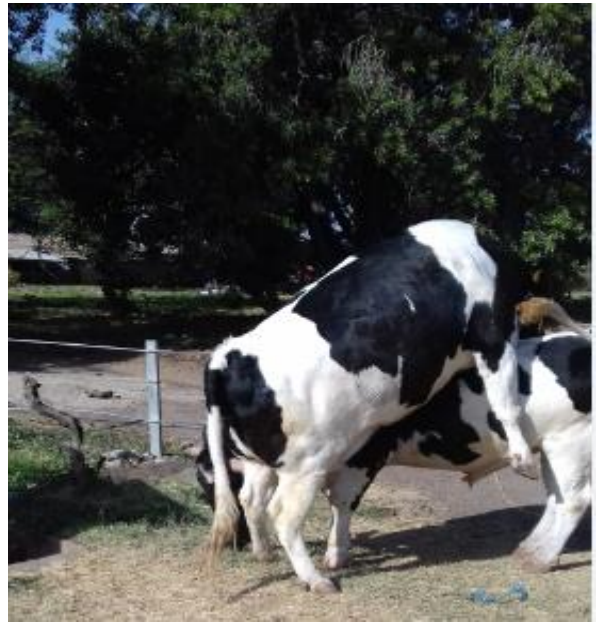
Partial view of research team, material and animal preparation and mobilizing for dairy owners’



Partial view of material and animal preparation and mobilizing for the dairy owners'



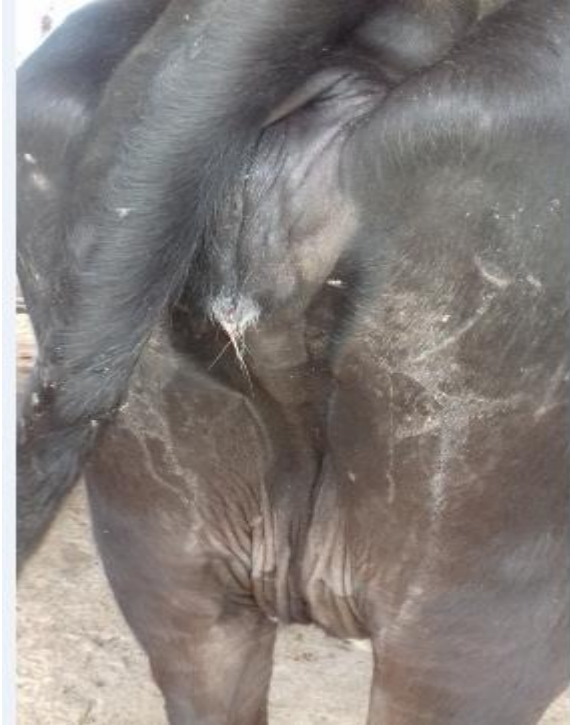
Partial view of rectal palpation and ultrasonography in the study progress



Partial view of different estrus expression characteristics after hormonal administration in the study progress



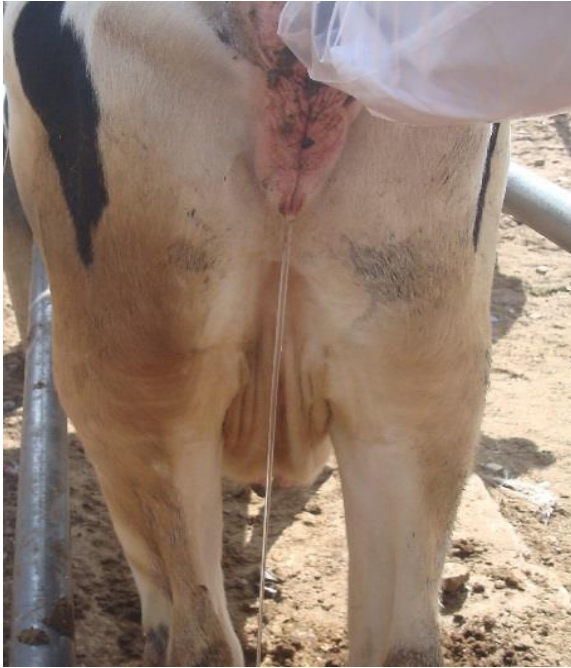
Partial view of different estrus expression characteristics after hormonal administration in the study progress



Partial view of different estrus expression characteristics after hormonal administration in the study progress



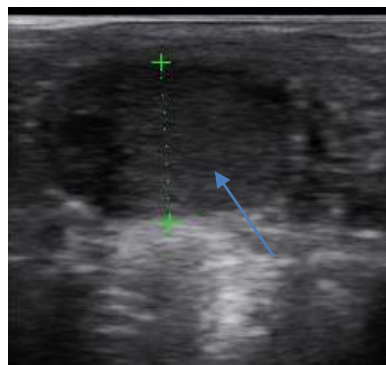
Partial view of different estrus expression characteristics and artificial insemination after hormonal administration in the study progress



Partial view of different estrus expression characteristics and artificial insemination after hormonal administration in the study progress



Follicle






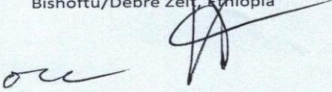
Corpus Luteum



35-days embryo

Ultrasographic scanning results of utro-ovarian during the study

Annex 3. Ethical approval sheet

<p>አዲስ አበባ ዩኒቨርሲቲ የእንስሳት ሕክምናና ግብርና ኮሌጅ ቢሾፍቱ/ደብረ ዘይት</p>		<p>ADDIS ABABA UNIVERSITY College of Veterinary Medicine and Agriculture Bishoftu/Debre Zeit</p>
<p>Animal Research Ethics Review Committee</p> <p><i>Ethical clearance certificate</i></p>		
<p>Certificate Ref. No: VM/ERC/04/08/11/2019</p>		
<p>Name of Applicant: Tilaye Demissie (DVM, MSc, PhD fellow)</p> <p>Address: College of Veterinary Medicine and Agriculture (Addis Ababa University)</p> <p>Title of the project: <i>In vitro embryo production potential of Boran and Zebu-Holstein cross dairy cows in Ethiopia</i></p> <p>Date of application: 13/11/2018</p> <p>Nature of the project: mildly invasive</p> <p>Target animal species: dairy cattle</p> <p>Number of animals involved: 40</p> <p>Study area: Bishoftu, Ethiopia</p> <p>Minutes No. and date of review: VM/ERC/08/11/018, 21/12/2018</p> <p>The above indicated research project is acceptable from ethical perspective, relevance, originality and technical competence points of view. Hence the project is ethically sound to be executed provided that:</p> <ol style="list-style-type: none"> 1. All procedures and conditions stipulated in the proposal are respected and any deviation or changes be reported to the committee 2. The project activities be open for occasional supervision by the committee whenever this is deemed necessary 		
<p><u>Dr Getachew Terefe</u> Chairman</p>	<p> Signature</p>	
<p>መልሱን በሚጽፉልን ጊዜ እባክዎን የኛን ደብዳቤ ቁጥር ይጥቀሱልን Please quote Our Ref. No. When replying</p>		
<p>ፋክስ } Fax 251-11-4339933</p>	<p>ስልክ } Tel. +251 114338450</p>	<p>ፖ.ሣ.ቁ } P.o.x. Box}34</p>
<p>ቢሾፍቱ/ደብረዘይት ሊትዮጵያ Bishoftu/Debre Zeit, Ethiopia</p> <p></p>		