THESIS REF. NO.________

ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

EFFECT OF BREED, PARITY AND BODY CONDITION ON FFICACY OF DOUBLE AND SINGLE PGF2α BOVINE ESTRUS SYNCHRONIZATION PROTOCOL IN BORAN AND CROSSBREED DAIRY CATTLE IN BISHOFTU, ETHIOPIA

BY

EJIGAYEHU DEMISSE

JUNE, 2018

BISHOFTU, ETHIOPIA

College of Veterinary Medicine and Agriculture

Effect of breed, Parity and Body Condition Score on Efficacy of Double and Single PGF2α Bovine Estrus Synchronization Protocol in Boran and cross breed Dairy Cattle in Bishoftu, Ethiopia

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

By

Ejigayehu Demisse
Department of Clinical Studies
MVSc in Veterinary Obstetrics and Gynecology (VOG)

Advisors: Dr. Alemayehu Lemma (PhD, associate professor)
Dr. Tamirat Degefa (PhD, Senior Researcher)

June, 2018
Bishoftu, Ethiopia

APPROVAL SHEET

ADDIS ABABA UNIVERSITY

College of Veterinary Medicine and Agriculrure

Department of Clinical Studies

Efficacy of Double and Single PGF Bovine Estrus Synchronization Protocol in Boran and Crossbreed Dairy Cattle in Bishoftu, Ethiopia

Submitted by:

Ejigayehu Demisse

Name of student -------------------------- ------------------------

Signature Date

Approval for submittal to dissertation assessment committee

……………..…….. ……………………. …………….

Chairperson Signature Date

…………………….. ……………… ………………

External Examiner Signature Date

………………….. ……………. ……..............

Internal Examiner Signature Date

Dr. Alemayehu Lemma

Major Advisor ----------------------- ----------------------

Signature Date

Dr. Tamrat Degefa ------------------------ ------------------------

Co-Advisor Signature Date

Dr. Fufa Abuma ------------------- -------------------------

Department head Signature Date

DEDICATION

This manuscript is dedicated to my mother, W/o BizuneshTufa for nursing me with affection and love and for her dedicated partnership in the success of my life.

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: Ejigayehu Demissie Tufa

Signature: ________________________

College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission: June, 2018

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

Above all, I praise the almighty God, for all he has done for me in every fraction of seconds. This work would have not been possible without the support and contribution of many people; therefore, I would like to thank my advisors Dr. Alemayehu Lemma and Dr. Tamrat Degefa for their constructive advice, provision of different materials, overall intellectual and practical guideline.

I would like to express the respect I have for you my beloved families for all your support, specially to my beloved Mom W/ro Buzinesh Tufa, elder sister Weynishet Gebre Egziabher, to Meseret Fikadu , Zinash Fikadu , Asrat sisay and to all my family member.

I would also like to express my respect to all of you my best friends of MVSc and MSc Batch of 2009 E.C entry and to all of you my friends.

Many thanks goes to my institute, Debrezeit Agricultural Research Center all Staff, to Holleta Agricultural Biotechnology Research Center, Addis Ababa University College of Veterinary medicine and Ethiopian public health Institute for all their contribution in the success of my work

LISTS OF TABLES

LIST OF FIGURES

ABBREVIATIONS

BCS	Body Condition Score
BoA	Beuro of Agriculture
CBPP	Contageous Bovine Pleuro Pneumonia
CIDR	Controlled Intra Vaginal Drug Releasing
CL	Corpusluteum
DZARC	DebreZeit Agricultural Research Center
FMD	Foot and Mouth Disease
FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin Releasing Hormone
ILRI	International Livestock Research Institute
LH	Luteinizing Hormone
LIVES	Livestock and Irrigation Value Chains for Ethiopian Smallholders
LSD	Lumpy Skin Disease
MGA	Melengestrol Acetate
MoA	Ministry of Agriculture
MOET	Multiple Ovulation Embryo Transfer
NEB	Negative Energy Balance
OV-Synch	Ovulation synchronization
$PGF_2\alpha$	Prostaglandin $F_2\alpha$
PIF	Protien Inhibiting Factor
SNNP	Southern Nation, Nationalities and People
TAI	Timed Artificial Insemination

ABSTRACT

From January 2017 to June 2018 study was conducted to evaluate efficacy of single and double bovine estrus synchronization in boran and cross breed dairy cattle in Bishoftu, Ethiopia. Estrus Synchronization is one of the reproductive management tools. Failure to detect estrus in timely and accurate manner is a major factor limiting reproductive efficiency. For this different factors may suggested. The current study was conducted with the hypothesis of, breed, parity; body condition score can influence estrus synchronization with the objectives of evaluating estrus response rate of double and single PGF2α, studying estrus characteristics and effect of breed, parity and body condition on the outcomes of single and double PGF2α. Randomized block design were employed, a total of 54 animals' heifers and cows of local and cross breed were selected, and 36 for double and 18 *single* PGF2α. Animals were grouped based on treatment, parity, body condition and breed. Insemination was done based on standing to be mounted signs of estrus and pregnancies were diagnosed after 21 days post insemination using ultrasonography. Results of the study showed that an overall estrus response of 72.2% and conception rate were 30%. Body condition of cows/heifers significantly ($p<0.05$) effect on estrus response, treatment protocol and parity also influence interval to estrus. Estrus characteristics such as restlessness (57.4%), standing to be mounted (53.7%) and mounting others (51.9%) were the most frequent observed signs, were standing o be mounted significantly influence($p<0.05$) ovulation.On conclusion; estrus response was influenced by body condition, but treatment protocol, parity and breed had no effect. Interval to estrus was influenced by parity and treatment protocol. On the other hand estrus characteristics like standing to be mounted had influence on ovulation rate. Moreover serum progesterone level before and after PGF2α treatment showed significant difference indicating that the treatment induces luteolysis. Duration of estrus, conception and ovulation rate has not shown any significant difference between treatment groups, breeds of study animals, parities and body condition categories. Based on this; for synchronization animals, optimal body condition is priory, single PGF2α treatment is preferable than double PGF2α since it is cheaper and has the same effect as double PGF2α in this study.

Key words: *Estrus synchronization, PGF2α, estrus response and conception rate*

1. INTRODUCTION

Livestock play a significant role in the economic, social and cultural plan in Ethiopia. Thus, it constitutes an essential link of the economy through the generation of incomes and the satisfaction of the food needs for the rural and urban populations. The domestic production of meat and milk remains very low; Indeed, the importations of dairy products in Ethiopia (mostly in local consumption) and demand of milk are increasing each year to more than domestic production compared with the cattle population (Alemayehu and Getu, 2015).In Ethiopia exotic and crossbreed cattle are the main contributors of milk to the growing dairy industry. Confronted with the problems of poor heat detection and less than adequate AI facilities, the optimum exploitation of the genetic potential of the crossbred cattle is a big challenge.(Mekonnin *et al* 2016).

Estrus Synchronization (ES) is one of the reproductive management tools that involve induction of estrous 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).

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)

Fertility is an important factor for the production and profitability in dairy herds, calving interval of 12 to 13 months is generally considered to be economically optimal, but often difficult to achieve. To meet this, cows must cycle and become pregnant within an average of 85 days postpartum. Besides, the incorporation of efficient and accurate heat detection, proper semen handling techniques and timely inseminations relative to ovulation time are key factors (Alemneh *et al*., 2015)

Failure to detect estrus in dairy herds, in a timely and accurate manner, is a major factor limiting reproductive efficiency. As dairy herds expand, having inadequate labor to pay attention to the important details of heat detection, artificial insemination becomes a larger problem, and also it is often more difficult to detect cows in estrus when they are constantly on slippery, concrete surfaces; often found in large, intensive farming systems (Mekonnin *et al*., 2016).

Bos-indicus and their crosses reach puberty at older ages than *Bos-taurus* heifers; differences in concentrations of reproductive hormones and altered sensitivities of their release for LH, estradiol, and progesterone have been noted; characteristics associated with follicular growth pattern and estrus are also different between *Bos-indicus* and *Bos-taurus* cattle (Esterman, 2010), on the other hands, Parity of the dairy animal can also affect estrous manifestation, where a significantly poor estrous expression rate has been observed in the prim parous(Mwaanga *et al*., 2011) .

In Ethiopia from past few years mass Synchronization of cattle in different region were carried out, However, manifestation of estrous and the conception rates of those synchronized animals were not satisfactory in improving the planned breeding program due to different factors related with synchronization. Thus, it was hypothesized that breed body condition and parity of animal are major factors that can influence estrus synchronization response; based on this hypothesis the current study proposed with the following objectives:-

- To evaluate estrus response rate of double and single PGF2α estrus synchronization protocol.
- To study estrus characteristics after synchronization treatment.
- To study effect of breed, parity and body condition on the outcomes of single and double PGF2α estrus synchronization protocols.

2. LITERATURE REVIEW

2.1. Estrus Characteristics

Estrous cycle is controlled by a cascade of hormones initiated by GnRH, Follicle Maturation proceeds under the influence of FSH while LH acts upon the ovarian tissues at the site of ovulation; transforming them into CL (Hixon, 1993). Further, these hormones regulate the different phases of estrus, follicular phase, estrus, and luteal phase, (Smith *et al.,* 2006).

Both estrogen and progesterone are produced following FSH and LH stimulation of the ovary. The uterus contributes to reproductive control, as it produces prostaglandin F2α (Williams *et al.,* 2002). The combination of hormone secretion and metabolism maintain the correct hormonal balance during the follicular phase, estrus, and luteal phase of the cycle. Pre-ovulatory follicle and the subsequently formed corpus luteum are the two primary ovarian structures that regulate the estrous cycle through secretion of estradiol and progesterone, respectively. Changes in a pre-ovulatory follicle and corpus luteum, patterns of secretion of LH, estradiol and progesterone, and changes in ovarian blood flow during the ruminant estrous cycle (Smith *et al.,* 2006).

The primary sign of estrus in cattle is standing to be mounted whereas secondary signs include frequent mounting, mucus vaginal discharge, and restlessness (Rorie *et al.*, 2002). Detection of estrus has been cited as the most important factor affecting the reproductive success in artificial insemination programs. However, proper control of the time of estrus is difficult, since peak estrus activity often occurs at night, and determination of the actual onset of standing estrus may be difficult without 24 hours observation (Aulakh, 2008).

Successful heat detection relies on regular observation of the breeding groups. Ideally, these should be observed at least two times a day, including once early in the morning and once in the evening. Each observation should last at least 20 minutes. The more females that are on heat simultaneously, the more heat activity will be exhibited. High stocking rates and concrete or slippery flooring (as opposed to straw yards or pasture) reduce manifestation of estrus behavior. The best time to inseminate a heifer or cow is slightly before the time of ovulation, which occurs approximately 24 –38 hours after the start of standing heat. This means that cows or heifers should be inseminated in the latter two-thirds of heat or within a few hours after heat has finished Vickers,(2014).

There were many factors that affect the expression of estrus behaviour in bovine species; include environment (temperature, season and light), age and body weight, hormonal imbalance, nutrition and level of production .Temperature has a sound effect on the expression of estrous in bovine, as in extreme cold and hot conditions the sexual activity is less as a result low conception occurs. It also affects the length of estrous (Imran *et al*., 2014) .Detection of estrous at appropriate time is the major significant factor of herd management. If it was neglected, leads to many difficulties linked to fertility. The intensity of estrous signs had an important result on conception rate (Imran *et al*., 2014). There are commercially available estrus detection aids that can be used in conjunction with visual observation to increase estrus detection efficiency. The Heat watch Estrus detection system is probably the only tool that can replace visual observation, since this system provides precise data on the onset, intensity, and duration of estrus (Smith *et al*., 2011), Some of common estrus detection aids are included in (table 1) .

It is generally believed that the detection of estrus in zebu cattle is more difficult than in Bos taurus. In zebu cattle, weaker estrus symptoms and low heat detection rate has been observed (Liewelyn *et al*., 1987). Because of these facts, mounting activity occurred less frequently in zebu than in taurine or their zebu crosses cattle. The duration of standing estrus has been reported to be shorter in zebu than in taurine or their zebu crosses cattle (Mukasa-Mugerwa *et al*., 1989; Bekana *et al*., 2005). Zebu cows have been described a shorter, less intense estrus, which occurs later relative to the estrogen stimulus (Mukasa- Mugerawa, 1989). It seems possible that pattern of follicular growth and estrogen secretion or metabolism may differ in a minor way from tourine animals. As with taurine or their zebu crosses cattle, the duration of estrus and estrus cycle length tend to be shorter in zebu heifers than in cows. In Mexico, studies with zebu cattle (Lamothe Zavalete *et al*., 1991) showed that the duration of estrus is shorter with increasing environmental temperature (>27^{0}C). In Brazil, the duration of spontaneous estrus in Nelore zebu cows has been found 10.7 hrs, with ovulation occurring at an average 11.6 hr after estrus (Valle *et al*., 1994). The average duration of the estrus cycle in those cows has been recorded to be 20.9 days.

The mean estrus cycle length of Ethiopian High land Zebu is about 18- 21 days (Mukasa–Mugerwa and Tegegn 1989).The Arsi zebu line Ethiopia showed a period of 25 days of estrus cycle, while their contemporary Friesian crosses showed 23 days. The luteal phase occupies approximately 14 days followed by a follicular phase of 4-5 days. In post-partum cows, short luteal phase of less than 10 days have been documented in Swedish breed with low plasma concentrations of progesterone as compared to the following normal phase. Similarly, previous studies in Ethiopia have shown lower plasma concentration of progesterone in the Ethiopian highland zebu cattle (Mukasa–Mugerwa and Tegegn 1989).

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,epididy-ectomized, and or penile-deviated animals are used as teaser animals and will mount females in estrus	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)

2.2. 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 anestrous 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).

2.3. 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 F2α and its analogues are the most common treatment used to shorten the luteal phase and synchronize estrus. Progestagens 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 (PGF2α 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 to14 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 PGF2α 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

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 synchronization10–17 or as a therapeutic agent 18–25 has been extensively reviewed in both dairy and beef cows. Furthermore, for PGF2α 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 estrus cycle i.e. .the first 5 days was found to be ineffective in causing a luteolytic response in cattle (Murugavel *et al*., 2003).

2.3.1. Ovsynch

The primary synchronization of ovulation protocol, titled Ov-synch protocol, was given by Pursley *et al*. (1995) which consists of first injection of GnRH followed 7 days later with an injection of PGF, 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 Ov-synch allows for satisfactory pregnancy rates without heat detection, it does not necessarily eliminate the need for heat check. Ov-synch-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 the Ov-synch protocol (Geary *et al.,* 2000).

2.3.2. Co-Synch

It is another option to Ovsynch 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 co-synch than Ovsynch. As with Ovsynch, pregnancy rates are maximized if early heats (± 24 hours of PGF) are visually detected and bred using the a.m.-p.m. rule (Pursley *et al.,* 1998).

2.3.3 Select Synch

Select Synch 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 PGF (Option 1(Geary *et al.,* 2000) or bred to estrus for 72 hours after PGF 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. The Select Synch 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).

2*.3.4. Double PGF2α oestrus synchronization protocol*

Double PGF2α protocol which was given at a 7, 11, or 14 day intervals was developed so that cows at a stage in the estrus cycle other than diestrus would have a functional corpus luteum when they received the second PGF2α dose. doses of PGF2α allowed AI to be performed earlier, because cows not in the diestrus stage when subjected to the first PGF2α injection were found to have a functional corpus luteum when the second PGF2α injection was given 7 days later. However, several authors report the improved reproductive efficiency of cows detected to be in estrus after the second PGF2α dose using the double regime in which PGF2α doses are given 11 or 14 days apart(Murugavel *et al*., 2003).

2.3.5. Single PGF estrus synchronization protocol

A single injection of PGF2α is given to cyclic females, and then these females are bred as they express estrus. The disadvantage of this program is that 20-25% of the females will not respond to the injection, but the advantages are the lower cost of one injection and that females are only handled once other than for breeding (Gugssa, 2015).

2.4. Estrus Synchronization in Ethiopia

Estrous 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).

2.5. Factors Affecting Estrus Synchronization

Many factors such as age, breed, and body weight, level of nutrition, season of the year, hormonal imbalance, lactation, suckling, and level of milk production have been documented to affect the duration the oestrous cycle (Shiferaw *et al*., 2003, 2005). Herd confrontation and stress has been well known to cause irregular oestrous cycle (Roberts, 1986). In the farm yard, the duration of oestrus is likely to vary according to breed, management and a variety of environmental factors: 12-16 hrs may take as the usual duration of the heat period, with a wider range of 3-28 hrs observed over all (Mukasa–Mugerwa and Tegegn 1989; Allrich, 1994; Shiferaw *et al*., 2003, 2005). Ovulation has been documented to occur some 10-12 hrs after the end of the heat period (Roberts, 1986)

2.5.1. Body condition of the animal

Body condition score on a scale of 1 to 5 where score; 1 that the cow is emaciated, while a score of 5 indicates that the cow is obese. Around the time of breeding an ideal BCS are 3, a BCS below that indicates that the animal is without enough energy reserves to support a pregnancy. Negative energy balance (NEB) is indicated by a loss in BCS, because the cow has mobilized body fat stores to meet the energy demand for milk production in early lactation (Culmer, 2012)

Body condition is factor that is closely related to reproductive efficiency. There is substantial evidence linking body condition to reproductive performance in dairy cows. The negative impact of low Body condition on female fertility could be due to a number of factors, including a longer time from parturition to onset of ovarian activity (Wiltbank *et al*., 2007) Body condition at calving is the single most important factor determining when beef heifers and cows will resume cycling after calving (F.Rick, 2006).

Body condition score on a scale of 1 to 5 where score 1 that the cow is emaciated, while a score of 5 indicates that the cow is obese, around the time of breeding an ideal BCS is 3, below this indicates that the animal is without enough energy reserves to support pregnancy. Negative energy balance is indicated by a loss in BCS, because the cow has mobilized body fat stores to meet the energy demand for milk production in early lactation.(Culmer,2012).

The effects of nutrition, often measured by BCS, on oestrous cycle events have been investigated (Diskin *et al*., 2003). Some reports in heifer showed that supplementation of the diet provide an additional metabolize energy per day improved calving rates after oestrus control (progestagen and prostaglandin treatment) from 50% to 69 % as compared to the usual farm ration (Gordon, 1983). Acute nutritional deprivation of heifers has immediate deleterious effect on follicular growth and ovulation (Diskin *et al*., 2003).Inadequate nutrition can have an adverse effect on pituitary function that is the synthesis or release of gonadotropin and may also influence the response of target organs to gonadotropins or gonadal hormones .One important things by which energy restriction impairs reproductive activity seems to be suppression of an increase in lutenizing hormone pulse frequency that has been necessary for growth of ovarian follicles inhibit pulsatile secretion by the hypothalamus the ability of an animal to sustain a high frequency mode of pulsatile LH release has been related to it is metabolic status (Schilo, 1992)

2.5.2. Parity

The interval after calving to first ovulation has been demonstrated to be longer in primiparous cows than multiparous cows. This relationship is associated with greater nutritional deficiency being imposed on younger cows due to the requirements for growth other than lactation. In recent finding, under good management the first ovulation after calving in primiparous cows was delayed as compared to multiparous cows (Tanaka *et al*., 2008). Wathes *et al*. (2001) reported better reproductive performance in multiparous cows others found either no difference or better performance in primiparous cows. Possible reasons for better fertility in primiparous cows include a reduced risk of metabolic disorders in early lactation (Gröhn and Rajala-Schultz, 2000).

2.5.3. Breed of the animal

There are considerable differences in the outcomes of estrus synchronization between zebu and taurine breeds mostly because of their deference in estrus cycle. Variation in the length the of estrus cycle, duration and intensity of the estrus within and between breeds has been reported (Michalk *et al*., 1983; Mac Far lane, 1991). The duration of estrus has been known to be 4.8 and 7.4 hours, respectively, in zebu and their cross breed. It has also been shown that crossbreeding improved the manifestation of estrus and reaches puberty at an average of 6 to 12 months later than Bos taurus cattle (Mukasa–Mugerwa and Tegegn., 1989). Subtle differences in ovarian follicular dynamics that have a potential influence on application reproductive technologies such as synchronization or superovulation have been recently discussed (Degefa *et al*, 2016). These differences in estrus cycle or duration of estrus cycle have made synchronization protocols for one not to necessarily work for the other.

2.5.4. Lactation/suckling and level of milk production

Lactation has been reported to have a suppressive effect on ovarian activity in that prolactin function is maximal during intense lactation, the prolactin inhibiting factor (PIF), including dopamine and GnRH associated peptide are suppressed by sensory stimuli from sucking. As Dopamine and GnRH have essential links with Gonadotropins, GnRH factor. Especially LH releasing factor is suppressed leading to no release of LH, subsequently the final follicle maturation, oestrus and ovulation cannot occur (Stabenfeldt, 1992). High producing cow show poor oestrus behaviour and short oestrus duration than low producing cow (Roberts, 1986). Heavy producer cows have been reported not to have oestrus cycles for 3 to 4 months following parturition (Roberts, 1986).

2.5.5. Stress

Stress has been known to be capable of delaying, shortening or completely inhibiting the expression of estrus in the cow, even in the presence of estrus-inducing concentrations of estradiol. Different forms of stress, whether arising from adverse feeding, management or environmental factors are known to influence the normal operation of the cow's endocrine system. It is evident that stress in the cow can result in increased concentration of progesterone of adrenal origin and many routine husbandry operations may bring about an increase in plasma glucocortocoids. In cattle, increased release of cortisol has been documented in response to a variety of acute stressors in cattle (Nanda *et al*., 1990)

3. MATERIAL AND METHODS

3.1. Study Area

Study was carried out from January - June 2018 at Debre Zeit Agricultural Research Center (DZARC) Bishoftu, located about 45 km east of Addis Ababa 08° 44 N & 38° 58' E (Lat/long) 1900 m.a.s.l. The average annual temperature 8.9▫C min & 28.3▫C Max (Mean= 19▫C) with an average annual rainfall of 851 mm (Long term average) (DZARC Agro-meteorology 2018). DZARC was established over 50 years ago on an area covering about 130 ha, and owns about 150 dairy animals of which 100 are Boran breeds whereas the remaining 50 are crosses. The animals are primarily maintained for various research purpose including nutrition, diseases, reproduction and breeding. The dairy farm is a free stall system, zero grazing where animals are allowed to exercise in yard. Milk produced from the farm is only sold for staffs working within DZARC. There is one laboratory for animal health under which is the reproductive biotechnology section, and another separate laboratory for nutrition and feed analysis. MOET and estrus synchronizations experiments were main focus of research.

3.2. Experimental Animal

Out of 60 animals, 54 cycling cows and heifers (16 Boran heifer and 13 Boran cows, 14 Cross heifers and 11 Cross cows) were included in this study. All animals were subject to clinical and gynecological evaluations of the reproductive tracts to check for abnormalities and to determine the reproductive status of individual animals using trans-rectal ultrasonography. Only cycling and apparently healthy animals without any reproductive disorder were selected as a candidate.

All cows were managed under similar housing, feeding and health management. Animals were fed teff straw and grass hay as a basal diet that was supplemented with commercially prepared concentrate feed (mixed from wheat bran, wheat middling, corn, Noug cakes, and mineral salts) and green fodder (fresh grass, alfalfa, elephant grass). Feeding was based on level of production, and stage of reproduction, while water was given daily *ad- libtum*. Animals were dewormed with broad-spectrum anthelmintic and vaccinated annually for anthrax, black leg, FMD, pasteurollosis CBPP and LSD.

3.3. Study Design

Ethical considerations in this study were taken seriously. None of the procedures involve undue stress to the experimental animals and animal handling has been with humane approach hence did not inflict any harm or unnecessary discomfort to the animals. All activities were carried out in accordance with the ethical guideline of the College of Veterinary Medicine and Agriculture (Annex 2) after receiving the ethical approval.

Randomized block experimental design was employed to study effect of breed, parity and Body condition, on efficiency of Double and single PGF2α Bovine estrus Synchronization Protocol; Experimental Animals were grouped based on Parity; as nulliparous (heifers) and cows (pluriparous), Breed**;** as Boran and Cross (Boran * holistein) and Body condition (optimal when ≥ 3 BCS and below optimal when 3 BCS, Klopii *et al*., (2011).

Before the start of the experiment, the ovaries of all animals were evaluated using a real-time B-mode ultrasound with 5 MHz linear array probe (SIUI, Altay Scientific S.P.A., Italy). Animals were randomly assigned into treatment groups: double PGF and Single PGF injection. Further, subgroups were created for Breed (Boran and B*H cross), parity (Cows and Heifers), and BCS (<3 sub optimal and ≥3 optimal). Only animals with active CL were considered. Data on presence or absence of CL, size of CL, estrus response, interval to estrus, and duration of estrus, ovulation rate, and conception rate were collected to evaluate the difference in treatment response. Individual animals were meticulously observed 3 times a day (7 am, 1 pm and 5 pm) to record estrus signs.

The number and size of corpora lutea (CL) and un-ovulated ovarian follicles present on each ovary were measured using trans-rectal palpation and ultrasonography to determine status of ovulation. Ovulation was defined as the disappearance of previously monitored pre-ovulatory follicle and the detection of a CL (Pursley *et al*., 1995;Ginther *et al*., 1989)

3.3.1. Double PGF synchronization protocols

A total of 36 animals were assigned in this experiment. On Day 0, animals were given 2ml IM injection of prostaglandin (Estrumate®, Schering-Plough ,Germemy) which was repeated 14 days later and AI was given after 72 hrs on detected standing heat (Figure 1).

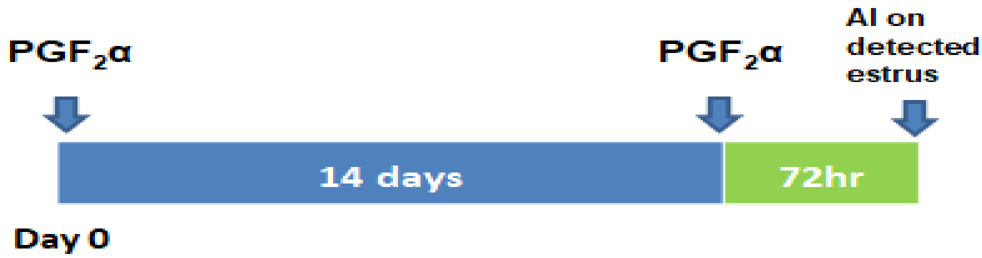

Figure 1. Double PGF Synchronization protocol

3.3.2. Single PGF2α synchronization protocol

A total of 18 were included in this experiment. On Day 0, animals were given a 2ml IM injection of Cloprostenol, (Estrumate®, Schering-Plough BPK; Figure 3). All cows were meticulously monitored to determine the onset, duration and intensity of estrus behavior. All signs exhibited were recorded. Cows that showed behavioral estrus were inseminated on standing estrus.

Figure 2. Single PGF2α estrus synchronization protocol

3.3.3. Record of estrus characteristics

Visual observation was carried out three times per 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 observed during heat

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 urinates frequently.
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	A 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.

3.3.4. Determination of progesterone

Blood samples were collected from jugular vein using vacutainer needle and plain vacutainer tubes for serum progesterone analysis. Collected blood sample were kept overnight on the rack and then centrifuged at 30x100rpm for 5 minutes to obtain the clear serum; then after serum was decanted in 2.0 ml Eppendorf tubes and stored at -20° C until processed. Serum was analyzed at the Chemistry Laboratory of the Ethiopian public health Institute, Addis Ababa.

3.3.5. Pregnancy diagnosis

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. Pregnancy was evaluated ultrasonically at 3 weeks later post AI. The ultrasound examination was carefully performed by imaging the whole uterus. A cow or heifer was diagnosed as pregnant when an embryonic vesicle was identified within the uterine horn.

3.4. Data Management and Analysis

All collected data were managed in Microsoft excel sheet. Using STATA version 13 (Stata Corp LP, Texas, USA). Data were summarized using descriptive statistics and frequency distribution. Effects of various factors such as breed, parity and body condition on estrus response were analyzed using Chi-square. Data on the onset 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 $p < 0.05$ to show statistically significant differences between variables, in this research, statistical analysis as performed where all tests were based on statistical significance at 95% confidence interval.

4. RESULTS

The objectives the current study were to evaluate the effect of breed, parity and body condition score on the efficacy (estrus response), estrus characteristics, ovulation rate, conception rates of double and single PGF2α estrus synchronization protocol. Details of finding were listed below.

4.1. Estrus Response Rate across Different Factors

Estrus response can be affected by different factors. The current study aimed to evaluate those factors that affect estrus response, following double and single PGF2α estrus synchronization protocols in Boran and boran *****Holstein cross heifers/cows. The overall induced estrus response rate was 72.2%, were 69.4% for the double PGF2α treatment and 77.8%% for single PGF2α treatment**,** this was not statistically significant between two groups *($p>0.05$)*. Estrus response rate was significantly different *($P<0.001$)* between animals with optimal body condition i.e. above 3 (90.3 %.) and below optimal condition (47.8%). Parity and breed did not influence estrus response, details of the analysis indicated in (Table 3).

Table 3. Estrus response rate across different factors

Factors	**Category**	**No**	**Responded**	**Percent (%)**	X^2	**P-value**
Treatment	Double	36	25	69.4%	*0.4154*	0.519
	Single	18	14	77.8%		
Breed	Boran	29	19	65.5%	1.4037	0.236
	Cross	25	20	80%		
BCS	Below op.	23	11	47.8 %	11.8859	**0.001**
	Optimal	31	28	90.3%		
Parity	Heifers(0)	29	19	65.5%	1.4037	0.236
	Cows(1)	25	20	80%		
Total		54	39	**72.2%**		

4.2. Interval to and duration of estrus after PGF2α injection

The overall mean (±SEM) interval to estrus and duration of estrus were 71.38± 3.1 hr. and 22.7± 2.7 hr., respectively. The effect of parity on interval to estrus was significant (*P<0.05*). Interval to estrus was highly influenced *(P< 0.05)* by the treatment (single versus double) and parity (heifers versus cows) (Table 4 and 5).

Table 4. Interval to estrus across different factors

Factor	Category	N	Mean± SEM(hrs)	p-value
Treatment	Single	14	78.86±5.86	**0.036**
	Double	25	67.20 ± 3.39	
Breed	Boran	19	74.53± 5.16	0.166
	Cross	20	68.40± 3.60	
Parity	Heifer	19	78.31± 4.44	**0.014**
	Cows	20	64.80± 3.93	
BCS	Below op	11	69.82± 5.07	0.621
	Optimal	28	71.00 ± 3.90	

Table 5. Duration of estrus across different factors

Factor	Category	N	Mean± SEM	p-value
Treatment	Single	14	22.29 ±1.16	0.55
	Double	25	23.04 ±4.21	
Breed	Boran	19	17.68 ± 1.41	0.97
	Cross	20	27.60 ±4.93	
Parity	Heifer	19	20.21 ±1.31	0.82
	Cows	20	25.20 ± 5.14	
BCS	Below op	11	17.45 ±1.89	0.88
	Above op	28	24.86 ±3.65	

4.3. Characteristics of Behavioral Estrus Response

Estrus characteristics were evaluated, for those in heifer/cows that receive the two synchronization protocol(single and double), of the total 54 animals, 57.4%, 53.7%, 51.9%, of animals showed restlessness, standing to be mounted, and sniffing respectively. Details of behavioral estrus characteristics were indicated in table below (Table 6).

Table 6. Behavioral estrus characteristics

Estrus Characteristics	**Animal examined**	**Responded**	**Percent (%)**
Redness of vaginal mucosa	54	27	50.0%
Standing to be mounted	54	29	53.7%
Chin resting	54	19	35.2%
Support chin of others	54	19	35.2%
Mounting others	54	28	51.9%
Mucus vaginal discharge	54	27	50.0%
Restlessness	54	31	57.4%
Soliciting with others	54	14	25.9%
Frequent urination	54	6	11.1%
Sniffing	54	28	51.9%
Bellowing	54	3	5.6%
Ruffled hair	54	12	22.2%

The association of ovulation and standing to be mounted was evaluated and there was a statistical significant difference ($P<0.05$) shown in the table below (Table 7).

Table 7. Relationship between standing to be mounted and ovulation

Factors	Category	No.	Ovulated	Percent (%)	X^2	p-value
Standing to be mounted	Yes	29	29	100%	6.3918	0.011
	No	25	20	80%		

4.4. Ovulation Rate

Even though ovulation rate was numerically difference between factors considered in this study, there was no statistical significant difference (P>0.05) (Table 8).

Table 8. Ovulation rate across different factors

Factors	Category	N	N	Percent (%)	X^2	P-value
Treatment	Double	36	32	88.9%	*0.4408*	0.507
	Single	18	17	88.9%		
Breed	Boran	29	28	94.4%	2.5175	0.113
	Cross	25	21	84%		
BCS	Below op	23	19	82.6%	3.1534	0.076
	Above op	31	30	96.8%		
Parity	Heifers	29	26	89.65%	0.0879	0.767
	Cow	25	23	92%		

4.5. Conception Rate

The overall conception rate was 30%, of which 25% was in double PGF and 35.7% in single PGF treatment, however effect of treatment on conception was not significant (P>0.05). Details of findings on conception rates were indicated in the table below (Table 9).

Table 9. Rate of conception across different factors

Factors	**Category**	**N. Examined**	**No.**	**Percent (%)**	X^2	**P-value**
Treatment	Double	16	4	25%	*0.4082*	0.523
	Single	14	5	35.7%		
Breed	Boran	14	4	28.6%	0.0255	0.873
	Cross	16	5	31.3%		
BCS	Below op	6	3	50%	1.4286	0.232
	Above op	24	6	25%		
Parity	Heifers	13	5	38.5%	*0.7822*	0.376
	Cow	17	4	23.5%		
Total		30	9	**30%**		

4.6. Evaluation of Progesterone

Serum progesterone level was measured before and after PGF2α in both double and single PGF2α protocol and the result revealed that there was a significant difference (P<0.05) (Table 10).

Table 10. Evaluation of level of progesterone before and after PGF2α

Variable	**Mean ±SEM**	**P- value**
Progesterone level before PGF [ng/dL]	1.740± 0.266	0.0159
Progesterone level after PGF [ng/dL]	1.082±0.1611	
Diff	0.657±0.298	

5. DISCUSSION

The present experiment demonstrates efficacy of double $PGF_{2\alpha}$ treatment as compared to single treatment to synchronize cows and heifers. This in agreement with earlier findings with similar treatment in *Bos taurus* (Stevenson *et al*., 2000; Tenhagen *et al*., 2005; Moriera *et al*., 2000) and Zebu (Mukasa–Mugerawa *et al*., 1989; Bekana *et al*., 2005; Meles, 2007).

Estrus response recorded in the present study was also consistent with other studies such as Patil and Pawshe, (2011) that, induced estrus in crossbred cows and heifers using PGF2α reported that 62.5% of the cows responded positively. Similarly, Kebede *et al*., (2013) who administered 5 ml of PGF2α to local cows and heifers in a study conducted in three districts of Bahir Dar Milk Shed reported high Estrus Response (89.3%), although the conception rate was low (13.7%). The authors concluded that PGF2α was very effective in both cows and heifers. Apparently, the perception of the farmers in the three districts was also that PGF2α was effective in triggering estrus but that the pregnancy rate was low due to severe feed shortage. (Malik *et al*., 2013) who studied the effects of the route of PGF2α administration (intramuscular vs intra-uterine) in Bali and Crossbred cattle also found a relatively high Estrus Response in Bali cows (41%) and Crossbred cows (45%) after the first intramuscular injection; the response increased to 80% and 83%, respectively, following the second injection. A recent study in Northern Ethiopia (Gebrehiwot *et al*., 2015) showed that the mean Estrus Response in local and Holstein Friesian cows was 91.67%.

The effect of different factors such as parity and body condition, affecting outcomes of estrus synchronization have been previously discussed (Rae *et al*., 1993). Conception rate between single and double PGF2α treatment was not different which agrees with Bayemi *et al*., (2015) who reported absence of evidence of the effect of treatments (single and double injection of PGF2α) on conception rates. Similarly, (Alemneh *et al*., 2015) also found no difference in conception rate based on treatment groups. Actually, it is universally known that synchronization drugs are not fertility drugs because the factors affection conception rates also come from differences in the quality of semen, time of insemination, and technique of insemination (Nebel *et al*., 2000, Bekana *et al*., 2005).

Response in the present study was most affected by body condition that cows and heifers with optimal body condition showing greater response than those with sub optimal condition. Acute nutritional deprivation leading to a negative energy balance (poor conditions) of heifers and cows has immediate deleterious effect on follicular growth and ovulation (Roche *et al*., 2000). It has been observed that flushing cows with high energy feed following administration of synchronization drugs results in very high response (Rahman *et al*., 2014).

The negative impact of low Body condition on female fertility could be due to a number of factors, including a longer time from parturition to onset of ovarian activity (Wiltbank *et al*., 2007) Body condition at calving is the single most important factor determining when beef heifers and cows will resume cycling after calving (Rick, 2006). 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 estrous 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.

On the other hand, Centurin-Castro *et al*., 2013 and Lemaster *et al*., 1999 reported the absence of any effect of body condition on estrus response when using exogenous estrogen implant to enhance estrus. According to Centurin-Castro *et al*., (2013), duration of estrus was not affected by BCS. Similar studies by Mani *et al*., (1992) also showed estrus response was not different between the feed-restricted and the maintenance groups. Result from other nutritional studies showed that BCS affects postpartum return to estrus, service per conception, pregnancy rate and calving interval (Ciptadi *et al*., 2012) although it was not fully described how synchronization treatment was influenced. Cows with higher BCS had positive associations with days to first estrus, interval to first service, and conception rate at first service and negative associations with calving interval and number of services per conception (Berry *et al*., 2003.).

There are conflicting reports regarding the effect of body condition on pregnancy although it is generally accepted that animals with poor body condition have lower conception rate. For instance, Mulliniks *et al*., (2012) reported that cconception rate of cows/heifers, was not significantly different and body condition score did not influence the overall pregnancy rates, indicating that young cows can have a reduced BCS and still be reproductively punctual. Contrary to this, Mani *et al*., (1992) reported a significant difference in conception rate between animals in good and poor body condition with those in good condition showing relatively higher rates of conception.

Ovulation rate in cows/heifers having body condition below optimal and above optimal groups was not different. This finding is contrary to report by Mani *et al*., (1992) whose finding shows lower ovulation rate for animal in poor body condition (feed restricted groups). On the other hand, Centurin-Castro *et al*., (2013) demonstrated that body condition score has effect on ovulation rate. The response in the taurine crosses was higher in the present study though the difference was not statistically significant. This is in line with that of Alemneh *et al*., (2015), whose report revealed that there were no difference among cross and local breed cows/heifers to estrus response in his single PGF2α. injection experiment.

The difference in the Estrus Response between breeds have been previously reported that cross breed animals respond better to PGF2α administration (Alvarez *et al*., 2000). Given that several studies have reported high estrus response to PGF2α(Bo *et al*., 2003) and (Galina and Orihuela, 2007) observed that the low intensity and short duration of signs of estrus in *Bos indicus* indicated that the work needed to correctly detect this period in these cows was a difficult one and also imprecise.

According to (Bo *et al*., 2003), it has been speculated that one of the reasons why the female *Zebu* presents weak signs of estrus is because the follicular diameter was generally smaller than in the *Bos taurus*. To this effect, it is likely that a direct relationship exists between the follicular diameter and the quantity of estrogen that is synthesized by the cells of the *theca interna* (Bridges and Fortune, 2003) and this possibly affects the intensity of the signs and sexual receptivity (Van Eerdenburg, 2002). More evidence-based research, however, needs to be done to ascertain these arguments However, Alvarez *et al*., (2000) reported difference existed in the response rates between breeds. There are breed differences in ovarian function that were found among *Bos indicus* (Brahman) cows, temperate *Bos taurus* (Angus) and tropically adapted *Bos taurus* (Senepol) cows maintained in a subtropical environment. *Bos taurus* and their crosses have been found to be relatively more sensitive to treatment to exogenous hormones used for management of estrus cycle (Degefa *et al* 2016).

This present finding corroborates with previous study by Mat *et al*., (2015). Hillegass *et al*., (2008) also concluded from his study that primiparous animals have a higher estrus expression compared to multiparous cows. Interval to estruses (from administration of PGF2α to onset of estrus) after administration of PGF2α was highly affected by parity and by synchronization protocol (single versus double injection). However, duration of estrus and the rate of ovulation were not be affected by treatment, breed, parity and body condition in this study. Older animals with parturition experience often respond poorly due to their blood progesterone levels (Roberts, 1986). Further, young females have usually been reported to have shorter length of estrous cycle than the adult animals. In another study by Meles, 2007, however, reported a much shorter interval to estrus (34.3±1.3hrs, which was also even shorter for cross breed (32±1.2 hrs) from the same group of animals. Relatively, the duration of estrus was much better in the present study (22.7± 2.7 hr).

The various behavioral signs recorded in the current study are similar in type and sequence to those reported by Bekana *et al*. (2005) and Meles, 2007, in the Ethiopian local breed with some differences in intensity, interval to estrus and duration of the manifestation. The most frequent signs observed in the present study were standing heat, mucus vaginal discharge and redness of the vaginal mucosa. According to Meles, 2007), the mean duration of standing estrus was 5.0±1.5 hrs in local and 4.0 ±0.4 in cross breed animals treated with double $PGF_{2\alpha}$. Similar studies by Bekana *et al*. (2005) and Mattoni *et al*. (1988) reported a 3.75±0.96 hrs and 7.7±4.5 hrs in Fogera breed of Ethiopia and small east African zebu cattle, respectively. 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). According to Centurin-Castro *et al*., (2013), body condition score in synchronized Beef Master Cows did not affect estrus characteristics(p>0.05). Parity and BCS has significant influence on signs of estrus, like swollen/red vulva, restlessness in does (Mat *et al*., 2015).Although ‘standing to be mounted by others’ behavior is considered as reliable behavioral expression during estrus, a lower rate (as low as 50-53% estruses) has been reported in Holstein cows (Lyimo *et al*., 2000). Previous study reported chin resting and restless behavior in 94.1% estruses, and sniffing and mounting attempt in 100% estruses in crossbred cows (Sood and Nanda, 2006) In the same farm, on indigenous Sahiwal cows, (Layek *et al.*, 2011) observed standing to be mounted by other cows followed by mounting to other cows, sniffing/licking of vulva, chin resting and restlessness in majority of estruses. Bellowing and urination were less frequently observed behaviors. Reddening of vulvar mucus membrane, mucus discharge appeared early and evident in all the estrous periods until the end of estrus with moderate to intense expression.

Majority of the Boran breed cows showed standing to be mounted and mounting on herd mates during estrus in agreement with those reported by Negussie *et al*., (2002) on Fogera cows (Bos-indicus). However these two estrus signs were expressed for short period of time in majority of the cows. Further the expression intensity of these two parameters was also weak and a single mounting lasted for not more than 4–6 s. Thus detection of estrus based on these signs could be difficult unless observed continuously. Similar type of weaker expression of mounting behavior (1. 3mounts/hr) was reported also by Orihuela, (2000).

Mounting behavior is highly dependent upon the number of cows in the sexually active group and the playing ground roughness, thus number of cows in a particular group could also influence the expression intensity of a particular behavior besides the breed specific characteristics. In addition to this 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) but the same may not be applicable in Boran cows as it is expressed in less intensity and for shorter duration as compared to Holstein Friesian or their crosses.

6. CONCLUSION AND RECOMMENDATIONS

The present study revealed that estrus response was influenced by body condition. But treatment protocol, parity and breed had no effect. Interval to estrus was influenced by parity and treatment protocol (single vs double).on the other hand estrus characteristics like standing to be mounted had influence on ovulation rate. Moreover serum progesterone level before and after PGF2α treatment showed significant difference indicating that the treatment induces luteolysis.

Duration of estrus, conception and ovulation rate has not shown any significant difference between treatment groups, breeds of study animals, parities and body condition categories.Based on the aforementioned conclusion the following recommendations are forwarded;

- ✓ Single PGF2α treatment is preferable than double PGF2α since it is cheaper and has the same effect as double PGF2α
- ✓ For synchronization animals, optimal body condition is primarily considered than breed and parity of animals.
- ✓ Because of estrus manifestation problem predominate in different breeds of tropical animals; AI using heat detection could reduce conception, to alleviate this use of TAI after synchronization can improve rate of conception
- ✓ Different factors, which may have effect on synchronization like lactation, level of milk production and stress has to be studied further.

7. REFERENCES

Alemneh T., Mogess W., Marew G. and Adera Z. 2015. Study on the conception rate of dairy cows artificially inseminated during natural heat and by synchronization in Fogera Woreda, North-West of Ethiopia. *African Journal of Basic & Applied Sciences* **7:** 5, 291-297.

Alemayehu K. and Getu A. 2015. The Status of Estrus Synchronization and Conception Rate in Ethiopian Cattle

Alvarez P., Spicer L. J., Chase C. C., Payton M. E., Hamilton T. D., Stewart R. E., Hammond A. C., Olson T. A. & Wettemann R. P. 2000. Ovarian and endocrine characteristics during an estrous cycle in Angus, Brahman, and Senepol cows in a subtropical environment. *Journal of animal science* **78:** 5, 1291-1302.

Aulakh B.S., 2008. In vivo Sex fixing in Dairy Animals to produce female progenies. Proceedings of the 15th congress of FAVA, pp. 243. Bangkok, Thailand.

Allrich, R. D. (1994): Comparative performance of F1 Friesian X zebu Heifers in Ethiopian anim.prod.**37,** 24-25.

Bekana, M., Gizachew, A. and Regassa, F. (2005): Reproduction performances of Fogera heifers treated with prostaglandin F2α for synchronizations of estrus. Trop Anima. Health. And prod **37,** 373-379.

Bo G.A., Baruselli P.S. and Martínez M.F., (2003): Pattern and manipulation of follicular development in *Bos indicus* cattle. Animal Reproduction Science. 78, 307.

Bridges, A. (2010): Basics of Estrous Synchronization in Beef Cattle. Available at http://ezproxy.library.usyd.edu.au/login?url=http://search.proquest.com/docview/815996343?accounti d 14757.

Beef Reproduction Task Force (BRTF). 2006. Applied Rep. Strategies in Beef cattle. Conference Proceedings. Breed. *International Journal of Agriculture and Veterinary Sciences* **1:** 1, 19-26.

Bayemi P. H., Leinyuy I., Nsongka M. V., Webb E. C., Nchadji J. M., Cavestany D. & Perera B. O. 2015. Effect of cow parity and synchronization method with PGF2+¦ on conception rates of Bos indicus cows in Cameroon. *Tropical animal health and production* **47:** 1, 159-162.

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. *J. Anim. Sci*. 65:645-650.

Centurin-Castro F., OrihuelaPorcayo J., AkLpez R. J., Magaa Monforte J. G., MontesPrez R. C. & SeguraCorrea J. C. 2013. Effect of body condition score on estrus and ovarian function characteristics of synchronized beef-master cows. *Tropical and Subtropical Agroecosystems* **16:** 2.

Ciptadi G., Nasich M., Budiarto A. & Nurgiartiningsih V. M. A. 2012. The Estrus Synchronization Response Following PGF 2 Treatment in Indonesian Madura Cattle with Different Body Condition Scores. *Pakistan Veterinary Journal* **32:** 4.

Culmer D. M. 2012. Detection of Ovulation in Dairy Cows byTwice-Daily Passive Monitoring of Reticulo-Rumen Temperature .

De Jarnette J.M., Day M.L., House R.B., Wallace R.A., and Marshall C.E. (2001). Effect of GnRHpretreatment on reproductive performance of postpartum suckled beef cows following synchronization of estrus using GnRH and PGF2*a*. *J. Anim. Sci*., **79**:1675-1682

Diskin M.G., Mackey D.R., Roche J.F. and Screenan J.M. (2003). Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle. *Anim. Reprod. Sci.*, **78:** 345-370

Degefa, Tamrat; Lemma, Alemayehu; Tegegne, Azage; and Youngs, Curtis R. (2016) :"Superovulation of Boran Cattle in Ethiopia: A Preliminary Report," *Animal Industry Report*: AS 662, ASL R3067. Available at: http://lib.dr.iastate.edu/ans_air/vol662/iss1/28

Edmonson, A.J., Lean, I.J.,Weaver, L.D., Farver, T.,Webster, G., 1989. A body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 72, 68–78.

Esterman R. 2010. Evaluation of follicle development and pregnancy rates in yearling heifers and lactating cows of *bos indicus* × *bos taurus* and *bos taurus* breeding that were synchronized with progestogen-based protocols.

F.Rick. 2006. Nutrition and Reproduction Interactions.

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). GalinaC.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 .

Gebrehiwot G., Gebrekidan B., Weldegebriall B., (2015): The effect of one shot prostaglandin on estrus synchronization of Local and Holstein Friesian cattle in and around Wukro Kilte Awulaelo District, Northern Ethiopia. J. Biology, Agriculture and Healthcare. 5, 99.

Gordon, I. (1983): Controlled Breeding in farm Animals. Paragon press oxford, 436 pp.

Gugssa T. 2015. Effects of prostaglandin administration frequency, artificial insemination timing and breed on fertility of cows and heifers in eastern Zone of Tigray Region, Ethiopia.

Ginther O. J., Knopf L. & Kastelic J. P. 1989. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. *Journal of Reproduction and Fertility* **87:** 1, 223-230.

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. Anim. Sci.,* **16**: 1-5.

Grohn Y.T., Rojala-Schultz P.J. (2000). Epidemiology of reproductive performance in dairy cows. *Anim. Reprod. Sci.,* **60–61**: 605–614

Hillegass J., Lima F. S., S Filho M. F. and Santos J. E. P. 2008. Effect of time of artificial insemination and supplemental estradiol on reproduction of lactating dairy cows. *Journal of Dairy Science* **91:** 11, 4226-4237.

Hansel W. and Beal W.E. 1979. Ovulation control in cattle. In: BARC Symposia III. Anim. Reprod. Allanheld, Osmun & Co., Montclair, NJ. p. 91.

Hansel W., and Convey E.M. 1983. Physiology of the estrous cycle. *J. Anim. Sci*. 57(Suppl. 2):404-424.

Hixon D. L. 1993. Estrous Synchronization in Lactating Cows.

Hopkins M.F. & Schrick N.F. 2012. Estrous Synchronization. Extension Dep. of An. Sci. Uni. of Tennessee. https://beefandlamb.ahdb.org.uk.

Hunter RHF (2003): Physiology of the Graafian Follicle and Ovulation. Cambridge University Press, Cambridge.

Imran S., Javed M., Yaqub T., Iqbal M., Nadeem A., Mukhtar N. & Maccee F. 2014. Genetic Basis of Estrous in Bovine: A Review. *International Journal* **2:** 4, 962-966.

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.

Lemaster J. W., Yelich J. V., Kempfer J. R. and Schrick F. N. 1999. Ovulation and estrus characteristics in crossbred Brahman heifers treated with an intravaginal progesterone-releasing insert in combination with prostaglandin F2alpha and estradiol benzoate. *Journal of animal science* **77:** 7, 1860-1868.

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 F2α for ovulation control in postpartum suckled beef cows. *Journal of animal science* **79:** 9, 2253-2259.

Legesse D. 2016. assessment of breeding practice and evaluation of estrus synchronization of dairy cattle in sidama zone, southern ethiopia.

Klopii M., HamoenA., and BewleyJ.,2011. *Body condition scoring of dairy cows*. Biotechnical Faculty, Department of Animal Science.

Kebede A., Zeleke G., Ferede Y., Abate T. and Tegegne A., (2013): Prostaglandin (PGF2α) based oestrous synchronization in postpartum local cows and heifers in Bahir Dar Milkshed. International Journal of Pharma Medicine and Biological Sciences. 2, 37.

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.

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. *Anim. Reprod. Sci*., **129(3)**: 140-145.

Liewelyn, C. A., Munro, C. D., Luckins, A. G., Jordt, T., Murray, M. and Lorenzini, E. (1987): Behavioral and ovarian changes during the oestrous cycle in boron (Boss Indices). *British veterinary Journal* **143**, 75-82.

Lyimo, Z.C., Nielen M, Ouweltjes W, Kruip T.A., and van Eerdenburg FJ. (2000): Relationship among estradiol, cortisol and intensity of estrous behavior in dairy cattle. *Theriogenology*, **53**: 1783-1795.

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.

Mani A. U., McKelvey W. A. C. & Watson E. D. 1992. The effects of low level of feeding on response to synchronization of estrus, ovulation rate and embryo loss in goats. *Theriogenology* **38:** 6, 1013-1022.

Mekonnin A. B., Tadesse G., Khar S. K. Bitsue H. K. 2016. Efficacy of a Modified Gnrh-PGF2 Combination for Estrous Synchronization in Dairy Cattle. *Global Veterinaria* **16:** 2, 200-205.

Macfarlane.J. S. (1991). The detectionand manipulation of oestrus in farm animals. In proceedings of 1989. Internationalconference on biotechnology in livestock in developing countries (Edinburgh) Pp. 70-90.

Malik A, A Wahid, Y Rosnina, M Bukar, A Kasim, and M Sabri, (2011): Effects of resynchronization with progesterone and prostaglandin F2 alpha on estrus response and pregnancy rate in beef cattle. J Anim Vet Adv, 10: 2474-2478.

Mattoni M, Mukasa Mugerawa E che. C (1988) The reproductive performance of east African (Bos indicus) zebu cattle in Ethiopia 1. Oestrous cycle length, duration, behavioural and ovulation time. Theriogenology **30,** 961-971

Michalk, B.K., B. Kebede and E.S.E.Galal.(1983) : Seasonality in the reproductive Behavior of local (zebu) and cross breed cattle in three location of Ethiopia.J.Agr.Sci.**5,** 119-129.

Moriera, F. and Hansen, P. J. (2003): Pregnancy Diagnosis in the cow. Dept. of nimal 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

Mat K. B., Panadi M. B., Victor S. S., Rusli N. D., Hasnita C. H.and Fuad I. S. A. 2015. Preliminary Study on Influence of Parity and Body Condition Score (BCS) on Estrus Response and Conception Rate of Saanen-Cross by Estrus Synchronization with CIDR. *Advances in Environmental Biology* **9:** 27, 307-316.

Murugavel K., Yaíniz J. L., Santolaria P., Lpez-Bjar M. & Lpez-Gatius F. 2003. Prostaglandin based estrus synchronization in postpartum dairy cows: an update. *Journal of applied research in veterinary medicine* **1:** 1, 51-65.

Murugavel K. 2004. Reproductive Performance of Dairy Cows Following Different Estrous Synchronization Protocols.

Mwaanga E. S., Choongo K., Simukoko H. Chama1 C. 2011. Parity Differences in Heat Expression of Dairy Cows Synchronized with GnRH, CIDR and PGF2á during Dry Season in Zambia. *packistan veterinary journal*.

Mulliniks J. T., Cox S. H., Kemp M. E., Endecott R. L., Waterman R. C., VanLeeuwen D. M. & Petersen M. K. 2012. Relationship between body condition score at calving and reproductive performance in young postpartum cows grazing native range. *Journal of animal science* **90:** 8, 2811-2817.

Nanda, A. S., Dobson, and Hand ward, W. R. (1990): Relationship between increases in plasma cortisole during transport induced stress and failure of estradiol to induce Luteinizing hormone surge in dairy cows. Research in veterinary science **49**, 25-28.

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 14<Sup (tb)> International Congress. Forsberg, N. **60/61**:713-723.

Negussie, F., Kassa, T., Tibbo, M., (2002):. Behavioural and physical signs associated with estrus and some aspects of reproductive performance in Fogera cows and heifers. Trop. Anim. Health Prod. 34, 319–328.

Orihuela, A., (2000): Some factors affecting the behavioural manifestation of oestrus in cattle: a review. Appl. Anim. Behav. Sci. 70, 1–16.

Patil S.R. and Pawshe C.H., (2011): Estrus induction and fertility following single dose of Prostaglandin F2 alpha in crossbred cows and heifers. Indian Journal of Animal Reproduction. 32(2), 61.

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:** 3, 695-704.

Patterson D.J., Wood S.L., and Randle R.F. 2000. Procedures that support reproductive management of replacement beef heifers. Proc. *Anim. Sci*., 1999. Available at: http://www.asas.org/jas/symposia/proceedings/0902.pdf. Accessed July 13, 2017

Patterson DJ, Kiracofe GH, Stevenson JS, Corah LR. 1989. Control of the bovine estrous cycle with melengestrol acetate (MGA): a review. *J. Anim. Sci.* 67:1895-1906.

Pursley J. R., Mee M. O. and Wiltbank M. C. 1995. Synchronization of ovulation in dairy cows using PGF2 and GnRH. *Theriogenology* **44:** 7, 915-923.

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. *J. Dairy Sci*., **81**: 2139-2144.

Pursley, J.R., Mee, M.O., and Wiltbank, M.C., 1995. Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology 44, 915–923

Rahman M.S., Khan M.K.I. and Bilkis T., (2014): Effect of flushed feeding and age on estrus synchronization and conception rate of Holstein × Local Crossbred cows after using analogue GnRH and Prostaglandin F2α hormone. Iranian Journal of Applied Animal Science. 4(3), 493.

Roberts. S. L. (1986): Veterinary obstetrics and genital disease 2nd Ed. Lithographed by Edwards Brothers Inc. Ann. Arbor. Michigan. Pp. 500 – 506.

Roche, S. F. Mackey, D. and Disking, M. D. (2002): Reproductive management of dairy cows, Anima – Repord. Sic. **61**, 703 – 712.

Roelofs JB, van Eerdenburg FJCM, Soede NM, Kemp B (2005): Various behavioral signs of estrus and their relationship with time of ovulation in dairy cattle. Theriogenology 63:1366–1377

Schillo K, (1992) Effects of dietary energy on control of lutenizing hormone secretion in cattle and sheep.J.Anim.sci. **70,**1271-1282**.**

Shiferaw Y., B-A. Tenhagen, M. Bekana and T. Kassa (2003). Reproductive performance of crossbred dairy cows managed under different production systems in the central highlands of Ethiopia.Tropical Animal Health and Production. **35,**551-561.

Shiferaw Y.,M. Bekana**,** B-A. Tenhagen and T. Kassa 2005 Reproductive disorders of crossbred dairy cows in the centralhighlands of Ethiopia and their effects on reproductive performance.Tropical Animal Health and Production. **37,**427-441

Sood, P. and Nanda, A.S. (2006): Effect of lameness on estrous behavior in crossbred cows. *Theriogenology*, **66(5)**: 1375-1380**.**

Stabenfeld, G. H. (1992): Reproduction/lactation in textbook of veterinary physiology.

Stevenson, J. S., Smith, J. F. and. Hawkins, and D. E. (2000): Reproductive outcomes of dairy heifers treated with combinations of prostaglandin F2α, norgestomet, and gonadotropin-releasing hormone. J. Dairy Sci. 83: 1-8.

Tenhagen, B.A.Kuchenbuch,S., Heuwieser, W. 2005. Timing of ovulation and fertility of heifersafter synchronizationof oestrus with GnRH and prostaglandin F2α. Reprod. Domest.Anim.; **40,** 62-67

Rorie R.W., Bilby T.R. and Lester T.D. 2002. Application of electronic estrus detection technologies to reproductive management of cattle. *Theriogenology* 57: 137-148.

Rae D. O., Kunkle W. E., Chenoweth P. J., Sand R. S. & Tran T. 1993. Relationship of parity and body condition score to pregnancy rates in Florida beef cattle. *Theriogenology* **39:** 5, 1143-1152.

Schafer D.W., Brinks J.S., and Le Fever D.G. 2007. Increased calf weaning weight and weight via estrus synchronization. Beef Program Report. Colorado State University. pp. 115-124.

Smith M.F., Perry G.A., Atkins, J.A., Jinks E.M., Pohler G. and Patterson D.J. 2006. Physiological Principles Underlying Synchronization of Estrus. Proceedings, Applied Reproductive Strategies in Beef Cattle. Department of Animal and Range Sciences, South Dakota State University. Pp. 29-52.

Smith M. F., Perry G. A., Atkins J. A., Jinks E. M., Pohler K. G. Patterson D. J. 2011. Physiological principles underlying synchronization of estrus.

Tanaka T., Arai M., Ohtani S., Uemura S., Kuroiwa T., Kim S., Kamomae H. (2008). Influence of parity on follicular dynamics and resumption of ovarian cycle in postpartum dairy cows. *Animal Reprod. Sci.,* **108**: 134–143

Valle, E. R. D. O, Encroach, R. De, O., Schenk, J. A. P. and J. V. E. Curvo. (1994): Duration of estrus and time of ovulation in Nelore Cows: Revista da Socioedade Brasilenia da zootecnia **23**, 852 – 858.

van Eerdenburg, F.J.C.M., Karthaus, D., Taverne, M.A.M., Merics, I., Szenci, O., (2002): The relationship between estrus behavioral score and time of ovulation in dairy cattle. J. Dairy Sci. 85, 1150–1156.

Vickers M. 2014. Artificial Insemination and Oestrus Synchronization of Beef Cattle. Plus + advanced information from BRP. Pp 1-12.

Wathes, D.C., Beever, D.E., Cheng, Z., Pushpakumara, P.G.A., Taylor, V.J., 2001. Life-time organisation and management of reproduction in the dairy cow. In: British Society for Animal Science. Occasional Publication No 28, pp. 59–69.

Williams S.W., Stanko R.L., Amstalden M., 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. *J Anim. Sci.*, 80:1173-1178.

Wiltbank M. C., Weigel K. A. & 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.

Yizengaw L. 2017. Review on estrus synchronization and its application in cattle. *Int J Adv Res Biol Sci* **4:** 4, 67-76.

8 ANNEXS

Annex 1. Different signs of behavioral estrus

 A Standing heat	 B Head butting
 C. Chin resting	 D. Sniffing

Annex 2. Ethical approval sheet

አዲስ አበባ ዩኒቨርሲቲ
የእንስሳት ሕክምናና
ግብርና ኮሌጅ
ቢሾፍቱ/ደብረ ዘይት

ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu/Debre Zeit

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/16/05/10/2018

Name of Applicant: Ejigayehu Demisse (DVM, MVSc fellow)

Address: College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: Effect of breed, party and body condition on efficacy of double and single PGF bovine estrus synchronization protocol in Bishoftu, Ethiopia

Date of application:	15/10/2017
Nature of the project:	non-invasive
Target animal species:	cattle
Number of animals involved:	36
Study area:	Ethiopia

Minutes No. and date of review: VM/ERC/05/10/018, 03/01/2018

The above indicated research project is acceptable from ethical perspective, relevance, originality and technical competence points of view. Hence the project is allowed to be executed provided that:

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

Dr Getachew Terefe
Chairman

Signature

መልስ ሲጽፉልን የኛን ደብዳቤ ቁጥር ይጥቀሱልን
Please quote Our Ref. No. When replying

ፋክስ
Fax 251-11-4339933

ስልክ
Tel. +251 1143…

ፖ.ሣ.ቁ
P.O. Box 34

ቢሾፍቱ/ደብረዘይት ኢትዮጵያ
Bishoftu/Debre Zeit, Ethiopia

Annex 3. Ultrasonography scanning of animals during experiment