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EFFECT OF BREED, PARITY AND BODY CONDITION ON OVARIAN RESPONSE AND CONCEPTION RATE OF BORAN AND ZEBU-HOLSTEIN CROSSBRED DAIRY COWS SUBJECTED TO OVSYNCH OR CO-SYNCH PROTOCOL

MVSc Thesis

By

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Department of Clinical Studies, MVSc in Veterinary Obstetrics and Gynecology

June, 2018

Bishoftu, Ethiopia

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A Thesis Submitted to College of Veterinary Medicine and Agriculture of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Veterinary Obstetrics and Gynecology

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APPROVAL SHEET

Addis Ababa University

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As member of the Examining Board of the final MVSc open defense examination, we certify that we have read and evaluated the Thesis prepared by: Alebachew Tilahun, titled: **Effect of Breed, Parity and Body Condition On Ovarian Response and Conception Rate of Boran and Zebu-Holstein Crossbred Dairy Cows Subjected to Ovsynch or Co-Synch Protocol** and recommended that it be accepted as fulfilling the thesis requirement for the degree of Master of Science in **Veterinary Obstetrics and Gynecology**.

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DEDICATION

This manuscript is dedicated to my mother, Dinkayehu Alehegn and my wife, Mintewab Andualem for nursing the author with affection and love and for their dedicated partnership in the success of his life.

STATEMENT OF AUTHOR

First, I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MVSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture 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.

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ABBREVIATIONS

CIDR	Controlled Internal Drug Release
CL	Corpus Luteum
CSA	Central Statistics Agency
DAGRIS	Domestic Animal Genetic Resource Information System
DIM	Days in Milk
DMI	Dry Matter Intake
EASE	Ethiopian Agricultural Sample Enumeration
EIBC	Ethiopian Institute of Biodiversity Conservation
FSH	Follicular Stimulating Hormone
FTAI	Fixed Time Artificial Insemination
GnRH	Gonadotropin-Releasing Hormone
IFG_I	Insulin-Like Growth Factor I
ILRI	International Livestock Research Institute
IPMS	Improving Productivity and Market Success
LF	Larger Follicle
LH	Luteinizing Hormone
LIVES	Livestock and Irrigation Value Chains for Ethiopian Smallholders
MGA	Melangestrol Acetate
MoA	Minister of Agriculture
NEB	Negative Energy Balance
NEFA	Non-Esterified Fatty Acid
OSMAI	Estrus Synchronization and Mass Artificial Insemination
PGF2α	Prostaglandin $F_2α$
RTS	Reproductive Tract Score
SNNPR	Southern Nations, Nationalities, and Peoples' Region
TAI	Timed Artificial Insemination
VWP	Voluntary Waiting Period

ABSTRACT

A study was carried out to evaluate the effect of parity, breed and body condition on ovarian response and conception rate of Boran (n=17) and Zebu-Holstein cross (n=18) dairy cows bred by Ovsynch or Co-synch timed artificial insemination. Ovarian response (ovulation and luteolysis) and pregnancy diagnosis were made by ultrasound and progesterone assay. For progesterone assay blood samples were collected at day 0, 5, 7, 9, 15 and day 21 of the experiments. The overall ovulation rate was 85.7%, of which 90% and 80% in Ovsynch and Co-synch, respectively. In this study, body condition, breed, and parity did not influence ovulation rate in both protocols (P>0.05). The overall conception rate was 60%, (55% in Ovsynch and 66.7% in Co-synch). Conception rate was relatively higher in Boran (70.6%) compared to crossbred (50%). However, the difference in conception rate was not statistically significant (P>0.05). Conception rate was significantly higher in primiparous (73.7%) compared to multiparous dairy cows (43.8%) (P <0.05). Conception rate was not influenced by body condition and breed in both protocols. The mean diameter of corpus luteum was 18.3±1.31 mm for animals with good body condition while it was 11.84±0.93 for those with low to moderate body condition and mean difference of corpus luteum was statistically significant (P<0.05). However, breed and parity did not influence mean size of corpus luteum. Twenty-seven cows (77.1%) had functional corpus luteum on day seven of the treatment (progesterone >1 ng/ml) and the progesterone was reduced to less than 1 ng/ml in 22 cows (81.5%) on day nine after PGF injection. In five cows (18.5%) the progesterone remained at levels greater than 1 ng/ml after PGF2α treatment (day 9). The result of this study demonstrated that conception was higher in Boran cows than Zebu-Holstein cross. Based on the result, it could also be concluded that both Ovsynch and Co-synch can give acceptable level of conception with the established TAI protocols.

Key words: *Boran, conception rate, Co-synch, Ethiopia, Ovsynch*

1. INTRODUCTION

Ethiopia owns the largest livestock population in Africa and one of the largest in the world, with 52 million cattle among others (CSA, 2013). About 85% of the Ethiopian populations are engaged in the agricultural sector, which is the backbone of the country's economy. Livestock and its products are important sources of food and income in Ethiopia (EIBC, 2004). The majority of them are indigenous breed which are well adapted to the environment in the tropics because they possess a high degree of heat tolerance and resistance to most of endemic diseases. Most reports on dairy cattle production in the tropical and subtropical areas recommend crossing indigenous with temperate breeds (EASE, 2003).

Zebu (*Bos indicus)* cattle are multipurpose animals with low potential for meat and milk production, which is estimated to meet only 35% of the human demand (Landiver *et al*., 1985; Mukasa-Mugerewa, 1989). Boran cattle breed is the most common indigenous breeds used for crossbreeding with exotic dairy animals for improving milk production in Ethiopia. It is classified in zebu type that originated from Southern lowlands of Ethiopia. The breed is extensively used for milk, meat, draft power and manure production (Alberro and Haile-Mariam, 1982). Boran breed is adapted to semi-arid tropical conditions due to high degree of tolerance to heat, and diseases prevalent in tropics (Mensah and Okeyo, 2006).

The reproductive efficiency of dairy cows has declined over the last several years and is considered lower than optimal (Lucy, 2001; De Varis and Risco, 2002). This is thought to be due to many factors including inefficiency and inaccuracy of estrus detection, improper timing of insemination, delayed ovulation, anovulation, negative energy balance, selection for high milk production and inbreeding (Lucy, 2001). The development of timed artificial insemination (TAI) protocols based on the use of GnRH and PGF2α precisely synchronizes the time of ovulation and eliminates the need for estrus detection (Pursley *et al*., 1995). Reproductive failure is a major source of economic loss in dairy and beef industry (Perry, 2005). Introduction of reproductive techniques such as estrus synchronization and AI are becoming instrumental to solve the effects of these limiting factors as well as to enable the application of more intensive systems of production to facilitate genetic improvement (Kouamo and Sawadogo, 2012). A strategy, which is widely used throughout the dairy industry for improving reproductive efficiency, is the use of estrus synchronization programs with TAI (Palgrave, 2010).

Estrus Synchronization is one of the reproductive management tools that involve induction of estrous in a group of females to breed relatively in around the same time (Schafer *et al.*, 2007; Rick, 2013). Estrus synchronization aimed to improve reproduction efficiency by reducing breeding length and calving seasons to increasing calf-weaning weights (Gupta *et al.,* 2009). Human errors and management costs could be minimized by using estrus synchronization tools. Discovery of ovarian steroids has led to the effective control of the length of the bovine estrous cycle and the timing of estrus and ovulation (Schafer *et al.*, 2007).

The development of effective schedule of synchronizing heat and ovulation has been based on consideration of physiological and hormonal mechanisms controlling the estrous cycle, the induction of estrous in heifers and postpartum cows. Estrus synchronization products such as PGF2α, MGA, CIDR and GnRH and protocols have changed over time. An understanding of how these products affect the bovine estrous cycle and how management decisions determined pregnancy success would have an effect on the success of any reproductive program (Perry *et al.,* 2004).

Estrous synchronization programs provide an organized and efficient approach to administer artificial insemination (AI). Synchronization programs facilitate AI by altering the length of the estrous cycle and (or) through manipulation of follicular growth, thereby making the occurrence of estrus more predictable or allowing for appointment breeding (timed AI) without detection of estrus (Dalton *et al.,* 2005). Most estrous synchronization program employs a method for controlling follicular wave development, promoting ovulation in anestrous cows, regressing the corpus luteum in cyclic cows, and synchronizing estrus and (or) ovulation at the end of treatment. In most herds, a non-intervention period is practiced where postpartum cows are observed estrus. Cows not observed in estrus are then treated (Day, 2004).

Failure to detect estrus could prove a major constraint to increasing the productivity of *Bos indicus* cattle in developing countries. Problem related to heat detection can be overcome by using a synchronization protocol that permits fixed time AI. Many literatures indicated that TAI programs are applied routinely in dairy and beef production in different parts of the globe (Stevenson *et al.,* 2003; Chebel *et al.,* 2003; Baruselli *et al.,* 2004; Wiltbank *et al.,* 2011). Synchronization with TAI protocols reduce the interval from parturition to first service and increased the proportion of cows becoming pregnant sooner after the voluntary waiting period (VWP) (Gutiérrez *et al.,* 2009; Herlihy *et al.,* 2011). Moreover, variations of protocols affect the interval from parturition to first estrus and conception at the time of breeding (Busch *et al.,* 2008). Furthermore, there is scarce information towards Ovsynch and Co-synch estrus synchronization in Boran and crossbred dairy cows in the study area.

Hypothesis: Combination of different estrus synchronization products, synchronization protocols can overcome heat detection difficulties and improve fertility in Boran and Zebu-Holstein crossbred dairy cows when fixed timed AI is used; and factors such as parity, breed and body condition may have effect on the success of synchronization and TAI.

The objectives of the experiment were;

- To study synchronization induced ovarian response in Boran and Zebu- Holstein crossbred dairy cows
- To evaluate the effect of breed, parity and body conditions on conception rate of Ovsynch or Co-synch protocol in Boran and Zebu-Holstein cross dairy cows.

2. LITERATURE REVIEW

2.1. Boran Cattle Breed in Ethiopia

Boran breed originally descended to Africa from West Asia during introduction of Zebu. The breed established its presence first in the semi-arid and arid pastoral Borana plateau of Southern Ethiopia. Pastoral movements and migrations led to the spread of the Ethiopian Boran to the Eastern rangelands as well as into Northern Kenya and South-Western Somalia. The Ethiopian Boran, Somali Boran and Kenya Boran have evolved from these migrations (http://eth.dagris.info/node/2322). Boran cattle are the most suitable breed for arid and semi-arid regions in the country due to their adaptive characteristics like tolerance to heat, tick infestation, feed and water shortage, hardened hooves and lighter bones that enabled them to endure long migrations (Solomon, 2001; Mekonnen *et al.*, 2010; OARI, 2010). It is widely used for milk, meat, draft power and manure production (Alberro and Haile-Mariam, 1982).

Different literatures showed that Boran breeds are superior to other highland cattle in Ethiopia: birth weight, weaning weight, faster growth rate, mature weight for cows, higher fertility, daily milk yield, milk yield/lactation, lactation length, milk fat, age at first calving, calving interval, and calving rate (DAGRIS, 2008). They are also superior in mature weight for bulls, providing of good source of beef for local and international markets in Ethiopia and other African countries such as Kenya, Tanzania, Uganda and Zambia (Coppock, 1994). They have large body size with good body conformation (large and long-legged, tall height, broad back and wide pin-bones, well developed dewlap and udder, and well-developed hindquarters) and body weight ranging from 318 - 680 kg in male and 225-454 kg in female (Mekonnen *et al*, 2010; OARI, 2010). However, effort made so far to increase the productivity on Boran cattle in Ethiopia was fully based on crossing with exotic breeds either through AI or natural breeding. As a result, crossbreeding of Boran with Holstein has resulted in improved growth, milk production and reproductive performance, and these traits exhibited an increasing trend with increasing exotic inheritance level (Haile *et al.*, 2009).

The main physiological differences of *Bos indicus* include delayed age at puberty, higher circulating concentrations of hormones such as estradiol, progesterone, insulin and IGF-I (Insulin-like Growth Factor I), despite having smaller ovulatory follicle size and corpora lutea, greater population of small follicles and smaller size of the dominant follicle at deviation; and greater sensitivity of follicles to gonadotropins than *Bos taurus* (Sartori *et al*., 2010). Some of the differences in genotypes include a reduced capacity for LH secretion in *Bos indicus* compared with *Bos taurus* breeds (Randel, 1984) and a greater sensitivity of *Bos indicus* cows to exogenous gonadotrophins (Munro, 1986). Moreover, the timing of the LH surge and ovulation occur earlier in relation to onset of estrus in *Bos indicus* breeds (Randel, 1984), while behavioral estrus was found to be shorter, less marked and frequently occurred in night in *Bos indicus* females (Galina *et al*., 1996). Smaller corpora lutea (CL) and lower peripheral concentrations of progesterone (P4) have also been reported in *Bos indicus* cattle (Randel, 1984; Segerson *et al*., 1984).

2.2. Physiology of Estrous Cycle and Ovarian Follicular Dynamics

Ovaries of cattle contain two different pools of follicles, the non-growing pool and the growing pool. The non-growing pool contains the primordial follicles, whereas the growing pool contains the primary, secondary and tertiary follicles (Kanitz *et al*., 2001). Entry of primordial follicles into the growth phase occurs throughout the reproductive life. The primordial follicles continuously leave the arrested pool and undergo the primordial to primary follicle transition. The oocytes increase in size and the surrounding squamous pre-granulosa cells become cuboidal and proliferate to form a layer of cuboidal cells around the growing oocyte (Fortune *et al*., 2000).

The estrous cycle of the cow is 21 days long on average, but it can range from 17 to 24 days in duration. Each cycle consists of luteal phase where the cycle is under the influence of progesterone and follicular phase where the cycle is under the influence of estrogen. The cycle begins with standing heat, or estrus. This time of peak estrogen secretion can last from 6 to 24 hours, with ovulation occurring 24 to 32 hours after the beginning of estrus

(Williams *et al.,* 2002). Ovulation marks the beginning of the luteal phase, and is the culmination of a process called oogenesis (Rick, 1999). The ability to produce estrogen is a hallmark for a follicle becoming selected (Beg *et al*., 2001) with factors such as aromatase, free insulin-like growth factor, and cocaine- and amphetamine-regulated gene transcript being differentially expressed in the selected follicle compared to subordinate follicles (Kobayashi *et al*., 2004). The selected follicles secrete inhibin and estrogen, which suppress secretion of FSH and result in atresia of many of the FSH-dependent follicles. Follicles that develop LH receptors on the granulosa survive the decline in concentrations of FSH and become dependent on LH (Zielak *et al*., 2008).

One follicle (dominant follicle) of the cohort grows more than the others (subordinate follicles) and is said to be selected for preferential growth. The follicular waves continue to develop during pregnancy (Ginther *et al.,* 1989). The existence of follicular waves has been recorded as early as 2-weeks of age in calves (Evans *et al.,* 1994) and follicles continue to grow and regress throughout the prepubertal period, however, ovulation does not occur until the animal attains puberty. Emergence of successive follicular waves during the estrous cycle have been associated with increases in circulating concentrations of FSH which preceded wave emergence by one day. Furthermore, declining concentrations of FSH after wave emergence have been implicated in the mechanism of selection of the dominant follicle (Adams *et al.,* 1992). Wave emergence has been detected, on average, on the day of ovulation days 0, 9 and 16 in cattle with 3 follicular waves. The dominant follicle of Wave 3 was the ovulatory follicle in cycles with three follicular waves (Ginther *et al.*, 1989).

During an anovulatory wave, the development of each follicle has been subdivided into growing (increasing diameter), static (no change in diameter) and regressing (decreasing diameter) phases (Ginther *et al.*, 1989). These ultrasonographically classified phases have been shown to be closely correlated with the follicle's ability to produce steroid and protein hormones (estrogen: progesterone and estradiol: androstenedione ratios, *a*- and dimeric-inhibin concentrations, IGF-binding proteins, mRNA for gonadotropins receptors and steroidogenic enzymes), indicative of follicular health (Sunderland *et al.*, 1996).

Mechanisms for the occurrence of dominant and subordinate follicles is that DF changes its dependence on FSH to LH, continuing to grow even during the FSH deprivation in detriment of the other follicles, which stop growing and undergo atresia (subordinates; Ginther *et al.*, 1996). Despite the gonadotropins (FSH and LH) playing a primary endocrine role on follicular development, local factors can also interfere on this process as inhibin and IGF-1 (Insulin Like Growth Factor - 1). Inhibin secretion by the DF, by negative feedback, decreases the FSH secretion, playing an important role on the follicular recruitment and development (Turzillo and Fortune, 1993).

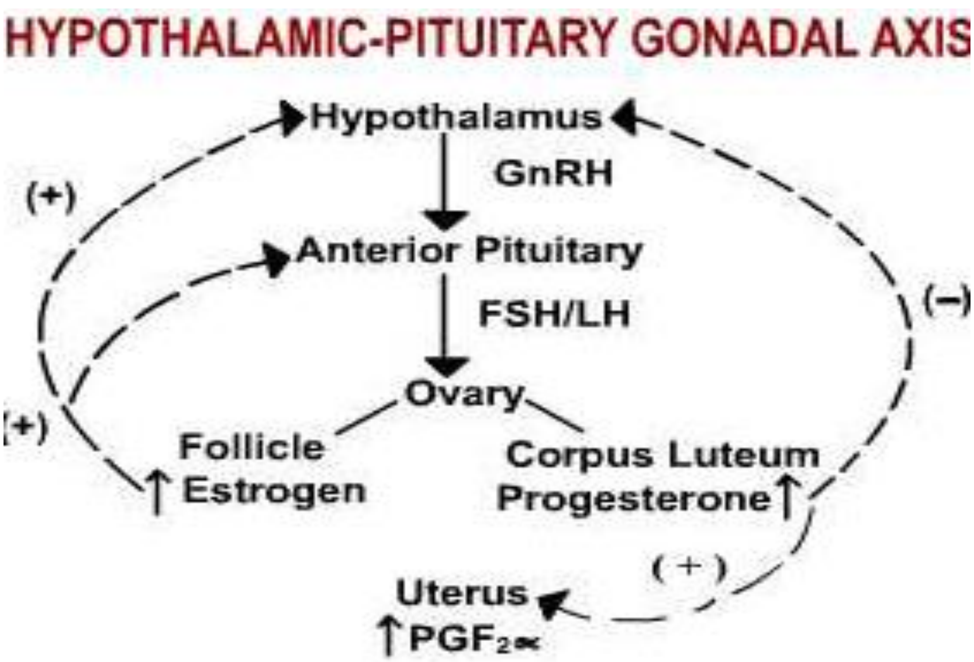
2.3. Hormonal Control of Estrus

The estrous cycle is consisting into three stages (follicular phase, estrus, and luteal phase) and is regulated by hormones secreted by the hypothalamus (GnRH), anterior pituitary gland (follicle stimulating hormone (FSH) and luteinizing hormone (LH), ovary (estradiol and progesterone), proteins and peptides of follicular origin (inhibin A, and follistatin) and uterus (PGF2α). The preceding hormones serve as chemical messengers that travel in the blood to specific target tissues, which contain receptors that are hormone specific and regulate the phases of the estrous cycle (Mihm *et al*., 2002; Smith *et al.*, 2006).

The hypothalamus is a specialized portion of the central brain, which produce gonadotropin-releasing hormone (GnRH) in response of circulating estrogen and cease GnRH production in response to progesterone. The anterior pituitary is located directly beneath the hypothalamus in a small depression of the sphenoid bone. It produces the gonadotropin follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in response to GnRH. The production of FSH and LH is inhibited by progesterone. The third portion of the reproductive axis consists of the ovaries, located in the pelvic cavity. Follicles are structures on the ovarian surface that contain ova (egg) and produce estrogen. Follicles range in size and maturity at different stages of the cycle, but usually only one is selected to ovulate. A corpus luteum (CL) responsible for progesterone is a structure that formed from the previous cycle’s ovulation point. 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. Preovulatory 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 preovulatory follicle and corpus luteum, patterns of secretion of LH, estradiol and progesterone, and changes in ovarian blood flow during the ruminant estrous cycle (Fig. 1) (Smith *et al.,* 2006).



Source: (Rick, 1999)

Figure 1. Reproductive axis-hypothalamus, pituitary, and the ovary

2.4. Estrus Signs and Estrus Detection Methods

The primary sign of estrus in cattle is standing to be mounted and secondary signs of estrus includes frequent mounting, watery mucus from vulva, and restlessness (Rorie *et al.*, 2002). Estrus (heat) detection has been cited as the most important factor affecting the reproductive success of 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).

There are a number of estrous detection aids available to assist producers including pressure mount detectors, tail chalk/paint, androgenized cows, and teaser bulls (rendered sterile by vasectomy, epididectomy, or penile deviation). However, the heat watch electronic estrous detection system is the most effective estrous detection aid, which provides precise information on the onset, intensity, and duration of estrus (Rorie *et al.*, 2002).

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 around dusk. 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 estrus behavior. The best time to inseminate a heifer or cow is slightly before the time of ovulation, which occurs approximately 30–38 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).

2.5. Bovine Estrus Synchronization

The history of estrous cycle synchronization and the use of AI in cattle is a testament to how discoveries in basic science can be applied to advance the techniques used for livestock breeding and management. After the discovery ovarian steroids, several scholars described experiments that have been led to the effective control of the length of the bovine estrous cycle and the timing of estrus and ovulation (Patterson *et al.*, 1989). The progression of bovine estrus synchronization is listed below (Table 1).

Table 1. Chronology of developments of estrus synchronization in animal reproduction

Year	Discovery
1927	Hammond reported that removal of the corpus luteum from the cow's ovary is followed within a few days by estrus and ovulation
1929	Corner and Allen isolated and synthesized progesterone
1937	Makepeace and others demonstrated that administration of exogenous progestins to control estrus and ovulation in rabbits
1948	Christian and Casida record the successful synchronization of estrous cycles in cattle by using daily injections of progesterone
1961	Hansel *et al*. first used orally active progestins to synchronize estrus and ovulation in cattle
1966	Dziuk *et al*. Estrus Control in cows by an implanted progesterone
1966	Babcock first suggested that prostaglandin might be a luteolytic agent
1969	Phariss and Wyngarden reported that prostaglandin is luteolytic in rats
1974	Goding demonstrate that PGF is the uterine luteolysis in the ewe.
1974	Roche reported Synchronization of estrus and fertility following artificial insemination in hefiers using PGF.
1974	Cooper did Control of the estrus cycle in heifers with a synthetic cloprostenol
1978	Schams *et al*. reported Profiles of LH, FSH and Progestrone in Postpartum Dairy Cows
1995	Pursley *et al*. done Synchronization of ovulation in dairy cows using PGF2α and GnRH

In Ethiopia, studies on estrous synchronization in dairy cattle started in the late eighties (Tegegne *et al*. 1989; Mukasa-Mugerwa *et al.*, 1989). The first field trial was conducted by the Improving Productivity and Market Success (IPMS) project in Tigray and SNNP regions with the objectives of testing a simple hormonal estrous synchronization regime and mass insemination to improve access to improve dairy genetics by smallholder farmers and to kick-start market-oriented smallholder dairy development in selected sites. Next to field test, the synchronization technology was adopted and scaled up by the MoA and regional BoAs in collaboration with international development partners (IPMS and Livestock and Irrigation Value Chains for Ethiopian Smallholders (LIVES) projects of ILRI) and the national research system. However, the project was incoherent to proceed. Finally, LIVES project initiated action research activities to appraise the 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. Farmers breeding practices and opinions on OSMAI, evaluation of OSMAI were assessed in four (Oromia, Amhara, Tigray and SNNP) regions of Ethiopia by different researcher Gizaw *et al.* (2016).

Table 2. Snapshot of bovine estrus synchronization progress in Ethiopia

Year	Research	Scholar
1989	Fertility of *Bos indicus* and *Bos indicus**Bos taurus Crossbred Cattle after Estrus Synchronization	Tegegne *et al*
1993	Evaluation of luteolysis and Estrus Synchronization Using a Prostaglandin Analog (Prosolvin) in Boran*Friesian Crossbred Heifers in Ethiopia	Tegegne *et al.*
1994	Superovulatory Response, Embryo Recovery and Progesterone Secretion in Boran (*Bos indicus*) Cows after Treatment With either Pergovet or Pluset	Tegegne *et al.*
2007	Estrous synchronization in cross bred and local zebu heifers using GnRH and PGF2α	Haliu
2010	Effect of estrus synchronization with prostaglandin F2α in Ethiopian highland zebu (*Bos indicus*) cows	Mukasa-Mugerwa *et al.*
2015	Effects of Prostaglandin Administration Frequency, Artificial Insemination Timing and Breed on Fertility of Cows and Heifers in Eastern Zone of Tigray Region, Ethiopia	Tadesse
2016	Superovulation of Boran Cattle in Ethiopia: A Preliminary Report	Degefa *et al.*
2016	Effects of genotype and FSH dose on estrus and ovarian response of Boran and Boran*Holstein Friesian Cows in Ethiopia	Degefa *et al.*
2017	Evaluation of estrus synchronization & mass artificial insemination service of dairy cattle in Mizan Aman area, Bench Maji zone	Tegegn and Zelalem

To avoid the problems associated with AI programs utilizing estrus detection, several research groups have developed different strategies to inseminate cows at an appointed time, eliminating the need for heat detection. The first affirmative results of this effort emerged in mid 1990s with the development of the Ovsynch protocol (GnRH-7 days-PGF-48h-GnRH-16h- timed AI) (Pursley *et al.*, 1995). Several methods were designed to control luteal and follicular phases, which permit timed AI (TAI) with satisfactory pregnancy per AI (P/AI). Currently, TAI programs are applied routinely in dairy and beef herds providing a systematic approach to avail AI (Baruselli *et al.*, 2004). Currently, combination of synchronization agents and TAI are exercised by different scholars abroad and in Ethiopia.

2.6. Principles of Estrus Synchronization

In animals, estrus synchronization 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, which result in subsequent estrus and ovulation. The first method involves long-term administration of a progestin to prevent subsequent regression of the corpus luteum (Britt, 1987). Estrus and ovulation occur within 2 to 8 days after progestin withdrawal. Whereas, the rest method involves injection of a luteolytic agent which shortened the normal life span of the corpus luteum. This is accompanied generally with estrus and ovulation within 48 to 120 hrs after injection (Murugavel *et al*., 2004). Therefore, Synchronization of estrus in cows is feasible by either prolonging or shortening of corpus luteum lifespan in dioetrus phase.

2.7. Management Considerations for Synchronization

The success of an estrus synchronization program is largely based on understanding the bovine estrous cycle, the biological actions of estrus synchronization products (progestins, PGF2α, and GnRH), 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 (body condition score of 3-4 in scale 5). With attention to detail and adequate feed, these programs can work effectively (Hopkins and Schrick, 2012).

Cows and heifers should be selected based on the following criteria. These are 1) Adequate time has elapsed from calving to the time synchronization treatments (a minimum of 40 days postpartum at the beginning of treatment is suggested), 2) average or above average body condition score (at least 3-4 on a scale of 1 to 5), 3) with minimal calving problems, 4) replacement heifers are developed to pre-breeding target weights that represent at least 65% of their projected mature weight; and 5) and reproductive tract scores (RTS) 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). Age at puberty is affected by a variety of factors, including genotype, body weight, nutrition, social environment, and season. Furthermore, replacement heifers should not receive growth-promoting implants since implants administered within 30 days of birth may impair normal development of reproductive organs in growing heifers. At weaning, older heifers should be selected as potential replacement females and each heifer should attain 65% of their mature body weight before breeding and 85% prior to first calving (Smith *et al.,* 2006).

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 will contribute to cash costs of synchronization. Estimated savings from fewer bulls needed for natural service and increased returns from age and weight of AI sired calves should be considered. Producers that find AI most cost effective due to capture of additional returns from AI sired calves (BRTF, 2006).

2.8. Approaches of Synchronization Protocol

Improvement of estrus synchronization protocols in cycling animals has involved the following approaches: 1) Obstruct ovulation following spontaneous CL regression (long-term progestin insertion), 2) Induction of corpus luteum regression (prostaglandin injection), and 3) a combination of 1 and 2. Most the current progress utilizes the third approach. The first approach requires long-term progestin insertion (7 to 14 days) and is effective at synchronizing estrus; however, fertility at the synchronized estrus is frequently reduced due to the occurrence of persistent follicles. 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 a dominant follicle and synchronize a new follicular wave (Smith *et al.,* 2006).

2.8.1. Prostaglandin based estrus synchronization protocol

Prostaglandin F2α and its analogs were conveyed in 1972 to have a luteolytic effect in bovine (Lauderdale, 1972). Identification of synthetic PGF analog for uterine luteolysis was quickly followed by the commercialization of a natural synthetic form (Lutalyse) (Hafs *et al*, 1975) and potent analogues (Estrumate) (Cooper, 1974). Injection of PGF and its analogs reduced luteal phase by prompting luteolysis of CL. Luteolysis consists of functional and structural regression of the corpus luteum. Functional luteolysis is characterized by a decline in secretion of progesterone (P4) (McCracken *et al.,* 1999). Luteolysis is temporally associated with an increase in estrogen concentration (Ginther *et al.,* 2010). This meant that a treatment was only effective in the presence of a functional CL from Day 7 to Day 17 of a normal estrus cycle (Estrus at Day 0). Conversely, this method is ineffective in anestrous females and variation among animals in the stage of the follicular wave at the time of PG injection directly contributes to the variation in onset of heat during the synchronized period (Macmillan and Henderson, 1984).

Prostaglandin F2α regulates a female's estrous cycle by causing regression of the CL. Synchronized regression of the CL will synchronize a decline in progesterone and result in the final growth of the DF to produce estradiol and behavioral heat. Females with a mature CL on their ovaries when they receive PGF treatment will usually exhibit heat two to five days later. Thus, in order for PGF to be effective, females must be cyclic and in a responsive phase of the estrous cycle. Prostaglandin will not regress an immature CL (Days 1 to 5), and the effectiveness of this treatment increases with each subsequent day of the cycle after Day 5. Prostaglandin F2α also has no effect after the CL has started to regress (Day 17+). Anestrous cows and prepubertal heifers will not respond to an injection of PGF since no CL exists (Day and Geary, 2005).

One shot injection prostaglandin system is used where heat detection is experienced and drug costs are a concern. If less than 3% of cows can be found in heat per day before injection, abandon the idea. Sixty to seventy percent of cows will be bred AI using this system. Whereas, two shot injection prostaglandin system is used to bring more cows in heat during the AI period (90%). Heat detection must be practiced. If extended heat detection is not a concern and drug costs are a problem, then animals can be heat checked and bred after the first injection of PG. Animals not showing heat following the first injection would then be injected a second PG injection 11-14 days later and then bred (Hopkins and Schrick, 2012).

2.8.2. Progestin based estrus synchronization protocol

The principle of injecting P4 and its analogs is to extend the normal estrus cycle by extending the period of diestrus (Macmillan, 2010). Exogenous administration of P4 suppresses LH release, alters ovarian function, suppresses estrus and prevents ovulation in cattle (Adams *et al.,* 1992). Both P4 and progestins have been incorporated to the estrus synchronization protocols in cattle via oral route such as melangestrol acetate (Patterson *et al.*, 1989) or by insertion of intravaginal P4 device or progestin ear implants (Martinez *et al.,* 2000). Progesterone based TAI protocols have incorporated an inducer of ovarian follicular wave emergence at the beginning of the protocol with exogenous P4/progestin source which is normally removed after 7, 8 or 9 days (Baruselli *et al.,* 2004). Due to the emergence of a new follicular wave during the protocol and the short treatment period, the incidence of persistent follicles is reduced and fertility after TAI is good following AI upon estrus detection (Santos *et al.,* 2009).

The initial research using orally active synthetic progestogens incorporated the use of medroxyprogesterone acetate (sold as Repromix) as a synchronization tool in the 1960's and led to the first results of successful synchronization of estrus in beef cows (Hansel *et al.,* 1961). Melengestrol acetate is an orally active progestin. When consumed by cows or heifers on a daily basis, MGA will suppress estrus and inhibit ovulation (Imwalle *et al.,* 2002). Melengestrol acetate fed at a rate of 0.5 mg/animal/day in a single daily feeding. The duration of feeding may vary between protocols, but the level of feeding is consistent and critical to success. Animals that fail to consume the required amount of MGA on a daily meal may prematurely return to estrus during the feeding period. This is expected to reduce the estrous response during the synchronized period. Therefore, adequate bunk space (60 linear cm/head) must be available so that all animals consume feed at the same time (Patterson *et al.,* 2003).

Control internal drug release (CIDR) is an intravaginal progesterone insert, used in combination with other hormones to synchronize estrous in beef and dairy cows and heifers. The CIDR was developed in New Zealand and has been used for several years to advance the first pubertal estrus in heifers and the first postpartum estrus in cows. The CIDR is a "Y" shaped device with flexible wings that collapse to form a rod that can be inserted into the vagina with an applicator. On the end opposite to the wings of the insert a tail is attached to facilitate easy removal. The backbone of the CIDR is a nylon spine covered by progesterone (1.38g) impregnated silicone skin. Upon insertion, blood progesterone concentrations rise rapidly, with maximal concentrations reached within an hour after insertion. Progesterone concentrations are maintained at a relatively constant level during the seven days of CIDR insertion in the vagina. Upon removal, progesterone concentration is quickly eliminated (Lamb *et al.,* 2006).

2.8.3. GnRH based protocol

Gonadotropin releasing hormone (GnRH) controls the follicular phase of the estrous cycle. Follicles grow in wave-like patterns, with each estrous cycle consisting of two or three follicular waves. The dominant follicle of each of these waves is capable of ovulating (releasing of ova). Gonadotropin releasing hormone is a naturally occurring hormone that induces a luteinizing hormone (LH) surge, which causes ovulation of the dominant follicle even in the presence of progesterone. During an estrous cycle with three follicular waves, there are three periods when a dominant follicle is present and can be induced to ovulate with an injection of GnRH. When a follicular wave is developing and a dominant follicle is not present an injection of GnRH will have no effect (Salverson and Perry, 2005).

A single injection of GnRH or its analogues (Buserelin, Cystorelin) to cows at random stages of their estrous cycles causes release of luteinizing hormone leading to synchronize ovulation or luteinization of most large dominant follicles (≥ 10 mm) (Sartori *et al.,* 2001). Consequently, a new follicular wave is initiated in all cows within 2 to 3 days of GnRH administration. Luteal tissue that forms after GnRH administration is capable of undergoing PG-induced luteolysis 6 or 7 days later. GnRH stimulates the release of both FSH and LH, causing ovulation of dominant follicles, leading to the formation of a CL and stimulation of further follicular development GnRH can be used to assist the timing of ovulation in relation to insemination. Other hormones are available for inclusion in the various programs. Many hormone injections require administration via a deep intramuscular injection. It is important to follow manufacturers' instructions carefully (Vickers, 2014).

2.8.4. Combination protocols

When PGF, GnRH, or progestins used alone, they will only synchronize either the luteal or follicular phases of the estrous cycle. Therefore, most estrous synchronization protocols combine the above methods to control both phases of the estrous cycle.

Numerous new synchronization protocols currently recommended for cows use gonadotropin-releasing hormone (GnRH) in combination with PGF. A naturally occurring hormone, GnRH is more popularly known by the commercial brand names of Cystorelin, Factrel, and Fertagyl. Each GnRH-based protocol uses the same basic framework, which involves an injection of GnRH followed 7 days later with an injection of PGF. The way animals are subsequently handled for heat detection and breeding is where the protocols begin to vary. To understand the benefits of GnRH-based synchronization protocols and how they work, first understand the concept of follicular waves in cattle (De Jarnette, 2004).

Ovsynch: The primary synchronization of ovulation protocol, titled Ovsynch 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 Ovsynch allows for satisfactory pregnancy rates without heat detection, it does not necessarily eliminate the need for heat check. Ovsynch-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 Ovsynch protocol (Geary *et al.,* 2000).

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).

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).

2.9. Factors Affecting Synchronization in Early Post-Partum Period

Body condition: Body condition score is an internationally accepted, subjective visual and tactile measure of body condition and temporal changes in BCS are used to monitor nutritional and health status of high producing cows during their productive cycle (Berry *et al.*, 2007). Low body conditioned cows at calving, which further suffer excess BCS loss early postpartum, are less likely to ovulate, at the end have reduced submission rate to AI, conception rate to first service, 6-week in-calf rate and also have an increased likelihood for pregnancy loss and increased calving to conception interval (Berry *et al.*, 2007). This can partly be attributed to impair oocyte competence associated with a low BCS (1.5–2.5; 5-point scale) (Snijders *et al*., 2000). Fertility in cows that are over conditioned at calving is also compromised as they have reduced dry matter intake (DMI) just prior to calving, take longer to increase DMI postpartum, tend to have greater fat mobilization and therefore a more severe NEB early postpartum than cows with an optimum BCS at calving (Roche, 2006).

Body condition score (BCS) reflects the nutritional status of the herd and hence used as a criterion to measure the response to hormonal treatment to stimulate the resumption of ovarian activity in weaned cows when the negative effects of suckling has been suppressed (Fernando *et al*., 2013). Blood glucose concentrations have a positive relationship with GnRH production by the hypothalamus. Even though, protein, minerals or vitamins have an equally significant role, most nutritional work has focused on energy as the limiting nutrient in establishing estrous cycles in the postpartum cow (Hill *et al*., 2014). Dairy cattle in negative energy balance influences follicular growth (Wiltbank *et al*., 2002) and the size of the ovulated follicle (Armstrong *et al*., 2001). A nutritional deficiency can affect the diameter of the dominant follicle and therefore ovulation (Diskin *et al*., 2003).

Nutrition: it is crucial for reproductive performance of cattle. Chronic dietary energy deficits as well as energy surpluses has a detrimental impact on reproductive capacity modulating the hypothalamic GnRH neuronal network and/or the pituitary gonadotropin secretion (Garcia-Garcia, 2012). Insufficiency in energy intake leads to loss of body condition, extended periods of anovulation, postpartum anestrus, and infertility. The metabolic condition of cows in NEB shifts to catabolic metabolism, which in turn causes increased plasma growth hormone (GH) and NEFA concentrations, decreased plasma IGF-I, insulin, and glucose as well as leptin serum concentrations (Do and Taylor-Robinson, 2015). Resumption of ovulatory cycles is connected with regaining of energy balance and the underlying mechanisms seem to be associated with metabolic signals and regulatory hormones primarily insulin and IGF-I, which link nutritional status with gonadotropin secretion, re-coupling of the GH-IGF system, and follicle maturation and ovulation. Feeding diets that promote increases in plasma glucose and insulin may improve the metabolic and endocrine status of cows in early lactation (Garcia-Garcia, 2012).

The effects of nutrition on reproduction are either mediated 1) directly via alterations in GnRH secretion at the level of the hypothalamus or on release of gonadotrophs (LH and follicle stimulating hormone; FSH) from the anterior pituitary or 2) indirectly through changes in metabolic hormones such as insulin, growth hormone (GH), insulin-like growth factor (IGF) - I or II and its binding proteins, and leptin. These metabolic hormones signal nutritional status and can alter follicular growth, oocyte quality, and subsequent embryo survival. Furthermore, these metabolic hormones can affect the sensitivity of the developing follicle to the gonadotrophs and thus, potentially impact follicular steroid production and that of the subsequent CL (Bridges *et al*., 2012).

Age: Heifers should have reached 24 months and above ages to conceive and give birth to their first calf and more productive in their rest of lives. Thus, a heifer must become pregnant at 15 months of age. In treated and control synchronization groups, the conception rate was higher in younger cows than older one. However, Ferdousi and Khan (2013) observed that older cows i.e. 9 years old Holstein X Local cows showed a higher conception rate than the younger cows. This difference might be due to the effect of flushed feed and age. Coach (2010) found that the nutrition has the positive effect on conception rate. Duration of postpartum anestrus averages 20 days longer for first-calf heifers than mature cows (Troxel and Whitworth, 2007). Induction of ovulation was limited in 2-year-old cows until body condition scores were ≥5.0 (9-point scale). In older cows, induction of ovulation increased linearly with increasing body condition (Perry *et al*., 2004).

Season: A seasonal effect has been detected on estrous behavior. Several study concluded that the length of estrus was greater in summer compared to winter or spring; moreover, cows were mounted more frequently per estrus in winter compared to summer or spring. Therefore, estrous detection may need to occur more frequently in winter compared to spring or summer; whereas, in summer estrous detection may need to occur for a longer duration at each check (White *et al*., 2002). On the contrary, others disagreed on this that there was no effect of season on the interval from the onset of estrus to ovulation at a mean of 31 hrs. In Florida, an increase in the temperature-humidity index (THI) decreased the number of mounts per estrus (Landaeta-Hernandez *et. al*., 2002).

Heat stress exacerbates the effects of NEB. During the time heat stress, lactating cows have a reduced appetite and higher BCS loss early postpartum compared to non-heat exposed cows. Furthermore, concentrations of glucose, IGF-I and cholesterol are lower, while concentrations of NEFA and urea are higher in blood and follicular fluid of heat stressed animals (Shehab *et al*., 2010). These changes, along with a decrease in dominant follicle diameter, and coupled with a more severe NEB in heat stressed cows make achieving high reproductive efficiency in subtropical and tropical climates a greater challenge. Thus, this highlights the importance of monitoring body condition score pre- and postpartum as an aid to nutritional and management decisions in order to ensure a mild, but not severe NEB occurs early postpartum and to minimize its carry-over effects into the remainder of the lactation (Roche, 2006; Chagas *et al*., 2007). It is also important to note that partitioning of nutrients is under genetic control; hence different nutritional and management strategies are required for individual animals. Recent reviews have expanded on different nutritional strategies to optimize BCS at critical stages of the productive life of the dairy cow and should be referred to for a more comprehensive analysis (Chagas *et al*., 2007).

Parity: parity effect is still controversial in the resumption of the ovarian cycle. 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). Several authors reported higher conception rates in primiparous than in multiparous cows with Ovsynch and TAI (Cartmill *et al*., 2001; Tenhagen *et al*., 2001; Peters and Pursley, 2002). Whereas, other studies did not demonstrate this effect (Jobst *et al*., 2000; Klindworth *et al*., 2001).

Follicle size at TAI in primiparous cows producing smaller follicles than multiparous cows. Acceptable pregnancy rates by fixed-time AI (FTAI), or breeding by appointment without checking detecting estrus, are possible in beef cows; whereas, it is inconsistent in dairy heifers (Patterson *et al*., 2007). This has been credited to an incapability to synchronize follicular waves in heifers with the same degree of success that has been achieved in cows (Lamb *et al*., 2006).

2.10. Impact of Estrus Synchronization

2.10.1. Advantage of estrus synchronization

Synchronizing estrus is crucial to minimize labor and time cost for detection of heat for artificial insemination. Furthermore, estrous synchronization can also benefit overall herd management. In general synchronization has the following importance; 1) exhibition standing estrus at a predicted time, 2) conceive earlier in the breeding season, 3) calve earlier in the calving season, and 4) wean calves that are older and heavier at weaning. In addition, some estrous synchronization protocols (progestin-based protocols) can induce a proportion of anestrous cows to begin estrous cycles. This will decrease the anestrous postpartum interval and allow for more chances for cows to conceive during a defined breeding season. A study conducted at Colorado State University indicated cows that conceived to a synchronized estrus calved on average 13 days earlier and weaned calves 41 pounds heavier than cows that were not synchronized (Schafer *et al.,* 2007).

2.10.2. Disadvantages of estrus synchronization

The expense of hormones and requirement of high level of management are the major limitation of synchronization. In addition, synchronization requires cycling cows at good body condition. Moreover, it requires labor and time during handling animals for drug injection and heat detection compared to opening gate and letting the bull to mate cows and heifers naturally. Furthermore, one can only synchronize the number of cows that can be inseminated at one time (Hopkins and Schrick, 2012).

2.11. Fixed Time Artificial Insemination (FTAI)

In cattle, estrus synchronization and artificial insemination (AI) availed to exploit the reproductive potential of cows by incorporating superior genetics (Leitman *et al*., 2009). Artificial insemination (AI) promotes genetic and economic gains through the use of superior genetic bulls. Despite the technological advances of AI programs, the application of AI programs based on estrus detection is hampered by postpartum anestrous and estrus detection (ED) failure (Bó *et al.*, 2007).

Artificial insemination is the most widely applied tool facilitating extensive utilization of frozen semen from genetically superior sires, cryopreservation has been an invaluable technique. In order to extend the time span of spermatozoa viability, their metabolic rate has to be slowed down thereby reducing the rate at which substrates are used and toxins are produced. As a general rule cooling of spermatozoa is the simplest method that can successfully depress spermatozoal metabolic rate and prolong sperm survival. The use of carbon dioxide and other metabolic inhibitors like proteinase inhibitors are also known to produce a similar but less successful effect (Colenbrander *et al.*, 2003; Cremades *et al.*, 2005).

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in Boran and Zebu-Holstein crossbred dairy cows in dairy farms of Arsi University (Asella) from December 2017 to June 2018. Asella town is the capital of Arsi zone, Oromia regional state. It is located about 175 km Southeast of Addis Ababa at 6° 59' to 8° 49' N latitude and 38° 41' to 40° 44' E longitude. The altitude of the area ranges from 2500 to 3000 m.a.s.l. The minimum and maximum temperature ranges from 8.4 to 22.6°C, and the relative humidity ranging from 43 to 60%. The average rainfall is 2000 mm (KARC, 2008).

3.2. Experimental Animals

Boran and Zebu-Holstein crossbred dairy cows owned by Arsi University were the study population. Lactating cows which has been 60 days in milk (DIM) and above without any previous history of assisted calving, endometritis, retained placenta, laminitis, and without any recent systemic diseases and have not been in estrus were included in the study. The reproductive tracts of all the selected cows were examined by manual palpation per rectum and as well as trans-rectally by ultrasound to confirm the absence of utero-ovarian pathology. After thorough reproductive examination, only cows free of reproductive abnormalities were included into the study. Finally, out of 100 dairy cows found in Arsi dairy farms, free from any abnormalities in ultrasound and history from records with postpartum greater than or equal to 60 were 40 animals (20 Boran and 20 Zebu-Holstein crossbred) dairy cows

Body condition score (based on a five-point scale: 1=thin to 5=fat) of all animals were determined at the time of enrolment according to Edmonson *et al.* (1989). Animals with body condition scores 2.75 to 3.75 were included. Cows were classified as having a low to moderate (BCS from 2.0 to less than 3.0) or good (BCS ≥ 3) (Sá Filho *et al*., 2011).

Table 3. Summary of experimental animals

Treatment Groups	**Breed**		**Total**
	Boran	Zebu-Holstein crossbred	
Ovsynch	9	11	20
Co-synch	8	7	15
Primiparous	10	9	19
Multiparous	7	9	16
Low to Moderate (<3)	6	9	15
Good (≥3)	11	9	20
Total	17	18	35

3.3. Management of Animals

The cows were housed in free-stall barns provided with shade and standings with adequate floor space and good ventilation. Most of the houses were made of wood, the floor was made of concrete. All cows were turned out for grazing for six hours daily. Approximately 3-4.5 kg roughages and 1.5-2.5 kg concentrates were given daily to each animal as supplement. The concentrate feed consisted of wheat bran, oil seed cakes (by product of oil extraction), molasses and salt. Lactating cows were milked manually twice daily at an interval of 8-9 hours. All animals were tagged with long plastic ear tags for identification.

3.4. Study Design

The study design was completely randomized block design and cows were first divided into two based on breed (Boran and Zebu-Holstein crossbred). Cows were blocked by parity and body condition and were randomly assigned to either Ovsynch or Co-synch protocol. The parameters evaluated include ovulation rate, conception rate, P4 level, size of follicle and CL.

3.5. Synchronization Protocols and AI

On day of start ovaries were scanned (using a real time B-mode ultrasound scanner, Mindray, China), for follicular size and CL. Then all cows received 100µg (2.5 ml Buserilin acetate) (Zydarelin®, Zydus Animal Health, Cadila Healthcare Ltd., Ahmedabad, India) intramuscularly on day 0 (day of start) and 500µg (2 ml cloprostenol) (PGF analog) i/m (Synchromate®, Bremer Pharma GMBP, Warburg, Germany) on day 7. Prior to PGF2α injection at day 7, the size of CL and follicles were measured and recorded. Then cows were randomly assigned into two groups: Ovsynch group (n=20) second dose of 100µg (2.5 ml Buserilin acetate) i/m on day 9 and timed-AI on day 10 (approximately 16–17 hours after the second GnRH) (Pursley *et al.*, 1995) (Fig 2); Co-synch (n=15) second dose of 100µg (2.5 ml Buserilin acetate) i/m on day 9 and timed-AI on the same day of the second GnRH) (Geary *et al.*, 2001) (Fig 3). Five animals showed estrus immediately after PGF injection (within 8-9 hours) on day 7 and were excluded from the experiment and inseminated following the am-pm rule to satisfy the interest of the farm.

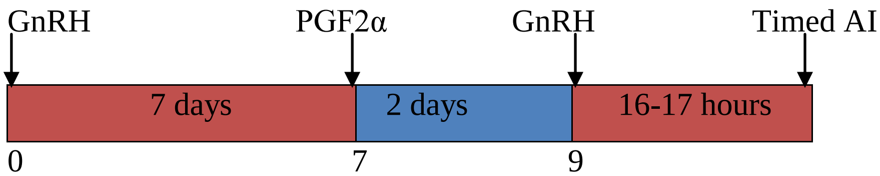


Figure 2. Ovsynch synchronization protocol

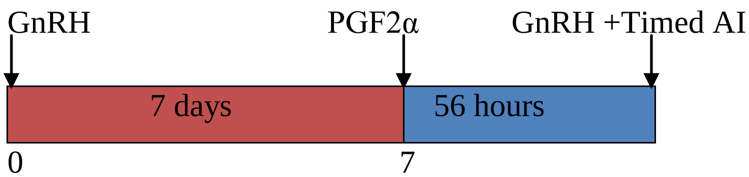


Figure 3. Co-synch synchronization protocol

Timed artificial insemination was conducted by the same technician and all the semen used were the same batch brought from the National Artificial Insemination Center, Kality, Ethiopia.

Generally, responses were evaluated through measuring ovulation rate, luteolysis rate and conception rate to different synchronization protocols.

3.6. Data Collection and Sample Processing

3.6.1. Detection of ovulation

To detect ovulation ultrasonographical examination were conducted after second GnRH treatment prior to AI and continued till day 11 twice a day in the morning at 3 o'clock and afternoon at 9 o`clock to confirm ovulation. Ovulation was identified when disappearance of a previously identified dominant follicle ≥9 mm was no longer observed during ultrasound examination (Ginther *et al.,* 1989; Pursley *et al.*, 1995). Persistence was defined as no disappearance of the dominant follicle (Pursley *et al*., 1995).

3.6.2. Pregnancy diagnosis

Dairy cows were checked for conception by day 21 using progesterone assay and at day 30 by ultrasonography post TAI. Conception rates were calculated by dividing the number of cows that were conceived to those inseminated in protocol

3.6.3. Progesterone assay

The luteolytic capacity of PGF2α was evaluated via progesterone analysis. Cows with concentrations of serum progesterone ≥1.0 ng/mL immediately before the PGF2α injection and then <1.0 ng/mL 48 hours later were considered to have luteolysis (Oyedipe *et al*., 1986).

Blood samples were collected in vacuum puncture of jugular vein just before administration of GnRH (day 0), Day 5, prior to PGF2α (day 7) and immediately before insemination (day 9 for Ovsynch and for Co-synch), day 15 and day 21 via 10 ml silicone plain tube by 20G Vacutainer needles. Then the samples were clearly labeled and transferred to laboratory. The samples were placed at room temperature till the serum started to separate. Then the samples were centrifuged at 2500 rpm for 6 minutes (Chen, 2014) Finally, Serum was harvested, frozen and stored in cryovials at−20°C in deep fridge until progesterone analysis was conducted.

The concentration of progesterone (P4) in the serum was measured in all samples by radioimmunoassay using a commercial kit (Roche Diagnostics Gmbh, Mannheim, Germany). All progesterone assays were performed at Ethiopian Community Health Institute, Clinical Chemistry Laboratory. The kit has lower and upper range of 0.030 ng/ml and 60 ng/ml respectively. The functional sensitivity of the assay was 0.15 ng/ml. A concentration higher than 1 ng/ml was considered to indicate the presence of a functional CL. Corpus luteum regression was defined by a cow having a serum P4 concentration of >1 ng/ml, and then declining to a level of <1 ng/ml (Oyedipe *et al*., 1986).

3.7. Data Management and Analysis

Data were collected, coded and entered in Microsoft excel spreadsheet and was analyzed in STATA (version 13). Dichotomous data such as ovulation rate and conception rate were analyzed using chi-square (X^2) test. Percentage was used to estimate amount of animals ovulated, conceived. In addition, a t-test was used to compare groups for follicle and CL diameters within the examination dates and were presented in mean (±S.E.M). Serum progesterone profile for Boran and Zebu-Holstein crossbred was sketched in scatter plot (with Error margin). Statistical differences between means and the association between response and explanatory variables were determined at (P <0.05).

4. RESULTS

4.1. Induced Ovulation Rate to GnRH-PGF-GnRH Treatment

This experiment was designed to evaluate factors affecting ovarian response following Ovsynch and Co-synch synchronization protocols in Boran and Zebu-Holestien crossbreds. The overall induced ovulation rate after second GnRH was 85.7%, of which 88.2% in Boran and 83.3% was in Zebu-Holstein crossbred dairy cows. Regarding to synchronization protocols, induced ovulation rate was 90% in Ovsynch and 80% in Co-synch. Ovulation rate was not associated with any of the factors considered in the study (Table 4).

Table 4. Effect of factors affecting ovulation rate

Factors		**No. examined**	**Ovulated**	**percent (%)**	X^2	**P-value**
Protocol	Ovsynch	20	18	90	0.700	0.403
	Co-synch	15	12	80		
Breed	Boran	17	15	88.2	0.1716	0.679
	Zebu-Holstein cross	18	15	83.3		
BCS	Lower-moderate (<3)	15	12	80	1.6762	0.195
	good (≥3)	20	18	90		
Parity	Primiparous	19	18	94.7	2.7632	0.096
	Multiparous	16	12	75		
Total		35	30	85.7		

4.2. Conception rate to Ovsynch and Co-Synch Protocol

The overall conception rate to TAI was 60%, while the conception rates for Ovsynch and Co-synch protocols were 55% and 66.7%, respectively. Conception rate was higher in primiparous (73.7%) than multiparous (43.8%) ($P < 0.05$). The details of conception rate in response to breed, synchronization protocol and body condition, were indicated (Table 5).

Table 5. The effect of breed, protocol, BCS and parity on conception rate

Factors		No. examined	Conceived	Percent (%)	X^2	P-value
Protocol	Ovsynch	20	11	55	0.4861	0.486
	Co-synch	15	10	66.7		
Breed	Boran	17	12	70.6	1.5441	0.214
	Zebu-Holstein cross	18	9	50		
BCS	Low-moderate (<3)	15	9	60	0.0000	1.000
	Good (≥3)	20	12	60		
Parity	Primiparous	19	14	73.7	3.867	**0.0471**
	Multiparous	16	7	43.8		
Total		35	21	60		

4.3. Follicular Size after GnRH Treatment

The mean (±SEM) follicular size was measured at day 7 after first GnRH treatment, the mean (±SEM) diameter of larger follicle was 9.99±0.92 mm in Boran and 10.89±1.43 mm in Zebu-Holstein crossbred dairy cows. There was no statistical significant difference (P>0.05) between mean(±SE) diameter of follicle and breeds. The mean(±SEM) diameter of larger follicle in different body condition and parity is presented below (Table 6).

Table 6. Follicular diameter of Boran and Zebu-HF crossbred dairy cows after GnHR

Factors		No. examined	Mean ± SEM	95% CI	t-value	p-value
Breed	Boran	17	9.99±0.92	8.00- 11.97	-0.5447	0.5917
	Zebu-Holstein cross	18	10.89±1.43	7.49- 14.28		
BCS	Low to moderate (<3)	15	10.16± 1.31	7.06-13.27	-3.354	**0.047**
	Good (≥3)	20	13.24± 0.98	8.272-14.78		
Parity	Primiparous	19	10.25±1.13	7.77-12.72	-0.0721	0.9432
	Multiparous	18	10.36±1.09	7.92- 12.80		

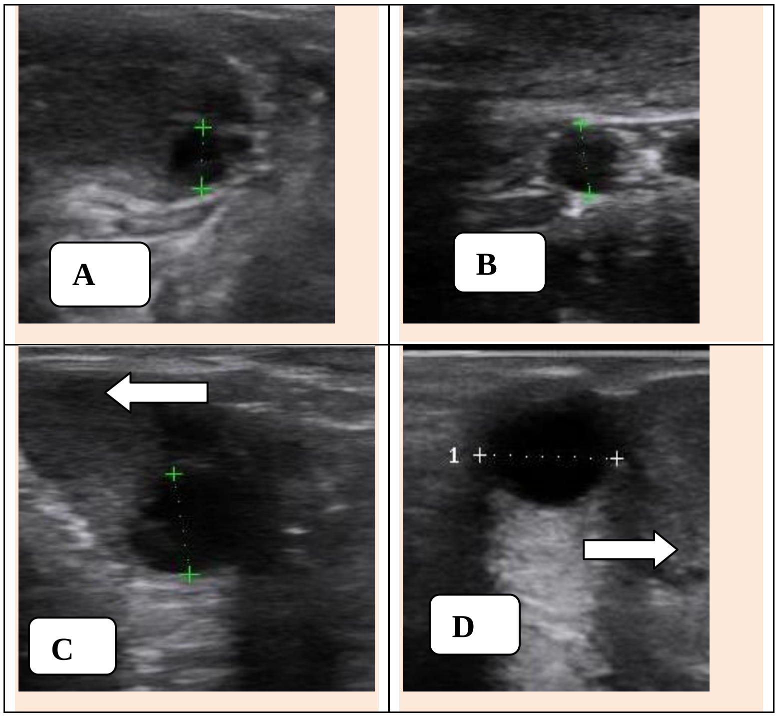


Figure 4. Image of ovarian follicle at day 0 (A, B) and day 7 (C, D). Note that CL at day seven (white arrow)

4.4. Factors Influencing Diameter of CL

In regard to body condition, mean (±SEM) diameter of CL was 11.84±0.93mm in dairy cows with low to moderate body condition score (<3) and 18.3±1.31mm in those with good body condition score (≥3) prior to PGF injection, the effect was statistically significant (P<0.05). Details of the effect of several factors affecting the mean (±SEM) of CL were measure at day 7 prior to PGF administration (Table 7). Images of Cl was indicated (Fig. 5).

Table 7. Factors affecting the mean (±SEM) diameter of corpus luteum

Factors		No. examined	Mean ± SEM	95% CI	t-value	p-value
Breed	Boran	17	16.99±1.69	13.32-20.66	1.6741	0.0535
	Zebu-Holstein cross	18	13.65±1.08	11.30-15.99		
BCS	Low to moderate (<3)	15	11.84±0.93	9.80-13.89	-3.8944	**0.0007**
	Good (≥3)	20	18.3±1.31	15.47-21.13		
Parity	Primiparous	19	14.69±1.38	11.75-17.64	-0.7581	0.4558
	Multiparous	18	16.32±1.57	12.77-19.87		

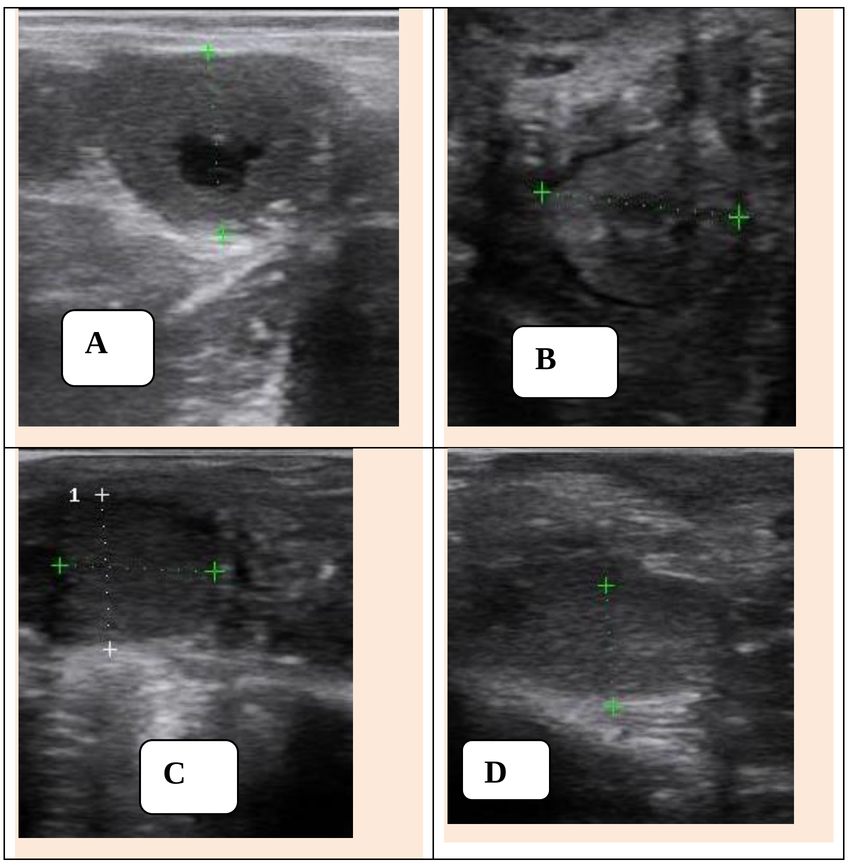


Figure 5. Image of CL (with lacuna in (A and B), without lacuna in (C and D) in day 7 of treatment

4.5. Serum Progesterone Concentration

Serum progesterone level were measured in Boran and Zebu-Holstein crossbred at day 0 prior to GnRH, day 5 after first GnRH, day 7 prior to PGF and day 9 after PGF injection. Based on the result, luteolysis was happened in 81.5% (22/27) of the animals after 48 hours of PGF injection as P4 level reduced to less than 1 ng/ml. Details on serum p4 level in day 0, 5, 7 and day 9 in Boran and Zebu-Holstein crossbred are shown below (Table 8).

Table 8. Effect of Exogenous PGF on Corpus Luteum regression

P4 level		No. examined	Boran	Zebu-Holstein cross	Percent (%)
P>1ng/ml	Day 0 (no.)	35	5	7	34.3
	Day 5 (no.)	35	8	7	42.9
	Day 7 (no.)	35	15	12	77.1
	Day 9 (no.)	27	2	3	14.3
Luteolysis (no.)	-----	27	13	9	81.5
Incomplete Luteolysis	----	27	2	3	18.5

Serum P4 level of day 0, 5, 7, 9, 15 and 21 was presented in scatter plot with line for Boran and Zebu-Holstein crossbred dairy cows. Note that progesterone at day 9 (day of AI) the P4 level was at lost point. Serum progesterone profile was sketched and presented below (Fig. 6).

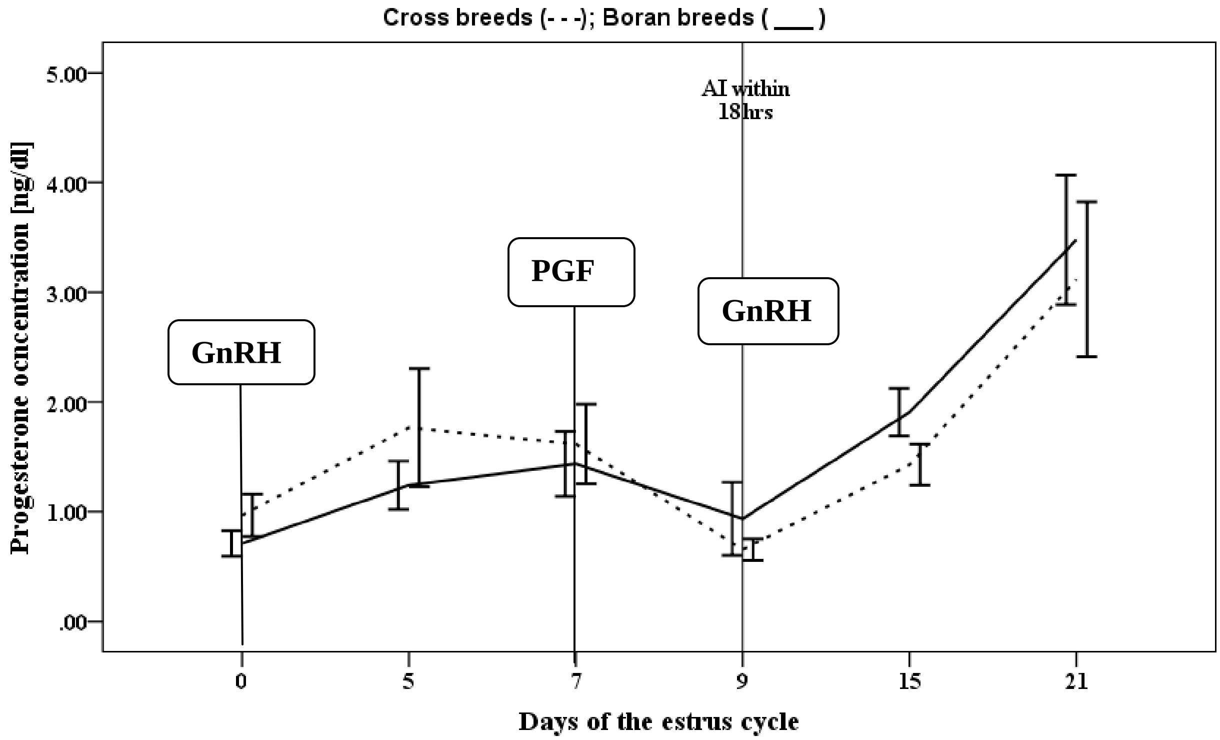


Figure 6. Serum progesterone profile of Boran and Zebu-Holstein crossbred dairy cows

5. DISCUSSION

The present finding revealed that ovulation rate was not influenced by parity (*P*<0.05). This is supported by the former work of Tenhagen *et al*. (2003) who also reported that parity did not affect ovulation. Whereas, this is in contradiction with the previous report of Lalman *et al.* (1997) who concluded that primiparous dairy cows has lower ovulation rate than multiparous *Bos taurus* x *Bos indicus* dairy cows. Lalman *et al.* (1997) justified that primparous dairy cows are still growing which affects ovarian activity comparing with multiparous dairy cows. Late onset of cyclicity is common in primiparous than multiparious cows (Huszenicza *et al*., 1987). This difference might be attributed to selection of animals with longer voluntary period (post-partum) a minimum of 65 days and above in our study might be enough for ovarian resumption in both primiparous and multiparous dairy cows.

Regarding to body condition, ovulation was not affect by body condition (*P*>0.05). This finding disagrees with the previous study by Diskin *et al*. (2003) who reported that a nutritional deficiency can affect the diameter of the dominant follicle and therefore ovulation. Body condition can be used as an indirect measure of nutritional status, and nutritional status influences estrous cycling status of suckled beef cows (Oyedipe *et al*., 1986). The difference might be due to insufficient sampled animals for quantifying the effect body condition on ovulation.

The effect of body condition on conception rate was evaluated. The effect was not statistical significant (*P*>0.05). This finding is in agreement with the previous findings of Perry *et al.* (2003) and Navanukraw *et al*. (2004) who also did not find influence of body condition on conception rate in synchronization protocol with TAI. Whereas, this finding disagrees with the former work of Moreira *et al*. (2000), Pancarci *et al*. (2002), Patton *et al*. (2007), Ciptadi *et al*. (2012), Tazangi and Mirzaei (2015) who reported that body condition influenced conception rate where they concluded conception was higher in high body condition score than low BCS animals. The reason for higher response to GnRH in cows with high BCS was attributed to an earlier postpartum resumption of cyclicity (Moreira *et al*., 2000). Low BCS has low fertility due to negative energy balance which is associated with low estrogen and LH secretion, low glucose level, low insulin and IGFI secretion, and that part of the low fertility syndrome could be related to low secretion of progesterone as well (Butler, 2000). The lack of association with BCS in the present study might be due to not too much variation in body condition (having medium and good BCS) of the experimental animals involved in the study.

Regarding to parity, **c**onception rate was higher in primiparous than multiparous dairy cows. The effect was statistical significant (*P*<0.05). This finding is supported by Tenhagen *et al*. (2003), Quintela *et al*. (2004), Paul *et al.* (2011), Grimard *et al.* (2013), Potdar *et al*. (2016) who confirmed that parity has a significant effect on conception rate in dairy cows where they concluded that conception rate was higher in primiparous than multiparous cows. While, this finding disagrees with previous findings of Diskin (1996), Wathes *et al*. (2001), Williams *et al*. (2002) who reported that conception rate was higher in multiparous dairy cows. Higher conception in primiparous cows 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, 2000).

Concerning the effect of breed, though conception rate was higher in Boran than Zebu-Holstein crossbred, the effect was insignificant (*P*>0.05). This finding disagrees with previous study of Khan *et al*. (2015) reported that conception rate was highest in native cattle and in Friesian cross and the lowest in Sahiwal cross, Rao *et al*. (1992), Marongiu *et al*. (2002) observed higher conception rate in indigenous cows than cross and exotic genotypic group. Studies which recorded high conception in indigenous breeds and crossbreds explained it with adaptability of these cattle to environmental conditions. Environmental and physiological adaptability of Friesian needs more attention in tropical than in temperate conditions (Usman *et al*., 2013; Potdar *et al*., 2016).

Concerning synchronization protocols, the overall conception rate was 60%, of which 55% and 66.7% in Ovsynch and Co-synch synchronization protocols, respectively. The result of

Ovsynch was higher than 35-40% reported by Cordoba and Fricke (2002), EL-Zarkouny *et al*. (2004), El-Tarabany and El-Tarabany (2015) and 30 – 45% by Alnimer *et al*. (2002), Bello *et al*. (2006), Galvao and Santos (2010) after synchronization with Ovsynch protocol in dairy cows. In addition, the current result of Co-synch is greater than previous findings of 33-49% reported by Stevenson *et al*. (2000), Lamb *et al*. (2001), Constantaras and Kesler (2004). The difference might be attributed to either agro-climatic variation or ultrasound screening at the beginning might minimize abnormalities in this study. Many reasons for variation in conception rates such as heat stress, humidity, season, and body condition have been reported (Moreira *et al*., 2000; Cartmill *et al*., 2001). In addition, factors such as endometritis, mastitis, lameness and other managerial factors causes post-partum infertility (Grohn and Rojala-Schultz, 2000).

Regarding to body condition, this study concluded that mean (±SEM) diameter of CL was affected by body condition (P<0.05), CL were larger in animals having good body condition score. This is in line with previous finding of Maina *et al.* (2008), Fihri *et al*. (2005) who reported that mean (±SEM) diameter of CL were affected by body condition, mean diameter of follicle and CL is higher in animals having good body conditioned animals. Follicular growth was affected only in cows fed a diet lacking essential nutrients and having a low body condition score at calving (Perry *et al*., 2004). Differences between underfed and adequately fed cows up to day 50 postpartum were more striking in terms of follicular growth (Grimard *et al*., 1995).

The present study revealed that breed did not influence mean(±SEM) diameter of CL (P>0.05) at day 7 prior to PGF administration. This is supported by Fihri *et al*. (2005) who reported that parity did not influence the size of corpus luteum. This finding contradicts with Alvarez *et al*. (2000) who reported Brahman (*Bos indicus*) breed have larger ovulatory follicle and CL than exotic Angus breed, Segerson *et al*. (1984) reported *Bos indicus* breeds have larger diameter of follicle and CL than *Bos taurus* (Angus and Senepol*).* Moreover, this finding disagrees with Moreira *et al*. (2000), Sartori *et al*. (2010) who concluded that Zebu (*Bos indicus*) breeds have a smaller CL and Bastos *et al*. (2010) who reported greater ovulatory follicles and CL in Holstein (*Bos taurus*) than Nelore (*Bos indicus*) in Brazil. This variation might be due to either genetic variation or larger follicular development in Boran (*Bos indicus*) during first GnRH injection. *Bos indicus* cattle have a larger follicle and CL than *Bos taurus* cattle when they are both kept in tropical or subtropical environments, where stressors like hot temperatures, humidity, ectoparasites and low quality forages are greater (Bó *et al*., 2003). In addition, *Bos indicus* has larger follicle and CL than *Bos taurus* cattle breed in Florid *Bos indicus* and Angus (*Bos taurus*) in summer where there is heat stress (Wolfenson *et al*., 2004).

Concerning CL regression, there were 81.5% of the animals showed complete luteolysis of corpus luteum as P4 level reduced to less than 1 ng/ml. This is comparable with the result of Moreira *et al*. (2000), Kim *et al*. (2003) who found 75% and 80% in lactating Holstein cows, respectively. Whereas, this finding is lower than Portillo *et al*. (2008) who reported 100% complete luteolysis in Angus, Brangus, and Brahman*Angus heifers, Brusveen *et al*. (2009) who reported 97.5% in lactating Holstein breeds using double PGF injection in Ovsynch. The difference might be due to premature ovulation of smaller follicle in first GnRH injection, from which smaller and unresponsive CL formed and leads to incomplete luteolysis during PGF injection. The CL does not completely regress in a percentage of cows receiving Ovsynch due to that 7-day old CL has lower probability of complete regression compared to the 13-day old CL (Moreira *et al*., 2000; Souza *et al*., 2007).

6. CONCLUSION AND RECOMMENDATIONS

The result of present study indicated that using fixed time artificial insemination conception rate greater than 50% can be achieved both in Boran (*Bos indicus*) and Zebu-Holstein crossbred dairy cows. Parity, breed and body condition did not influence ovulation in both Ovsynch and Co-synch synchronization protocols. Conception rate was higher in primiparous than multiparous dairy cows and mean diameter of CL was also affected by body condition. Breed and body condition did not influence conception in both protocols. Based on the result, 77.1 % (27/35) cows had functional CL (P4>1 ng/ml) at day 7 prior to PGF injection and from which luteolysis was happened in 81.5% (22/27) of the animals after 48 hours of PGF injection as P4 level reduced to less than 1 ng/ml.

In light of the aforementioned conclusions, the following recommendations were forwarded;

- In farms where heat detection would be assumed to be problem fixed time artificial inseminations might be used but studies with large number of dairy cows should be conducted.
- The present study was conducted in better managed farm that has well documented record and same result might not be expected at small holder dairy farms where management, exact record of age and parity may not be known. Studies should be conducted at small holder dairy cows

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8. APPENDIXES

Appendix 1. Ethical approval sheet

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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/17/05/10/2018

Name of Applicant: Alebachew Tilahun (DVM, MVSc fellow)

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

Title of the project: Effect of breed, parity and body condition on ovarian response and success of TAI in Ovsynch and Co-synch protocols in pure Boran and crossbred dairy cows

Date of application:	15/10//2017
Nature of the project:	non-invasive
Target animal species:	cattle
Number of animals involved:	35
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:

3. All procedures and conditions stipulated in the proposal are respected and any deviation or changes be reported to the committee.
4. The project activities be open for occasional supervision by the committee whenever this is deemed necessary

Dr Getachew Terefe
Chairman

Signature

The Federal Democratic Republic of Ethiopia
Addis Ababa University College of Veterinary Medicine and Agriculture

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Please quote Our Ref. No. When replying

ፋክስ Fax 251-11-4339933 | ስልክ Tel. +251 114338450 | ፖ.ሣ.ቁ P.o.x. Box}34 | ቢሾፍቱ/ደብረዘይት ኢትዮጵያ Bishoftu/Debre Zeit, Ethiopia

Appendix 2. Picture during TAI in field work