ESTRUS RESPONSE OF BORAN AND BORAN-HOLSTEIN CROSSED CATTLE TO PGF2α AND PREGNANCY RATES TO SEXED AND CONVENTIONAL SEMEN, HOLETA, ETHIOPIA

Addis Ababa University, College of Veterinary Medicine and Agriculture in partial fulfillment of the requirements for the Degree of Master of Science in Veterinary Obstetrics and Gynecology

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As Member of Examining Board of the final MVSc open defense examination, we approved that we have read and evaluated the Thesis prepared by: **Galma Boneya Arero**, Entitled: **Estrus response of Boran and Boran-Holstein Crossed cattle to PGF2α and pregnancy rates to sexed and conventional semen** 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

I dedicate this thesis manuscript to my father Boneya Arero for his ambition to get his talent through his children and to my mother Elema Guyo and my senior sister Guyatu Boneya for nursing me and their children with affection and love.

TABLE OF CONTENTS **PAGES**

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LIST OF FIGURES **PAGES**

LIST OF TABLES **PAGES**

LIST OF APPENDIXES **PAGES**

LIST OF ABBREVATIONS

AI	Artificial insemination
BCS	Body condition score
CL	Corpus luteum
CIDR	Controlled internal drug release
CR	Conception rate
CSA	Central statistical Agency
DMI	Dry matter Intake
DNA	Deoxy ribo nucleic Acid
E2	Estrogen
ES	Estrus synchronization
FSCR	First service conception rate
FSH	Follicle Stimulating Hormones
FSH-R	Follicle Stimulating Hormones receptors
FTAI	Fixed time artificial insemination
GH	Growth hormone
GnRH	Gonadotropin releasing hormone
IGF	Immunoglobulin F
IGFBP	Insulin growth factors binding protein
IM	Individual motility

LH	Luteinizing hormone
LH-R	Luteinizing hormone receptors
MA	Mass activity
MGA	Melengesterol acetate
NEB	Negative energy balance
NEFA	Non-esterified fatty acid
P4	Progesterone
PGF_2	Prostaglandin F 2 alpha
TAI	Timed Artificial insemination

ABSTRACT

The study was conducted majorly to evaluate pregnancy rate to sex sorted semen and high grade non sex sorted imported semen in a 2X2 factorial design in Boran and Boran X Holstein cross cows and heifers. The effect of some animal related factors which may affect pregnancy rate and heat characteristics of different breeds and party were evaluated. A total of 71cows/heifers consisting of 36 Boran and 35 Boran x Holstein of which 49 were cows and 22 heifers were purposively selected and synchronized using PGF2, twenty five Boran (69.4%) and twenty seven Boran x Holstein cross (77.1) were responded to PGF2α and exhibited estrus. When response to PGF2 was compared by parity, 65.3% cows (n=32) and 90.9% heifers (n=20) exhibited estrus. Parity has statistically significant ($P<0.05$) effect on estrus response in which heifers showed higher response rate than cows whereas, breeds, body condition score and treatment (response at single or double PGF2) did not show significant effects on estrus response ($P>0.05$). Average duration of estrus was significantly longer in Boran x Holstein cross breed than Boran breed. However, average interval to onset of estrus and duration of standing estrus were not significant differ ($P>0.05$) by both breed and parity. A multivariable logistic regression model including the breed, parity, body condition score, treatment, semen type and their interactions was evaluated. Parity and semen type showed significant variation ($P<0.05$) on the pregnancy rate. On pregnancy detection, 54.1% Boran and 57.6% cross breed cattle were found pregnant of which 46.8% were cows and 72.2% were heifers to both types of semen. The pregnancy rate was 66.6% to conventional semen and 46.1% to sexed semen. It could be concluded that sexed semen could be a better option to improve number of replacement heifers. Similarly, high grade imported semen may help speeding up genetic improvement when used on Boran heifers. However, data from large number of animals should better be generated. Heifers were well responded to than cows; duration of estrus was longer in Boran breeds than crossbred and heifers was higher conception rates than cows.

Keywords: Boran, Cross breed, Estrus response, Ethiopia, Pregnancy rate, PGF2, sexed semen

1. INTRODUCTION

Ethiopia ranked first and owns the largest livestock population among African countries and one of the largest in the globe with 60.39 million cattle (CSA, 2017). 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. Among indigenous cattle, Boran cattle are the most suitable types of breed for arid and semi-arid regions in the country due to their adaptive characteristics like tolerance to heat, ticks infestation, feed and water shortage, and hardened hooves and lighter bones that enabled them to endure long migrations (Mekonnen *et al.,* 2010).

Having good characteristics made Boran breed not restricted only to arid and semi- arid area, but also opportunities to be distributed throughout the country for genetic improvement with others exotic breeds. Boran cattle breed is the best indigenous breed among local breeds in our country that are exposed to assisted reproductive technology other than AI in the process of breed improvement with the most promising findings (Degefa, 2016). The country has made great effort to improve the productivity of local breeds through artificial insemination (AI) programme to crossbreed locally adapted cattle breeds with improved exotic dairy breeds. However, the success of such programme is not satisfactory due to numerous factors, including substandard nutrition, poor husbandry practice and infrastructure status. High reproductive efficiency in livestock is economically a very desirable characteristic but, it is suboptimal due to failure of estrous cycle/anestrus, infertility and Poor detection of estrus cycle (Chakravarthi and Sri Balaji, 2010).

Infertility is one of the major problems that influences the production and profitability in dairy herds (Dogruer *et al.,* 2010). In our country infertility is very common in which calving interval of 12 to 13 months is generally considered to be economically optimal, but often difficult to achieve. To meet this goal 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 and servicing techniques, and timely insemination relative to ovulation of the egg are also key factors (Alemneh *et al.,* 2015). Furthermore, a long postpartum anoestrous period is a very common problem in cows reared in a tropical environment (Million *et al.,* 2011).The reduced conception rates is another problems in the dairy herd which leads to low production of offspring, which mean few replacement heifers and bulls, which lead to greater economic losses. To overcome these problems, synchronization of the estrous cycle to implement AI becomes an attractive alternative method to improve reproductive performance and economics (Ribeiro *et al.,* 2018).

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 (Gupta *et al.,* 2009). Exogenous hormones and their analogues are used to manipulate the bovine estrous cycle to reduce the amount of labor and time expended on estrus detection. These days, prostaglandin is used to synchronize estrous in dairy cattle operations to boost the efficiency of AI by inducing the regression of the corpus luteum (Diaz *et al.,* 2005). it's the first method of heat synchronization that depends on the presence of a functional CL particularly in the diestrus stage of the estrous cycle (day 7 to17 of the cycle) (Oaxaca *et al.,* 2009). Its effectiveness usually affected by heat stress, asynchronous ovarian events exhibiting incomplete or delayed luteolysis, and weak or delayed estrous (Lamb *et al.,* 2001).

In cattle, there are economic requests of dairy farmers that the offspring are desired to be of a particular sex. Specifically, dairy farmers are engaged in the production of milk and herd replacement thus, prefer female calves and tend to consider male calves as byproducts (Naniwa et al., 2019). In dairy production, one of the top costs is cost of dairy replacement heifers in maintaining herd size and structure. So in there should be a possibility to make a reasonable and justifiable decision to be able to produce and raise dairy replacements heifers. This should be among others through the use of assisted reproductive technologies (ARTs) that utilize sexed sperm (gender selected semen). The process of sperm sorting often leads to some damage to sperm structure and leads to low pregnancy of sexed semen. Hence, optimizing time when sperm stay in reproductive tract could better improve pregnancy per AI in animals bred with sex sorted sperm.

The availability of genetically desirable replacement heifers are among factors that significantly influences the sustainability of many dairy enterprises. Similarly, in Ethiopia, costs of dairy heifers are severely increasing from time to time. One way of tackling such problems should be use of sex-sorted semen which easily allows farmers to significantly skew the sex ratio of their animals' offspring. Several researches were conducted on estrus synchronization of dairy cattle in different regions of Ethiopia that was implemented with conventional semen AI. However, manifestation of estrous and the conception rates of those synchronized animals are not satisfactory in improving the planned breeding and no research was conducted to evaluate pregnancy rate of sex-sorted semen in a country. In Ethiopia application of sexed semen to produce specific sex (female) for herd replacement and increasing milk production may benefits many dairy farms. However, conception rate to AI of conventional semen was very low (27%) (GebreMedhin et al., 2009).When compared to non-sorted frozen semen, sex sorted semen has reduced conception rates, which may further reduce conception rate to AI in Ethiopia. Therefore, aim of this research was to study factors affecting estrus response rate, and conception rates of sexed and high quality imported non-sorted sex semen in local (Boran) and crossbred (Boran x Holstein) cows and heifers.

Therefore, the objectives of this paper were:-

- To study factors affecting estrus response to PGF2α in Boran and Boran x Holstein cows and heifers.
- To evaluate heat characteristics of Boran and Boran x Holstein cows and heifers.
- To compare conception rate of sexed and non sex sorted semen in Boran and Boran x Holstein.

2. LITERATURE REVIEW

2.1. Follicular growth in cattle

Ovarian follicle growth takes a period of 3–4 months and can be categorized into gonadotropin-independent and gonadotropin-dependent stages (Webb *et al.,* 2004). Gonadotropin dependent follicle growth in cattle occurs in waves (Sirois and Fortune, 1988). Each wave of growth involves emergence, selection and dominance followed by either atresia or ovulation. Emergence of a follicle wave is defined as growth of a cohort of follicles <5 mm in diameter and coincides with a transient increase in FSH secretion (Adams *et al.,* 1992; Sunderland et al. 1994). Selection is the process by which the growing cohort of follicles is reduced to the ovulatory quota for the species (in cattle, it is generally one), and selection occurs in the face of declining FSH concentrations (Sunderland et al. 1994). The selected follicle survives in an environment of reduced FSH because of the development of LH receptors in granulosa cells (Bao *et al.,* 1997) and increased intra-follicular bio-available insulin like growth factor-I (Canty *et al.,* 2006). The increased bio-available IGF-I is achieved by reduced IGF-I binding proteins (IGFBP) because of increased IGFBP protease activity. Dominance is the phase during which the single selected follicle actively suppresses FSH concentrations and ensures suppression of all other follicle growth on the ovaries (Sunderland et al. 1994). The fate of the dominant follicle is then dependent on the prevailing LH pulse frequency during the dominance phase. In the presence of elevated progesterone (luteal phase of cyclic animals), LH pulse frequency is maintained at one pulse every 4 h and the dominant follicle undergoes atresia; in the follicular phase (pre-ovulatory period in cyclic animals), the LH pulse frequency increases to one pulse per hour and this mimic final maturation of developing follicle, undergoes estradiol concentrations increment and positive feedback on gonadotropin-releasing hormone, Luteinizing hormone and, Follicle stimulating hormone which leads to ovulation of ova (Sunderland et al. 1994). Normal follicle waves have an inherent lifespan of 7–10 days in duration from the time of emergence of a wave until emergence of the next wave (indicating either ovulation or physiological atresia of the dominant follicle). In cyclic heifers during normal 21 day oestrous cycle there are normally three waves (sometimes two waves and rarely one or four waves) (Murphy *et al.,* 1991).

2.2. Ovarian follicular dynamics and wave in cattle

Fundamental reproductive processes in farm animals are characterized by emergence, selection and dominance followed by either atresia or ovulation of the DF governed by the prominent role of FSH and LH. In bovine the beginning of gonadotropin dependent follicle development is typified by the emergence of 5–20 cohort of follicles correlated with a transient increase in FSH concentrations and FSH receptors (FSH-R) localized within the granulosa cells (Ginther *et al.,* 2002; Crowe and Mullen, 2013) . This enables FSH to perform its required downstream signaling effects including promoting cellular growth and proliferation leading to an increase in aromatase enzyme activity that converts androgen to estrogen in the granulosa cells of ovarian follicles known as two cells/two gonadotropin model. The increase in diameter of the DF selected from the cohort of follicles leads to an increase in estradiol and inhibin concentrations in follicular fluid, and actively suppresses FSH, thus preventing further follicle wave emergence until the DF either undergoes atresia or ovulated (Crowe and Mullen, 2013).

Luteinizing hormone receptor (LH-R) is localized in the theca and granulosa cells of healthy follicles at different stages of follicle development (Camp *et al.*, 1991). As the follicle grows the theca cell LH-R increases, and granulosa cells acquire LH-R in the follicle undergoing selection to become the DF and estradiol producer since it is dependent on sufficient LH pulse frequency (Bao *et al.,* 1997; Braw-Tal and Roth, 2005). The binding of LH to its receptors in the theca cells drives the conversion of cholesterol to testosterone through a series of catalytic reactions. Testosterone, once produced in the theca cells, diffuses out into the granulosa cells where it is converted to estrogens by the aromatase enzyme (Crowe and Mullen, 2013). During the follicular phase of the estrous cycle, when progesterone concentrations are basal, the large concentration of estradiol produced by the pre-ovulatory DF induces a GnRH surge from the hypothalamus. The resulting LH surge is of sufficient amplitude and frequency to stimulate final maturation and ovulation of the DF (Sunderland *et al.,* 1994). The increased estradiol concentrations also induce expression of estrous behavior, required for successful mating (Crowe and Mullen, 2013).

Each estrous cycle in cattle has either 2 or 3 follicular waves, which consist of a group of growing antral follicles of >4 mm in diameter from which a dominant follicle is selected, while the remaining follicles become subordinate and undergo atresia (Ginther *et al.,* 1989). In both 2- and 3-wave estrous cycles, emergence of the first follicular wave occurs on the day of ovulation (Day 0; 1 day after estrus), whereas the second wave emerges on Day 9 or 10 in 2-wave cycles, and on Day 8 or 9 in 3-wave cycles, with a third wave emerging on Day 15 or 16. Duration of the estrous cycle is approximately 20 days in 2-wave cycles and 23 days in 3-wave cycles. The dominant follicle present at the time of luteolysis will become the ovulatory follicle, and emergence of the next wave is delayed until the ensuing ovulation (Kastelic *et al.,* 1990).

Follicular wave dynamics and duration of the estrous cycle may be slightly different in high-producing dairy cows (Sartori *et al.,* 2004). The numbers of animals with 2- versus3-wave cycles are more or less equally distributed in beef cattle, whereas more 2-wave cycles have been reported in lactating dairy cattle (Sartori *et al.,* 2004). There appears to be no clear breed- or age-specific preference for one follicular wave pattern over the other, nor is there any apparent difference in fertility. Two studies suggested that conception rate to first service is reduced in beef and dairy cattle in which the ovulatory follicle came from the second compared with the third follicular wave during estrous cycle (Townson *et al.,* 2002). However, in 2 other studies, pregnancy rate did not differ among lactating dairy cows with different wave patterns of follicle development (Bleach *et al.,* 2004) or in beef cattle in which the length of progesterone exposure of the ovulatory follicle was investigated (Dias *et al.,* 2012).

2.3. Bovine estrus cycle

Estrus cycle can be defined as the rhythmic changes that occur in the reproductive system of a female animal starting from one estrus phase to another. It's a point in which females are most receptive to mating. In mature cows the normal duration of estrus are 21 days whereas 20 days for virgin heifers under normal condition. The entire estrus cycle can be follicular \ and luteal phase. The ovarian follicles develop in follicular phase and the corpus luteum in luteal phase. FSH (follicle-stimulating hormone) is the principal hormone controlling the follicular phase. The LH surge is responsible for ovulation of the matured Graafian follicle.

Estrus phase a period lasts from 6 to 30 hours the animal shows the signs of estrus or heat. At this time, vagina become red and production of mucus are greatly increased and clear mucus may be visible hanging from the vulva or wrapped around the tail and sometimes it reach hock, the corpus luteum (CL) has been lysed before this period, restlessness, frequent micturition, bellowing, swelling of the vulva, etc. The animal tries to mount other animals and stands to be mounted by other animals called as standing heat. After the estrus phase, the ruptured follicle starts to convert into corpus luteum and the animal enters into luteal phase. This phase is divided into metestrus and diestrus. The duration of metestrus is 3–4 days, whereas diestrus can last from 10 to 14 days. In metestrus the estrogen level starts decreasing and progesterone increases. Though, ovulation occurs in metestrus phase in cattle about 10 to 15 hours after the end of estrus. The uterine contraction subsides and endometrial glands start growing in metestrus. Diestrus is one of the stages of estrus cycle in which CL begins to develop, lasts about 12 to 14 days. The CL is producing high level of progesterone during the early days of diestrus starting from day 5 after estrus, as the capacity for steroid production increases maturation of corpus luteum will occur (Bridges, 2010).

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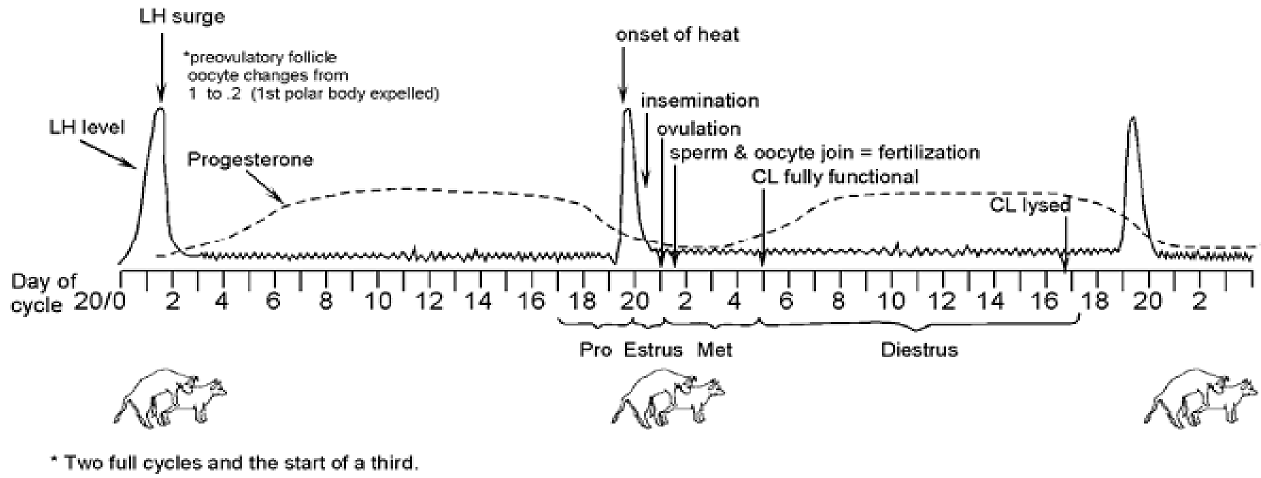

Figure 1: Stages of estrous cycle in cows

Source: Yizengaw, 2017

2.4. Estrus detection and signs

Estrus detection is one of the most important factors affecting the production and reproductive efficiency in dairy cattle especially in farms using reproductive technology mostly artificial insemination (Reith and Hoy, 2018). Visual detection of estrus is a challenging job. The expression of standing estrus is only shown by about 50% of cows in estrus and lasts for a short period of time of about 5 to 7 h (Roelofs *et al.*, 2005). Many studies have investigated estrus detection by visual observation. Most studies have focused on the most pronounced sign of estrus and standing heat. Estrus detection efficiency by visual observation of standing heat mentioned in different studies vary a lot from 90 to less than 50% (Roelofs *et al.,* 2006;Van Eerdenburg *et al.*, 2002). Many years back, a lot of veterinarians has been performed a clear trend toward the use of technological methods for effective and accurate detection of estrus in dairy cattle. Mount detector are used to record standing events, these devices are attached to the sacrum of the cow and indicate whether a cow has been mounted or not whereas: pedometers, accelerometers, cameras, temperature measurements, impedance or conductivity measurements of mucosal vagina and hormone analyses, are based on detection of increased activity during proestrus (Reith and Hoy, 2018).Between studies, different detection rates of different aids are mentioned. However, a combination of several estrus detection aids might give the best results in terms of estrus detection efficiency. Peralta *et al.,* (2005) used three times daily visual observations, a mount detection device, and an activity transponder. On its own, visual observation yielded the highest detection (49.3%) followed by the mount detection device (48%), and the activity transponder (37.3%). The combination of the three systems increased the detection rate to 80.2%. Another study combined visual observations by the herdsman with either a hormone-treated steer or tail paint. Observations by the herdsman alone gave a detection rate of 77%, which was the same as for the combination of visual observations and tail paint. The detection rate increased to 89% when the visual observations were combined with the hormone-treated steers. Using a combination of different traits as activity, milk temperature, milk yield, and conductivity gave detection rates between 67and 94% in different studies (Firk *et al.,* 2002).

2.5. Behavioral signs of estrus

Ovarian functions are regulated by endocrine hormones. The axis are hierarchically as follows hypothalamus secrete gonadotropin-releasing hormone (GnRH), anterior pituitary secretes FSH and LH, ovaries secretes progesterone, estrogen and inhibin and the uterus secretes (prostaglandin F2α (PGF2α) (Aungier *et al.,* 2015). Estradiol secreted by the pre-ovulatory follicle in turn promote a GnRH surge and allow animals ready to exhibit estrus during which progesterone levels are low. Elevation of estradiol leads to expression of behavioral estrus and the release of LH to cause ovulation. Estrous behavior can be categorized on the basis of primary (mostly standing to be mounted) and secondary signs (other signs of estrus rather than standing to be mounted).

Several studies indicated that, standing to be mounted was the primary sign of estrus and most characteristic external sign for determining when a cow is really in estrus (heat) and considered as time of sexually receptive for AI. But, due to many factors an advancing decrease in the number of animals showing standing estrus is well documented in several findings (At-Taras and Spahr, 2001). Many studies revealed that, <50% of the cows showed primary sign of estrus (standing to be mounted) on the day of estrus (Peralta *et al.,* 2005) this might be due to herd size or other factors. Duration is significantly affected by daily milk production. Wiltbank *et al.,* (2006) noted that the relationship between milk yield and duration of estrus were $r = -0.51$that characterized by correlation coefficient. This may be the result of a low level of serum estradiol concentration on the day estrus in for high milk producing cows' (Lopez*et al.,* 2004) because of increased metabolic clearance rate of steroid hormones in hormone are removed through metabolic way (Wiltbank *et al.,* 2006).

2.6. Estrus Synchronization in cattle

Estrus synchronization is one of the reproductive technologies which imply manipulation of the estrous cycle or induction of estrus to bring a large percentage of a group of females into estrus at a short-predetermined time. It’s one the most crucial technique for improvement of reproduction in livestock production. It is one of the ways to regulate the estrous detection. Today, these technologies remain the most effective and widely applicable reproductive biotechnology available for dairy cow operation used in dairy farm (Chakravarthi and Sri Balaji, 2010).

Synchronization of estrus are used to fix the breeding time within a required period and they schedule the parturition period at the most suitable season of the year, when the newborns can be reared in suitable environment with ample food for enhancing their survive ability. But it needs proper levels of nutrition, body condition and health with advanced level of estrus detection and technician with enough knowledge. However, lack of any of these areas can spell huge risk for an estrous synchronization program. Improvements in facilities and management may be necessary before implementing an estrous synchronization program and hormonal treatments are effective only if an animal are at least 50 days postpartum (Gizaw *et al.,* 2016).

2.7. Methods of Synchronization Protocol

Having enough knowledge on the hormonal profile, on synchronization protocol and stages of estrus cycle on ovaries is very much important for the selection and successful improvement of the estrus synchronization program (Patterson *et al.,* 2002). Improvement of estrus synchronization protocols in cycling animals now a day commonly utilizes the combination of progestin and prostaglandin which allows proper and effective synchronization of estrus, regardless of stage of estrus the cycle without compromising fertility. The addition of GnRH to this combination would help ovulation of a dominant follicle and synchronize a new follicular wave (Smith *et al.*, 2005).

2.7.1. 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). GnRH 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. Artesian or ovulation of the matured follicle and start to develop the emergences of a new wave of follicular growth an average of 2.5days ovulation of the dominant follicle depends on the status (growing, static or regressing) of the dominant follicle at the time of GnRH injection. Ovulation of a growing dominant follicle occurred 100% of the time following GnRH administration, however, ovulation of dominant follicles in the static stage or regressing stage occurred thirty three percent(33%) and zero (0%) of the time, respectively (Helmer and Britt, 1985). 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). Administration of GnRH on day 1 is used to program or adjust growth of follicle in cyclic females. But, in anestrous female, it induces ovulation this is to provide progestin pre exposure. Injection of prostaglandin on day 8 induces regression of CL that is present to cause a decline in progesterone. The four systems for synchronization of estrus with GnRH -PGF2α combinations are; select synchronization (select synch), ovulation synchronization (Ovsynch), combination synchronization (co-synch) and hybrid synchronization (hybrid synch) (Patterson *et al.,* 2003).

GnRH– PGF2α System

This combination represents the simplest GnRH –PGF2α based system. A common name for the GnRH-PGF2α system is “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 injection (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 (DeJarnette *et al.*, 2001). When GnRH is injected at day 1 and Prostaglandin is injected day 8, some cows exhibit estrus up to 48 hours before PGF day 6. The "early" heats are fertile and cows can be inseminated 8-16 hours after detection. Animals were inseminated 8 to 12 hours after being observed in standing estrus (DeJarnette *et al.*, 2004).

With this system, a minimum of 5 days of estrus detection after PGF2α and 2 days preceding PGF2α is required to detect most heats. The peak estrous response occurs 2-3 days after PGF2α with a range of days 1–5. Overall, estrus response rates in well-managed herds average approximately 70 to 75% with no adverse effect on conception rates (60 to 70%), resulting in synchronized pregnancy rates that average between 45 and 50%. Pregnancy rate was 41(Stevenson *et al.*, 2000); 45 (Patterson *et al.*, 2001); 47 (Kojima *et al.*, 2000) and 61(Dejarnette *et al.*, 2001a) percent following select synch system.

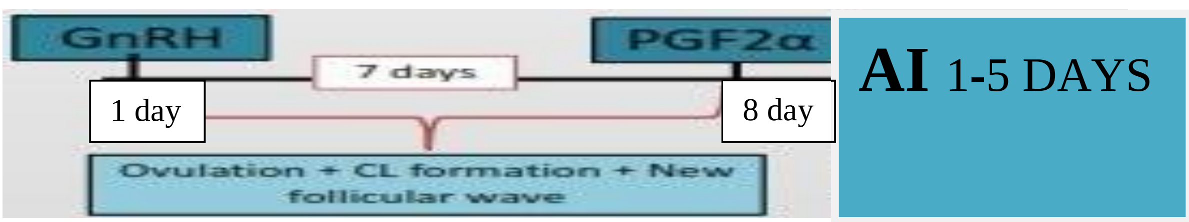

Figure 2: Select synchronization protocols

Source: https://www.google.com/search?q=dairy+cow+synchronization+protocols&tbm=isch&ved=2ahUK.

GnRH – PGF + GnRH System

The Ovsynch program is comprised of an injection of GnRH on day 1, an injection of prostaglandin on day 8, a second injection of GnRH was administered at 24 hours (Thatcher *et al.*, 2006); 48 hours (Pursley *et al.*, 1995) or 54 hours (Twagiramungu *et al.*, 1995) after the PGF2α injection. This second GnRH injection induced ovulation of the dominant follicle recruited after the first GnRH injection (Păcală *et al.*, 2010).

Injection of GnRH during presence of dominant follicle alters follicular growth by inducing ovulation in the ovaries. Afterwards, the uses GnRH injection are to form a new or additional CL.

During that time, estrus usually does not occur because of absence of mature dominant follicle until a PGF2α injection regresses the natural CL and the secondary CL (formed from the follicle induced to ovulate by the first GnRH injection) (Vasconcelos *et al.,* 1999).

Vijayarajan et al., (2009) reported 100 per cent ovulatory response between 24 and 32 hours after the second injection of GnRH in ovsynch treated lactating dairy cows. 40 to 55% (Ammu *et al.*, 2012);. 59 % (Geary *et al.,* 1998); 60 to 62 percent (Cecyre *et al.*, 2002) and 61.00 to 90.00 % (Muneer et al. 2009) conception rates have been reported following induction of ovulation using ovsynch protocol in lactating dairy cows. Report from many authors indicated that there was high pregnancy rate in early luteal phase when cows were started on the ovulation synchronization protocol compared with first 3 to 4 days or after day 10 of the oestrous cycle (Moreira *et al.*, 2000b).

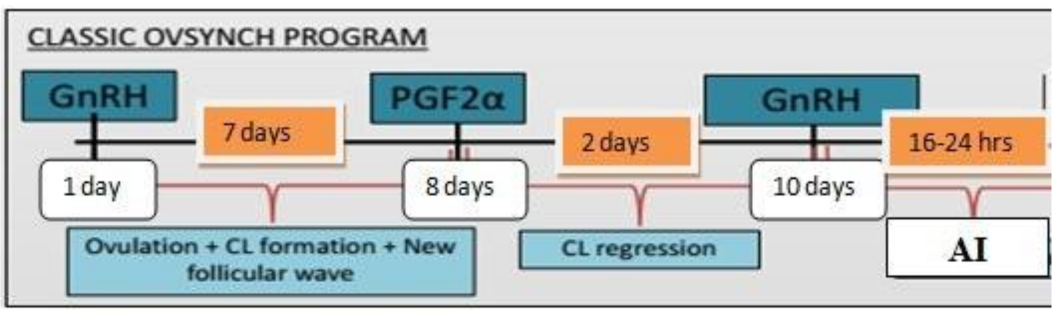

Figure 3: Ovulation synchronization protocols (ovsych-protocol)

Source:
https://www.google.com/search?q=dairy+cow+synchronization+protocols&tbm=isch&ved=2ahUK-

GnRH – PGF- GnRH + AI System

A common name for this system is “Co-Synch” The co-synch program utilizes the same strategy as Co-Synch was an alternative to ovsynch in which, second GnRH injection was given at the time of AI (Geary *et al.*, 2001). Larson et al., (2004) reported that the cows were bred at 54 hours after the injection of PGF2α following co-synch protocol. Overall, pregnancy rates have averaged 48%. It’s easy to apply this protocol in comparing with others because of AI and injections were done at the same time. In co-synch, the pregnancy rates were between 40 and 50 per cent (Dejarnette *et al.*, 2004). No estrus detection is required with co- synch. The Combination-Synchronization program is an injection of GnRH on day 1, prostaglandin injection occurs on day 8, during this time mature CL become lyse and then a second injection of GnRH with AI on appeared day 10.

The advantages are tight synchronization of estrus, most females respond to the program and it encourages estrus in non-cycling cows that are at least 30 days postpartum (Harrison *et al.*, 1990). Co-synch eliminates one animal handling by breeding cows "Coinciding" with the second GnRH injection. Most field trials indicate only a small reduction in conception rates when co-synch is compared to Ovsynch.

Figure 4: Combination synchronization protocol

Source:
https://www.google.com/search?q=dairy+cow+synchronization+protocols&tbm=isch&ved=2ahUKEwjxuLilrsTpAhUW-4UKHRLuAE4Q2-

Hybrid-Synch

Hybrid synch was a combination of select synch and co-synch systems (Stevenson *et al.*, 2000). Estrus detection and Al carried out until 72 hours after the PGF2α injection and then mass-AI along with GnRH injection were done to those cows that did not exhibited oestrus until 72 hours (Larson *et al.*, 2004; De jarnette *et al.*, 2001b and De jarnette *et al.*, 2004).

The Hybrid-Synch program is similar strategy with co-synch protocol (GnRH on day1: PGF2 on day 8 and then estrus detection and breeding from day 8 to 11. For animals that did not shows estrus from day 8 to 11 AI should be given on day 11and given a second injection of GnRH. In comparing with other protocols like ovsynch and co-synch it has lower cost and easy to handle but its higher cost when compared with select synch. The primary advantage is that Hybrid-Synch appears to have the highest conception rates among all GnRH prostaglandin program (Hafez, Y., 2015). Few studies reported the rates of Pregnancy after hybrid synch protocol (Stevenson *et al.*, 2000) reported 34%; (Dejarnette *et al.*, 2001b) reported 46%; (Larson *et al.*, 2004) reported53% and (Dejarnette *et al.*, 2004) 52 per cent.

2.7.2. Progesterone Based Protocol

The principle of injecting P4 and its analogs is to extend the normal estrus cycle by extending the period of diestrus. Exogenous administration of P4 suppresses LH release, alters ovarian function, suppresses estrus and prevents ovulation in cattle. Both P4 and progestins have been incorporated to the estrus synchronization protocols in cattle via oral route such as melangestrol acetate or by insertion of intra-vaginal P4 device or progestin ear implants (Martinez *et al.,* 2000).

Synchronization of estrus with progestogens maintains high levels of progesterone in the reproductive system of female animals, even after the regression of the corpus luteum. Estrus will occur 2 to 5 days after removal of progestin. The commonly used progestin protocols are melengesterol acetate (given orally), Syncro-Mate-B (implanted to ear) and CIDR (Placed inside vagina). In general, if progestin was administered for long period time to cattle, the rates of estrus synchrony is high, but it compromises fertility even for well synchronized animals (Moreira *et al.,* 2000a).

Melengesterol acetate (MGA) feeding

MGA is one of the progestin products that are given orally for better synchrony. When consumed by cows or heifers on a basal diet, MGA will suppress estrus and inhibit ovulation (Imwalle *et al.,* 2002). MGA is given through feed such that females receive 0.5 mg per animal per day for 14 days. After removal of MGA from the feed, cyclic females start to exhibit estrus. This estrus is sub-fertile, and it is not recommended to breed. For these reason, an injection of prostaglandin is given 15-19 days after removal of MGA from the feed (Jaeger *et al.,* 1992).According to Brown *et al.,* (1988) MGA was given through fed for 14d and at 16 to 18 d PGF2 administration after the last day of MGA feeding. This system was designed to place cattle in the late luteal phase of the estrous cycle at the time of PGF2α administration because progestin is to prolong stages of estrous cycle which prevent ovulation.

The rate of synchronization of estrus following MGA-PGF2α is usually greater than that following the use of PGF2 α alone (Patterson *et al.,* 2002). Female animals that are failed to consume fed the required (adequate) amount of MGA on a daily meal may prematurely return to estrus during the feeding period. At this time the expected rates of estrous response may be reduced. Therefore, enough bunk space must be available so that all animals consume feed at the same time (Patterson *et* *al.*, 2003).

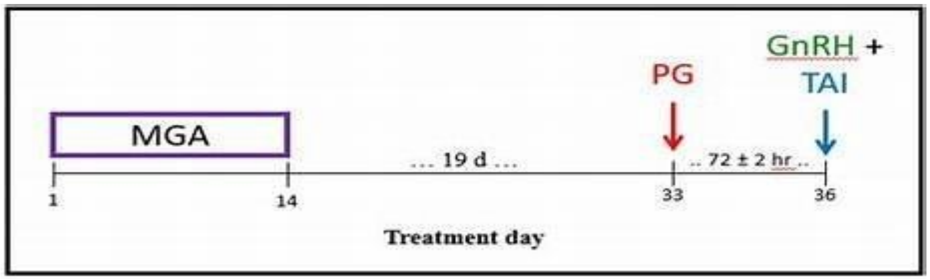

Figure 5: Melengasterol acetate protocol

Source:

https://www.google.com/search?q=dairy+cow+synchronization+protocols&tbm=isch&ved=2ahUKEwjxuLilrsTpAhUW-4UKHRLuAE4Q2

Syncro-mate-B (ear implant)

SMB treatment is implanted in ear. If applied at late stages of the estrous cycle (> d 14) in cow gives conception rate are reduced. The critical time for SMB treatment application is between d 8 and d12 of the estrous cycle to maximize estrus response or to get better synchrony (Ravikumar and Asokan, 2008). Syncro-mate-B breeds a high percentage of cows in a short period, increases probability of estrus detection and (Dinoprostromethamine) 25 mg intramuscularly at 11 days insemination. The initial norgestoment/progestogen ear implant has an inhibitory effect on the release of LH and FSH, which is necessary for the estrus cycle. Norgestoment inhibits LH secretion which inhibits the functional of the corpus luteum. Treatment with synchromate B synchronizes estrus in postpartum cow. This product is administered through the norgestoment implant in the ear after 9 day; a shot with estradiol valerate/norgestoment solution is injected intramuscularly. The cow will start to show heat about 48-60 hours after plant removal.

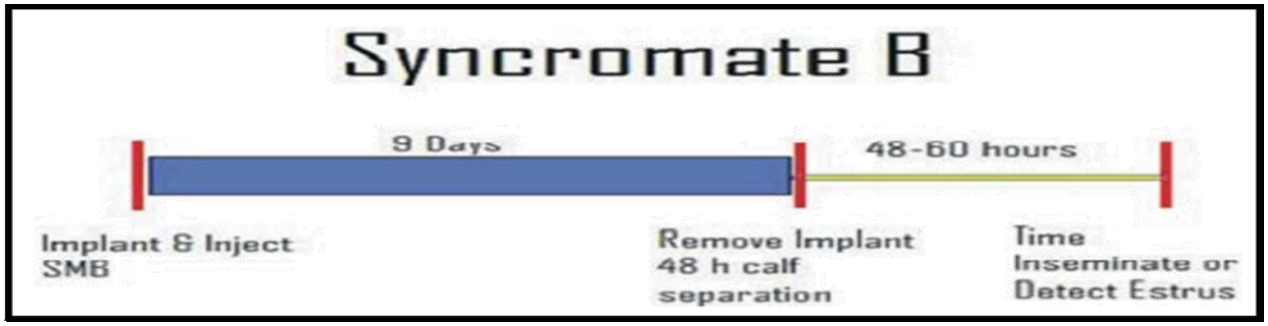

Figure 6: Synchro-Mate B protocol

Source:http://www.ansci.wisc.edu/jjp1/ansci_repro/misc/websites09/tues/synchromate%20b/syncromate%20 B%20Jackie%20R.html.

Application of CIDR

CIDR is molding device, thin layer of silicon and progesterone mixture around a nylon spine under high temperature that are inserted through vagina of cattle. The CIDR contains 1.38 gram progesterone and is designed to maintain elevated blood concentrations of progesterone at least 2 ng/ml for up to 10 days. Being relatively thin, the CIDR is easily inserted into the vagina and has good retention capacity (2.5% loss rate is normal). A flexible nylon tail is attached to the device to allow for easy removal from vagina. The CIDR Cattle Insert is used to induce an exogenous source of the hormone progesterone during the 7-day administration period. Removal of the CIDR Cattle Insert on treatment day 7results in increasing level of plasma progesterone levels, which results in synchronization of estrus in those animals responding to treatment.

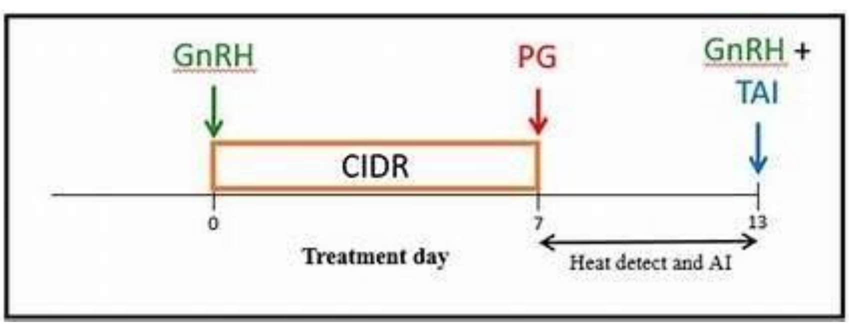

Figure 7: CIDR protocol (control internal drug release protocol)

2.7.3. Prostaglandin based Estrous Synchronization Protocol

Prostaglandin (PG) is a naturally occurring hormone. During the normal estrous cycle of a non-pregnant animal, PGF is released from the uterus 16 to 18 days after the animal was in heat. This release of PGF functions to destroy the corpus luteum (CL). The CL is a structure in the ovary that produces the hormone progesterone and prevents the animal from returning to estrus. The release of PGF from the uterus is the triggering mechanism that results in the animal returning to estrus every 21 days. Commercially available PGF2α (Lutalyse, Estrumate, Prostamate, lutaprost) gives the herd owner the ability to simultaneously remove the CL from all cycling animals at a predetermined time that is convenient for heat detection and breeding (Patterson *et al.,* 2003).

The major limitation of PGF2 α is that it is not effective on animals that do not possess a CL. This includes animals within 6 to 7 days of a previous heat, pre-pubertal heifers and postpartum anestrous cows. Despite these limitations, prostaglandins are the simplest method to synchronize estrus in cattle. Level of estrus response and fertility with these protocols is good with cyclic females at luteal stage, such as virgin heifers, but cannot induce estrous cycles in non-cycling cows (Bader, 2003).

One shot prostaglandin

Option 1: shows a single injection of PGF2α. It's recommended to given to cyclic females, and then these females are bred as express estrus. The demerit of this protocol is that 20 -25% of the females will not exhibit to the injection, but lower cost of injection and easy to handle animals once other than for breeding are the advantages of this program (Islam, 2011).

Option 2: Second one shot option requires detection of estrus before any PGF2α treatment is administered. The producer detects estrus for 5 days and breeds each cow as exhibits estrus. The cows that have not exhibited estrus by the fifth day are given an injection of prostaglandin, which should induce to come into estrus in about 3 to 5 days (Day and Geary, 2005).

This option represents the greatest savings in cost and labor associated with treatments because no repeated injections and unnecessary to involve all animals. In addition, detecting estrus for 5 days gives the producer some idea of the total number of cows that are cycling. During this 5-day period, approximately 20 to 25 percent of the cows should show estrus (4 to 5 percent per day). All cows that are cyclic should show estrus within five days after the PGF2α injection. This is the most popular protocol that uses only PGF2α to synchronize estrus and can result in more than 90% of cyclic cows being bred during the first 10 days of the season. If 4 to 5 percent of the cows are not exhibiting estrus each day, then the cows are probably not cycling. This will allow time to evaluate the effectiveness of the estrous synchronization program. The disadvantage of this program is that it requires 5 days of accurate detection of estrus before prostaglandin treatment is administered. This program is recommended because of the opportunity to determine the reproductive status of the herd before animals are treated for synchronization (Islam, 2011).

Two shot prostaglandins

The two injection programs for synchronization with PGF2α are designed to increase the proportion of females with a CL that is responsive to regression with PGF2α.

Option 1: uses two injections of prostaglandin spaced 14 days apart. Even if, animals show estrus, animals will not breed until scheduled day reach. Most of the time all cycling cows should come to estrus after second injection regardless of what stages of the estrous cycle were in when the first injection was administered. Remember the non-cycling cows will not generally respond to prostaglandin products. The advantage of this option is that more cows should come into estrus at any given time than with the one shot options. The disadvantage is that it involves the cost and labor of administering two injections of prostaglandin to all cows (Day and Geary, 2005; Păcală *et al.*, 2009).

Option 2 the second two-shot prostaglandin injection option is give the first injection, and breed all females exhibiting estrus and then give the second injection to only females that were not breed. This option lowers expense and handling, but results in two synchronized groups instead of one and a longer breeding period. Timed insemination instead of estrous detection may be used, but conception rates are generally lower than with estrous detection. Short-term calf removal may improve the response in cyclic postpartum cows (Day and Geary, 2005; Păcală *et al.*, 2009).

2.8. Factors affecting estrus response

2.8.1. Cow related factors

Breed

Estrus response are varies between breeds, cows and even the same cows from different estrus periods and the level of estrus expression has a low heritability (0.21) (Roxström *et al.,* 2001a, 2001b) Differences between genetic lines and breeds have been reported (Heres *et al.,* 2000). According to (Orihuela A, 2000) the duration of sexual receptivity and estrus intensity (mounts per hour) are higher for *Bos Taurus* than for *Bos indicus* cows, whereas, in general, dark-colored breeds show more intense estrus behavior than cows with white or red hair coloration.

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.,* 2008). 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 (Centurion-Castro *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).

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. Mufti *et al.,* (2010) reported that conception rate was higher in 1st delivered cows than that of the older cows attained the 4th or more parities. Conception rate is generally lower in older cows. In a Virginia study, conception rate remained constant (50%) during the first 3 lactations. Coach (2010) found that the nutrition has the positive effect on conception rate. According to (Troxel and Whitworth, 2007) Duration of postpartum anestrus averages 20 days longer for first-calf heifers than mature cows. 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).

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. Some authors reported higher conception rates in primiparous than in multiparous cows with Ovsynch and TAI (Peters and Pursley, 2002). Follicular size at TAI primiparous cows producing smaller follicle 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. 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).

Milk production

An increased level of production in dairy cows can lead to reduction of time and intensity of estrus expression (Lopez *et al.,* 2004). Kurykin *et al.,* (2017) reported that a delay in the resumption of cycling in cows after calving, weak estrus expression, and low detection rates of estrus have been the major factors limiting reproductive performance on dairy farms. Similarly, (Dobson *et al.,* 2008) stated that the pattern of estrus expression-standing to be mounted in dairy cows with high milk production has changed over the past half century. Many studies had shown the tendency of the dairy cows with high breeding value for milk yield of a delayed return to a normal ovarian cycle, reduced heat expression, increased silent estrus, poor conception rates, and inappropriate timing of insemination.

Although there is no correlation between estrus expression and milk yield (van Eerdenburg, 2008), duet to the metabolic clearance of steroid hormones related to high milk production which means removal of hormones due to metabolism (Sangsritavong *et al.,* 2002) probably reduces behavioral manifestation of estrus. In effect, in a study on 267 lactating dairy cows, Lopez *et al.,* (2004) reported that cows that produce higher milk yield (39.5kg/day) had a low level of serum estradiol concentration on the day of estrus, and duration of estrus was greatly reduced compared with lower producers (39.5 kg/day). In the same sense, in a study on 5,883 estrus events (López- Gatius *et al.,* 2005), each 1 kg increase in milk yield was associated with a 1.6% decrease in walking activity at estrus. Walking, however, is not the same as behavioral estrus score. Some behaviors are correlated with walking activity, but others are not Cows that produce more milk may have a deeper NEB and a deeper NEB will increase the NEFA level, which is correlated with lower walking activity (Adewuyi *et al.,* 2006).

Hormonal treatments

The importance of progesterone priming in the efficiency of estrus expression has been observed after use of synchronization programs (Vailes *et al.*, 1992). Progesterone increases the number of hypothalamic estradiol receptors during the luteal phase and, consequently, the sensitivity to estradiol (Blache *et al.*, 1994). This can result in a positive effect on expression of specific estrus signs (mounts, chin resting, and sniffing) compared to only estradiol treatment in ovaryectomized cows (Vailes *et al.*, 1992).Usually, no differences in duration of estrus have been observed between estrus induced by PGF2alpha and estrus occurring spontaneously (Roelofs *et al.*, 2005). Nevertheless, some authors observed a decrease of the accuracy of estrus detection after use of prostaglandins (Slenning and Farver, 1990).

2.8.2. Environment related factors

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 neurona 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).

Season

A seasonal effect has been detected on estrous behavior. Several study concluded that the estrus length was greater in summer compared to winter or spring this expected that heat stress is higher due to environmental factors; 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 very low effect of season of the year 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) reduced the estrus behavior (Landaeta-Hernández *et al.*, 2002). Heavy rain, strong wind, or high humidity reduce or suppress estrus behavior

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).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-El-Deen *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).

Circadian variation

Some studies suggest no variation in estrus during the day (Xu *et al.,* 1998). According to others studies, estrus behavior seems to be more frequent during the evening or nocturnal period (Mattoni *et al.,* 1988) or early morning and daylight hours (Amyot and Hurnik, 1987)Some observed variations can be related to usual management activities like feeding, cleaning, or milking.

The herd size

When the herd is larger, the number of social interactions between the animals will be greater. Most of the time (60%), mounting activity is initiated by heavier cows. The possibility to form sexually active groups (SAG) is higher when the size of the herd is increased. The degree of estrus expression and, therefore, the possibility to detect an estrus can be dramatically favored by the number of cows in estrus at the same time. The simultaneous presence of other animals in estrus allows the cow the opportunity to share estrus behavior so that estrus expression may also be result of sexual stimulation by other animals in estrus (Roelofs J, *et al.,* 2005).

2.9. Major factors affecting conception rates after AI

2.9.1. Sorting process

The decreased conception rates in sexed semen are attributed to the stress associated with the sorting process. The stress put on sperm cells includes the diluting of the semen sample, dyeing the sperm cells with a DNA binding agent (Hoechst 33342), mechanical forces including being sent through the flow cytometer at 60 miles per hour at 40 pounds per square inch (De Vries and Nebel, 2009) light from the laser used to illuminate the DNA, pressure from the collection process, and finally, centrifugation to purify the sample (Cerchiaro *et al.,* 2007). Sex-sorted semen does not survive cryopreservation as well as conventional semen (Garner and Seidel, 2003).

Reduced fertility, when using sorted sperm, has been attributed to damage of spermatozoa caused by the sexing process (Seidel and Garner, 2002). This includes staining and incubation of spermatozoa with Hoechst 33342, sperm dilution, exposure to high pressure and laser light, the rapid projection into the collection tube and also centrifugation to concentrate sorted sperm. After sorting, spermatozoa are partially capacitated resulting in a shorter life span and consequently in reduced fertilizing capacity (Vazquez *et al.*, 2003).

Additionally the nature of the damage to sperm has not been explained adequately by research, but there are many possibilities, including stretching the sperm tail as droplets are formed at the nozzle opening, and continued binding of the Hoechst 33342 to sperm post fertilization, slowing down progression of the first cell cycle between fertilization and the first cleavage (Seidel *et al.,* 2012). An unsettling point is that the damage appears to be only partially compensable by increasing sperm numbers per dose (Seidel, 2014).The technology of semen cell sorting is continuously being improved but the viability and quality of sperm during sorting procedure will be reduced (Razmkabir, 2018).

2.9.2. Number of sperm deposited

Conception rates in sex-sorted semen are also affected by the number of sperm cells per straw. The standard dose for a straw of sexed semen is approximately 2 x 10^6 sperm cells(Garner and Seidel, 2008;Healy, House and Thomson, 2013). Conventional straws have approximately 15-20 x 10^6 sperm cells per standard dose (Healy *et al.*, 2013). The lower number of sperm cells in sexed straws is because of the cost of the equipment and expertise required for the sorting process, the time needed to create a dose of sexed semen, and the variability in bulls' semen viability to survive the sorting process. According to (DeJarnette *et al.*, 2009)sex-sorted semen can enhance the differences in sire fertility rates. The reduced number of sperm cells in a dose exposes a sire's fertility which can be easily missed when more sperm cells are present (Dalton, 2016).

(DeJarnette *et al.,* 2010)found that by increasing the number of sexed semen cells from 2.1 x 10^6 to 3.5 x 10^6 did not increase conception rates (DeJarnette *et al.*, 2010). Both dosages of sex-sorted semen had conception rates that were approximately 75% of conventional semen. When semen doses were doubled or tripled (4 x 10^6or 6 x 10^6), pregnancy rates only increased slightly (5-7%). Increasing the number of sexed sperm cells present does not compensate for the damage that occurs during the sorting process (Hall, 2011).

(Seidel Jr *et al.*, 1997) established the concept of low dose insemination with sexed sperm. They obtained a 22.4% calving rate in heifers that were inseminated with $1–2×10^5$non-frozen, sexed sperm. Soon thereafter, numerous field trials were conducted with fewer numbers of non-frozen and frozen/thawed sexed sperm. For most bulls in these field trials, pregnancy rates with $1–1.5×10^6$sexed frozen/thawed sperm inseminated into either the uterine horns or body were 70–90% of unsexed controls containing $20–40×10^6$frozen/thawed sperm inseminated conventionally (Seidel J *et al.*, 1997).

2.9.3. Site of semen deposition

Faulty insemination technique is a major factor causing low conception rate in many herds. Correct semen placement is critical. This study indicates that the major difference between Al technicians with high conception rates and those with low rates was semen placement. During mating, the bull deposits several billions of spermatozoa into the anterior vagina. However, because the cervix is a major obstacle for sperm transport, the number of spermatozoa that finally reach the uterine body usually does not exceed 1%. In artificial insemination, semen is generally deposited directly into the uterine body, thus bypassing the cervix and permitting the use of a considerably reduced number of sperm (López-Gatius, 2000).

Many studies have compared conventional semen deposition near anterior part of uterus near uterine horns with deposition into the uterine body. According to (López-Gatius, 2000; Williams *et al.,* 1988) and (McKenna *et al.,* 1990) sites of semen deposition did not affect the efficiency of conception. Furthermore, (Diskin *et al.,* 2004) reported an inseminator and site of conventional semen deposition effect (interaction), with evidence of either an increase, decrease, or no effect of uterine horn deposition on P/AI for individual inseminators. In a competitive insemination study, (Dalton *et al.,* 1999)reported a slight advantage in accessory sperm number attributed to conventional semen deposition near the utero-tubal junction compared with deposition into the uterine body. In Nelore cows, (Meirelles *et al.,* 2012), using conventional semen, reported increased fertility following deep intrauterine AI in the horn ipsilateral to the dominant follicle, as compared to seminal deposition in the uterine body (Dalton, 2016).

In contrast, (Carvalho *et al.,* 2012) reported deposition of conventional semen in the uterine horns failed to improve fertilization rates in super ovulated Holstein cows. Because of the lower numbers of sperm used with sex semen, it is reasonable to hypothesize that placement of the semen within the reproduction tract would be critically important and that deep intrauterine horn insemination would lead to higher CRs. However, (Seidel, 1999;and Seidel and Schenk, 2008) found evidence that deposition of sexed semen in the uterine horns was superior to uterine body deposition.

2.9.4. Confirmation of estrus and inseminator skill

Incorrect estrus detection is the most common and expensive cause of failure of AI programs. Cows are often falsely identified as being in estrus and inseminated when conception cannot occur (Roelofs *et al.,* 2010).

Inseminating the cow is the final, but by no means least important, step in the process of estrus detection. Although professional inseminators palpate the reproductive tract of numerous cows every day, most are not trained to examine the uterus and ovaries and therefore to confirm estrus. This poses a serious practical limitation to the success of estrus detection procedures and AI. Multiple AIs are undertaken in cows that are not ready for service or are pregnant (Roelofs *et al.*, 2010). The situation is further worsened by the fact that the insemination of pregnant cows can cause embryonic mortality or abortion. The occurrence of estrus during pregnancy has been extensively reported. Pregnant cows stood willingly to be mounted by another cow or bull at all stages of pregnancy (Thomas and Dobson, 1989) and more than 40% of cows with high milk progesterone levels may be inseminated (Nebel *et al.*, 1987).Thus, the first goal of any estrus confirmation program should be to positively identify estrus and to reject cows for insemination that are not ready for service or are pregnant.

Examination of reproductive organs either by hand or by ultra-sonography through rectum allows for a correct diagnosis of estrus when the animal is ready for service (Roelofs *et al.*, 2010). A proper examination of the reproductive tract does not seem to impair uterine or ovarian physiology. The physical properties of the uterus and vaginal fluid (López-Gatius *et al.*, 1996); (Hässig *et al.*, 2006) during estrus may also be used as a reference for detecting estrus. A cow can be classified as ready for service when the corpus luteum is manually or by ultrasound estimated to be either less than 10mm or non-detectable, the largest follicle shows some fluctuation upon slight pressure and has an estimated diameter of 12 to 25 mm, the uterus is highly turgid and contractile to the touch, and vaginal discharges are copious, fluid, and transparent (López-Gatius and Camóón-Urgel, 1991)Vaginal fluid can easily be obtained at the time of insemination by gentle suction from the cranial vagina using a plastic inseminating sheath and a 50-mL syringe and examined for transparency, fluidity, and blood or pus contents (López-Gatius *et al.*, 1996).

In a study of estrus confirmation by trans-rectal palpation performed on 6084 normal repeat dairy cows following the first AI (López-Gatius and Camóón-Urgel, 1991) the 150-day non return rate for rejected cows with a corpus luteum greater than 15mm in diameter was 87%. Data from a subsequent larger study (Sturman *et al.*, 2000) confirmed the benefit of examining the reproductive tract per rectum at the time of insemination to inseminate cows at estrus only. In Israel, inseminators are trained to check the tone and symmetry of the uterus by trans-rectal palpation and to examine vaginal fluid. In this country, Israel, it is common practice to evaluate several factors indicative of estrus before insemination to avoid the insemination of pregnant cows, of cows that are not at the appropriate stage of the estrous cycle, or of those with purulent discharge. During the period of study performed in Israel (1996 –1997), inseminators rejected about 16% of the cows submitted for re insemination and the 95% accuracy of rejection was 44% pregnant cows. The main reasons for rejection were the condition of the vaginal fluid and tone of the uterus. In the same study, it was confirmed that the criteria used for submitting cows for re insemination in areas around New York State were similar to those used in Israel. Both diminished efficiency of AI and failure to detect estrus correctly should warrant the re-evaluation of inseminator training to confirm estrus. Examination of vaginal fluid and uterine palpation before insemination have been shown to improve pregnancy rates, reduce abortions, and reduce the unnecessary use of semen, all of which contribute to the improved reproductive performance and consequent profitability of dairy herds (Roelofs *et al.,* 2010).

2.9.5. Fertility of Bull

Historically, the assessment of male fertility has focused on the quantity and quality of sperm delivered to the female. Heat dissipation is an essential mechanism for the maintenance of normal body temperature and normal functioning of body organs (Verma and Husain, 1990). High ambient temperature has an adverse effect on health and sexual behavior of animals like puberty, testicular degeneration and reduces percentages of ejaculate, normal and fertile spermatozoa in males, due to disturbances of sexual activity. Regarding semen quality (Hansen, 2013) reported that fertility increases with increasing numbers of structurally intact and motile sperm

Scrotal circumference and testosterone have highly significant positive correlation with ejaculate volume, MA (Mass activity) and Individual motility (IM) and has a negative correlation with head, mid-piece, and tail abnormalities. The results indicated that SC and testosterone are useful indicators of sperm production and important criteria for selection of high genetic potential young Murrah bulls (Kumar *et al.,* 2014) studied in Aberdeen Angus, Hereford, Poll Hereford and Charolais breeds. (Singh *et al.,* 2014) found that no significant correlation between First service conception rate (FSCR) and scrotal circumference.

Similarly, no consistent relationship existed between sperm concentration and scrotal circumference. The testicular thermoregulation mechanism plays an important role in normal spermatogenesis, which maintains the fertility of mature bull, alteration of thermoregulation mechanism have a deleterious effect on spermatogenesis (Hansen, 2013).

2.9.6. Timing AI

To date, several groups have studied the appropriate timing of AI relative to the onset of estrus or ovulation in cows bred with non-sorted sperm (Dransfield *et al.*, 1998; Pursley *et al.*, 1998;Hockey *et al.*, 2010).The general consensus is that later AI (>12 h after the onset of estrus) usually results in greater fertilization rates but lower embryo quality when compared to insemination closer to the onset of estrus (Saacke, 2008). For example, a large field study that included 17 herds and 2,661breedings demonstrated that inseminating cows with non-sorted sperm >24 h after the onset of estrus resulted in a dramatic reduction in the frequency of pregnancy compared to inseminations performed between 4 and 12 h after the onset of standing estrus (Dransfield *et al.*, 1998).

In a small field trial, (Schenk et al., 2009) reported increased P/AI in heifers receiving AI18 –24 h after the observed onset of estrus, as compared to those inseminated at 0 –12 h. It is therefore reasonable to expect that decreasing the insemination-ovulation interval may be critical for achieving greater conception rates with sex-sorted sperm following TAI (Schenk *et al.*, 2009).

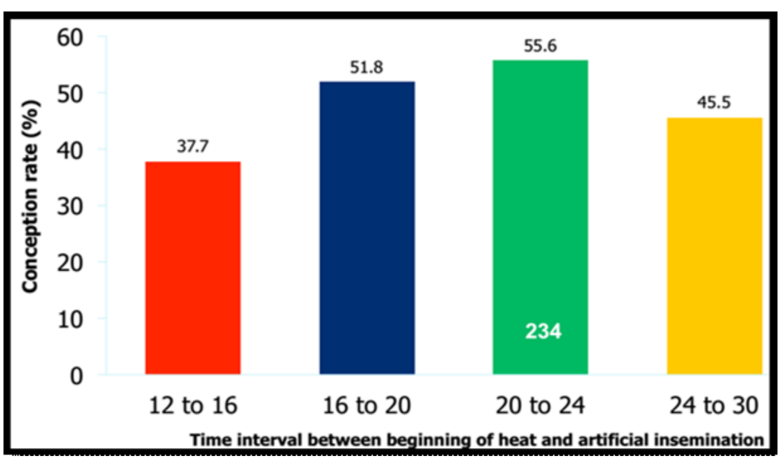

Figure 8: Time interval (hours) between beginning of heat and insemination related to conception rates in heifers
Source: Jordan et al, (2014).

2.9.7. Environmental factors

The decrease in conception rates during summer seasons can range between 20 and 30%, with evident seasonal patterns of estrus detection (De Rensis and Scaramuzzi, 2003). Elevated environmental temperatures negatively affect the cow's ability to display natural mating behavior, as it reduces both the duration and intensity of estrous expression (Orihuela, 2000). A reduction in estrous behavior has been argued to be the result of reduced DMI and the subsequent effects on hormone production (Westwood *et al.,* 2002).

Moreover, reduced estrous behavior may be attributed to man's domestication of bovine breeds, which has attempted to change the cow from a "seasonal" to "year round" breeder. The cow's natural selection for seasonal breeding has been argued by some to be reduced due to improved feed quality and availability, improved health monitoring, and care for the calf thus reducing the need to express various components required for successful reproduction (uterine health, embryo quality, hormone concentrations) on a strictly seasonal basis. However, the encompassing effects of heat stress on reproduction are persistent and exacerbated in the summer months, and year-round breeding continues to be problematic for producers (Mellado *et al.,* 2014).

Some studies indicate that high temperature has a negative effect on the reproduction process of dairy cows owing to undetected estrous cycle, decreased estrous period intensity (De Rensis *et al.,* 2015) effects on the physical activity and low fertility (López-Gatius, 2013). High environmental temperature has a detrimental effect on embryo survival (García-Ispierto *et al.,* 2006) as well as on the survival and motility of sex-sorted sperm (Mellado *et al.,* 2014). Cerchiaro *et al.,* (2007) reported that the pregnancy rate of sexed AI in heifers was lowest in summer (44.2%) and significantly lower than that in spring (53.9%), autumn (50.8%), and winter (50.7%). Similarly, (Mellado *et al.,* 2014) reported that the pregnancy rate of sexed AI in heifers in summer (33%) was only approximately half of that in winter (64%). The pregnancy rates in dairy cows are generally lower than those in heifers, but show the same trend in that summer is the worst season:16% in summer and 21% in winter in a recent study (Mellado *et al.,* 2014).

2.9.8. Thawing procedure

Frozen semen in straws has become the universally accepted unit of storage and transfer of bovine genetics to cattle procedures which depends on preserve the functional activity of spermatozoa (viability and fertilizing ability)(Bearden *et al.,* 2004).High viability and motility of spermatozoa are important factors for successful artificial insemination (AI) because a significant correlation between post-thawing sperm viability and subsequent conception rate has been reported (Correa *et al.,* 1996).The freezing and thawing of semen inevitably reduces the proportion of motile spermatozoa and causes ultra-structural, biochemical and functional damages (Senger, 1980). It has been shown that an increase in post-thaw viability will result in increased fertility of the semen (Rastegarnia *et al.,* 2013).Thawing procedure is just as important as the freezing procedure in terms of its impact on the survival of spermatozoa (Nur *et al.,* 2003).

Many researchers have been conducted to determine the optimal thawing temperature, duration and increased to know the adequate thawing rate that may give highest percentage of viable spermatozoa after post thawing process(Pace *et al.*, 1981; Correa *et al.*, 1996) However, a number of studies have shown that thawing temperatures as high as 60-80 ^{0}C could further improve post-thaw motility (Dhami *et al.,* 1996). In some countries, pellets of sperm thawed at +55 ^{0}C. Some researchers propose to use for thawing frozen semen of bulls higher temperatures-from +50 ^{0}C to +75 ^{0}C or even 100-150^{0}C (Dhami *et al.,* 1996). Many studies have been conducted to assess the influence of high thawing temperatures on sperm survival and motility, using different thawing rates, for bulls (Senger, 1980 ; Al-Badry, 2012).

Al-Badry, 2012conducted research in Iraq, frozen Semen was thawed in the following procedures: 5°C for 30 min, 37°C for 20 sec, 37°C for 30 sec and 60°C for 8 sec and motility, live, morphology and Post-thaw livability of sperm cells was assessed by determining the percentage of progressively motile sperm at 0, 2 and 4 h of incubation at 37°C. Results revealed that motility, live, abnormality, intact acrosoma and Post-thaw viability of sperm cells, were significantly ($p<0.05$) higher for the

37°C for 30 sec and 60°C for 8 sec than the other thawing methods and post-thaw semen can maintained at water bath 37°C until 3 h. Also thawing procedures at 37°C for 30 sec it is recommended to use in Iraq because it was showing good quality of post thawing semen and easy to use in field.

3. MATERIAL AND METHODS

3.1. Study Area

The study was carried out at Holeta dairy farms from January to June 2020. Holeta is a town of Welmera district and situated in Oromia special zone surrounding Addis Ababa. The town is located 29 kilometers west of Addis Ababa at 9°30' N and 38°30' E with altitude ranging from 2300-3800m above sea level which is actually parts of central highlands of Ethiopia. The average annual minimum and maximum temperatures were 6° and 22°C, respectively. The annual rainfall ranges from 900-1100 mm. The area receives bimodal rainfall with two rainy seasons in a year. The short rainy season occurs between March and May and the main rainy season is during June to September, while the dry season is from October to February. Holeta dairy farm consist three breeds; local (Boran breed), jersey and crossbred (Boran X Holstein) which are managed under semi-intensive farming system. Grass hay constituted the major proportion of the feed supply. Whenever there was a basal diet is grass, teff (Erogrostis teff) straw was substituted. The amount of concentrate offered depended on the volume of milk from each cow. Animals were allowed to graze during daytime (from 7:30am to 3:00pm) and housed at night in a barn. Cows were machine milked twice a day. Animals on the farm were regularly vaccinated against common infectious diseases such as Lump skin disease, Anthrax, Blackleg, and Foot and mouth disease. Regular preventive treatments were administered against prevalent endo- and ecto- parasites.

3.2. Study animals

A total of 71dairy cattle (35 Boran and 36 Crossbred) were included into this study. All cows were greater than 60 days postpartum. The parity ranges between 1-8 and body condition score range from 2 to 5 on a scale of 1 to 5. Rating the body condition was done subjectively based on fat cover and flesh over the ribs, loin and tail head (Roche *et al.,* 2009). Prior to the start of the experiment, status of reproduction of cows and heifers was confirmed based on farm record. Animals with record of reproductive problems were not included into candidate. In animals selected to be included, reproductive organs were palpated per rectum followed by ultra-sonography to assess the presence of mature corpus luteum on either of ovaries.

3.3. Study design and Synchronization protocol

Candidate animals were divided into two by breed and within breed into two by parity (cows and heifers) (two by two experimental design). On the day of start**,** all animal received 2ml (i/m) PGF2 alpha (Lutaprost® 250, Pharmadix Corp. SAC., Peru). All animals with mature CL on ovary at PGF2 alpha were meticulously observed any sign of estrus four times a day for 5 consecutive days (intentionally 7 am, 1 pm and 5 pm and mid night 12 am, Time of observation was not evenly distributed, 3x during day and only one time in the night. It would be better if 06:00, 12:00, 18:00, 24:00 on 24h basis). Any symptoms of estrus (for example, bellowing, sniffing, clear vaginal discharge, mounting other cattle, allowing other cattle to mount them, and other related signs) were recorded. Animals with no CL on ovary at start of PGF2 alpha received a second 2ml (i/m) at 11 day after the first PGF2.Animals were proportionally allocated (except in heifers group) to be inseminated by sex sorted semen or non-sorted semen. Insemination was done 12hr after onset of estrus.

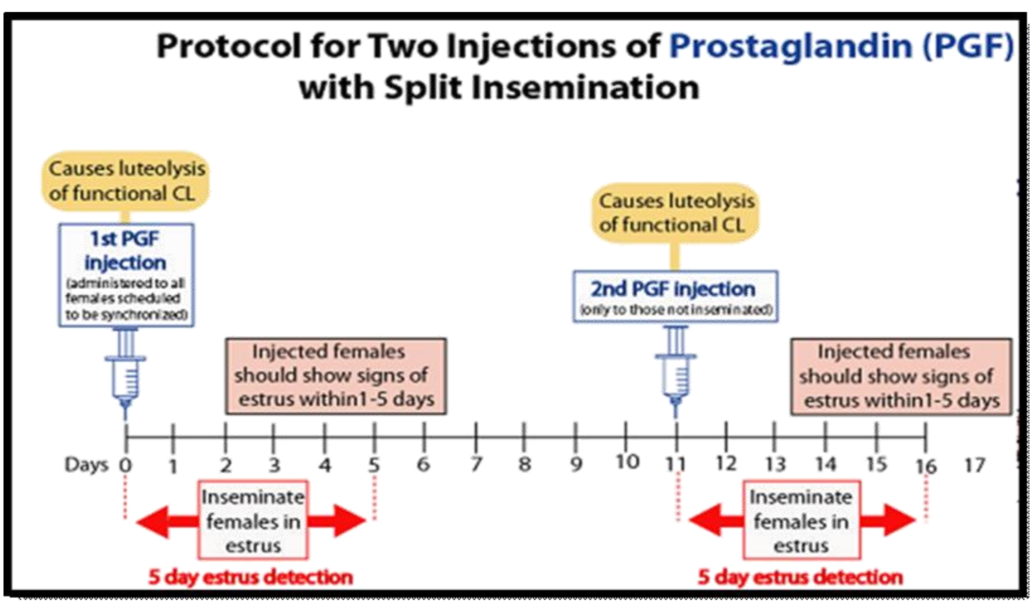

Figure 9: Schematic graphs showing single and double prostaglandin (PGF2-alpha) protocol

Source:

Naoman, 2019:

3.5. Pregnancy diagnosis

Pregnancies were confirmed through rectal palpation of the fetus at d 60 post inseminations. Pregnancy rate was calculated by dividing number of animals found pregnant by numbers inseminated.

Pregnancy rate (PR) = $\frac{Numbre\ of\ cows\ or\ heifers\ pregnant}{number\ of\ cows\ or\ heifers\ inseminated} X100$

3.6. Data Collection and Analysis

The collected data were, coded and entered in Microsoft excel spread sheet and All analyses were performed using R software (Team, 2015). Generalized Linear Model was used to analyze the effects of breeds, parity, body condition score and treatment on estrus response and additionally, effects of semen type on conception rate (CR). Independent t-test was used to analyze mean interval to estrus after injection, mean of standing estrus, duration of estrus. P-value, chi-square, odds ratio and confidence interval associated with above mentioned factors were evaluated on estrus response and conception rate whereas, mean of interval to estrus, standing estrus and duration of estrus were compared among breeds and parity. Percentage was used to estimate amount of animals responded and conceived.

4. RESULTS

4.1. Estrus response rate

Heifers were significantly (P= **0.036)** more responded (90.9%) to PGF2α than cows (65.3%). Boran cattle were slightly less responded to PGF2α (69.4%) than crossbred cattle (77.1%). However, the difference was not statistically significant. The details of the factors considered and estrus response rate were indicated in Table 1.

Table 1: Effect of factors affecting estrus response rate

Factors	Category	Estrus proportion	Percent (%)	95%CI	Odd ratio	X^2	p-value
Breeds	Boran	25/36	69.4	-1.9-1.3	0.49	0.463	0.465
	Cross	27/35	77.1				
Parity	Heifers	20/22	90.9	-1.1-3.2	0.87	0.01	**0.036**
	Cows	32/49	65.3				
BCS	<3sub-opti	25/37	67.5	-0.49-3.0	2.6	0.9	0.327
	≥ 3optimum	27/34	79.4				

4.2. Treatment to Estrus Onset Interval and Duration of Estrus

Boran breed shows slightly longer interval from PGF2α to estrus onset than crossbreed. Similarly the interval from PGF2α to estrus onset was longer for heifers than cows. However, these differences were not statistically significant (P>0.05). The average duration of standing estrus was longer in cross breeds than in Boran. Similarly, the duration of standing estrus was longer in heifers 9.0h than in cows 8.6h. The duration of estrus was not significantly different (P < 0.05) among both breeds and parity (Table 2 and 3). The duration of estrus was significantly longer (P < 0.05) in cross breed than in pure Boran breeds (Table 2). The average duration of estrus recorded in the cows and heifers was

14.6h and 15.87h, respectively. The difference in duration of estrus was not significant (P > 0.05) within parity.

Table 2: The mean values of heat characteristics among breeds

Heat characteristics	**Breeds**	**Proportional to estrus**	**Percent (%)**	**Mean values(h)**	**t-value**	**p-value**
Interval to estrus	Boran	25/36	69.4	75.96	1.26	0.2136
	Cross	27/35	77.1	68.25		
Standing estrus	Boran	25/36	69.4	8.3	-1.709	0.0938
	Cross	27/35	77.1	9.4		
Duration of estrus	Boran	25/36	69.4	13.96	-3.25	**0.0020**
	Cross	27/35	77.1	16.70		

Table 3: The mean values of heat characteristics among parity

Heat characteristics	**Parity**	**Proportional to estrus**	**Percent (%)**	**Mean values(h)**	**t-value**	**p-value**
Interval to estrus	Cows	32/49	65.3	67.4	1.77	0.08617
	Heifers	20/22	90.9	79.25		
Standing estrus	Cows	32/49	65.3	8.6	-0.711	0.4804
	Heifers	20/22	90.9	9.0		
Duration of estrus	Cows	32/49	65.3	14.6	-1.44	0.1553
	Heifers	20/22	90.9	15.87		

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4.3. Estrus behaviors

The estrus expression rates of Boran and Boran ×HF crossbred cows and heifers synchronized with single and double PGF2 alpha are summarized in Table 4. Estrus characteristics were evaluated, for those in heifer/cows that receive the two synchronization protocol (single and double), of the total 52 animals responded, 82.6%, 78.8%, 76.9%, of animals showed vaginal mucus discharge, mounting others and standing to be mounted, respectively.

Table 4: Details of behavioral estrus characteristics after hormonal injection on responded animals

Estrus behaviors	**Animals responded**	**Animals Shows signs**	**Percent (%)**
Bellowing	52	26	50%
Restlessness	52	30	57.6%
Standing to be mounted	52	40	76.9%
Vaginal mucus discharge	52	43	82.6%
Mounting others	52	41	78.8%
Raised tail	52	39	75%
Loss of appetite	52	27	51.9%
Swelling of vulva	52	39	75%
Sniffing	52	39	75%
Chin resting	52	33	63.4%
Micturition	52	17	32.6%

4.4. Pregnancy Rate and Factors Affecting Pregnancy Rate

The overall pregnancy rate was 56% (28/52). The pregnancy rate of the Boran and crossbreds was 54.1% (n=13) and 57.6 %(n=15) respectively. Pregnancy rate was significantly higher ($P<0.05$) heifers 72.2 %(n=13) than cows 46.8% (n=15). The odds of being pregnant was 8.2 times higher in heifers (95% CI of OR: 0.21, 4.01) compared with cows. The details of factors that affected the pregnancy were indicated in table 5.

Table 5: Effect of Breed, Parity, Body condition score, Treatments and Semen type on Conception Rate

Factors	Category	Conception proportional	Percent (%)	95% CI	Odd ratios	X^2	p- value
Breeds	Boran	13/24	54.1%	-2.51-0.39	0.344	0.06	0.152
	Cross	15/26	57.6%				
Parity	Cows	15/32	46.8%	0.21-4.01	8.284	3.23	**0.029**
	Heifers	13/18	72.2%				
BCS	<3 sub-optim	13/24	54.1%	-0.42-2.91	3.476	0.55	0.143
	>3optimum	15/26	57.6%				
Treatment	Single	22/40	55%	-0.96-2.37	2.020	0.05	0.409
	Double	6/10	60%				
Semen type	Conventional	16/24	66.6%	-4.73- -0.67	0.067	9.87	**0.009**
	Sexed semen	12/26	46.1%				

5. DISCUSSION

The aim of the present experiment was to evaluate the factors affecting estrus response rate, and estrus characteristics of Boran and cross breed cows and heifers. The study was conducted majorly to evaluate pregnancy rate to sex sorted semen and high grade non sex sorted imported semen in a 2X2 factorial design in Boran and Boran X Holstein cross cows and heifers. The overall estrus response rate (73.2 %) in present study was higher than that of Million, et al (2011) who reported 67.3% of estrus response rate in Boran and Cross breed at Holeta Agricultural Research Center. Păcală, et al., (2009), reported 68% of animals exhibited estrus following PGF2α administration. Murugavel and his colleagues (2010) reported 70 to 90% estrus rate within 2 to 5 days when PGF2 was administered to cows with a functional corpus luteum. The result of estrus expression rate in present experiment was little bit higher than Ejigayehu, (2018) reported 72.3% of estrus expression rate of Boran and Cross (Boran x Holstein) after single and double PGF2α injection in Bishoftu Agricultural Research Center. This difference of estrus response might be due to the type of PGF2 protocol used and effectiveness and type of drug used in comparison with other reported studies.

The present result revealed that the estrus response rate was not influenced by breeds. This result was not supported by Bó et al., (2003) who reported poor estrus expression of *Bos indicus* breed in tropical region. Similarly, Gugssa, (2015) reported the estrus response rate was higher in crossbreds (91.1%) than local breeds (83.3%). Difference might be management of animals between dairy farms or genetic factors of animals. Parity was statistically significant ($p<0.05$) effect on outcomes of estrus synchronization. These have been previously discussed by Rae et al., (1993). In the current result, among 17 cows that did not manifest estrus, 13 of them was primiparous suckling cows with reproductive stages greater than 60 days. The significant difference between parity might be due to delayed resumption of ovarian cyclicity after post-partum in cows and also lower estradiol hormonal concentration due to metabolic clearance rate of suckling cows (Opsomer *et al.*, 2002).

The results of this study indicated that the difference in average interval to onset of estrus after first PGF2a injection was not significant by breed. The present results strengthened with finding reported by Patil and Pawshe, (2011). However, the average time required to manifest estrus after injection of prostaglandin F2 alpha was significantly differ between cows and heifers. On the other hand, the effect of PGF2a varies depending upon the stage of the luteal phase at the time of injection. When prostaglandin F2α is given through the mid between 10 to 14d and late 15 to 19d phase of luteal estrus synchrony is increased.

Duration of standing estrus in Boran and cross breed observed in the current studies was reported 8.3h and 9h respectively. This result was higher than mean of standing estrus reported by Dransfield (*et al.*, 1998). In present study, the difference in mean duration standing estrus was not statistically significant (p>0.05) by breeds (Boran and cross) and parity (cows and heifers).

The average duration of estrus recorded in the Boran and Cross was 16.7 h and 13h, respectively. In agreement with the present study, a significant breed effect on the duration of estrus was reported (Rae *et al.*, 1999). Plasse., et al.,(1970) reported that duration estrus in *B. taurus* females varied from 4 to 48 hr with means reported between 13.60 and 19.30 hr, while in *B. indicus* cows the mean duration of estrus was short (6.70 h) with rage of 2 to 22 hr. According to Orihuela (2000), cattle breed differs with estrus duration which may in turn affect the mounting duration between individuals in a herd. Also, zebu cattle do not exhibit obvious estrus signs like exotic breeds; perhaps because estrus is short and subdued (Woldu *et al.*, 2011). The exact reason for breed difference in duration of estrus in present study are unclear but may be related with physiology of crossbreds produce high circulating estradiol due to a low metabolism of steroid or difference in the interval from estradiol surge to subsequent expression of between Boran and Boran x Holstein.

There was no significant difference in the duration of estrus between cows and heifers in this study. The present result of duration of estrus in heifers (15.4) was a bit similar with Aoyagi, (2003) who reported the duration of standing estrus in heifers to be 16.0 h, on average. Study made by Million *et al.*, (2011) indicated that the Boran breed tended to have a longer interval to estrus and shorter duration of estrus than Boran*Holstein crossbred cattle treated under similar conditions. The length of estrus recorded in the present studies in cows (16.7h) was almost the same with result of studies by Pawshe (1990) that the length of synchronized estrus was 18.25+0.4h.

Pregnancy rate was higher in Boran x Holstein (57.6%) than Boran (54.1%), (P> 0.05). This finding disagrees with the previous study by Alebachew, (2018) who reported that conception rate was higher in Boran than zebu-Holisten cross. However, in agreement with present finding, Khatun *et al.,* (2014) reported that conception rate lower in local cows (52.9%) than Friesian cross (62.3%) cows in Bangladesh. Marongiu *et al.,* (2002) observed higher conception rate in indigenous cows than cross and exotic genotypic group. This might be due to better synchrony of ovulation and fertilization as the existing follicles were influenced the next wave of follicles during induction.

Body condition was not statistical significant (P>0.05) effect on conception rate in present study. This finding is in agreement with the previous findings of 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; Ciptadi *et al.,* 2012; Tazangi and Mirzaei, 2015) who reported that body condition influenced conception rate and concluded conception was higher in high body condition score animals. The reason for higher response to GnRH in cows with high BCS was probably attributed to an earlier postpartum resumption of cyclicity (Moreira *et al.,* 2000).

Pregnancy 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 pregnancy rates. Similarly, (Alemneh *et al.,* 2015) also found no difference in conception rate based on treatment groups. Actually, differences in the quality of semen, time of insemination, and technique of insemination all may affect pregnancy rates (Nebel *et al.,* 2000, Bekana *et al.,* 2005).

In the present study, pregnancy rate was significantly higher in conventional semen 62.5% than sexed semen (46.1%). The preset result was higher than work in some previous studies (Djedović *et al.,* 2016) reported 55% conception rates for conventional and 44% for sexed semen. Another study on farm records in Australia (Healy *et al.,* 2013) revealed 52% pregnancy rates for sexed semen and 58% for conventional semen. In a study in Denmark (Borchersen and Peacock, 2009) the conception rate using sorted semen was 5% points lower than with conventional unsexed doses for Danish Reds, 7% points for Jerseys, and 12% points for Holsteins. Again Based on Danish A.I. field data, Borchersen and Peacock (2009) found that the conception rate using sorted semen was 12% lower than that of conventional doses for Holsteins. In contrast to present study conception rate of 69.7% (30/43) for sexed semen and 66.5% (1545/2325) for unsexed semen following AI was reported in China (Lu *et al.,* 2010). Generally the reduced conception rate of sexed semen in present study might be due to for several reasons, this includes potentially reduced lifespan of sex-sorted sperm in the female reproductive tract (Maxwell *et al.*, 2004), fewer numbers of sorted sperm/straw (DeJarnette *et al.*, 2008) and possible pre-capacitation induced by the sorting procedure (Lu and Seidel Jr, 2004).

A significant difference ($p<0.05$) was observed in an overall conception rate of heifers (72.2%) than in cows (50%). In the present result the mean conception rate of heifers was 80% under conventional semen and 69.2% under sexed semen. These values were higher than Norman *et al*. (2010) reported conception for heifers was 56% under conventional semen and 39% for sexed semen; the corresponding conception rates for cows were 30 and 25%, respectively. De Jarnette *et al.,* (2011) reported the conception rate for sexed sperm per inseminate was 46%, and the control conception rate with conventional semen was 61% in heifers. In nulliparous Australian Holsteins, Healy *et al*. (2013) reported empirical conception rates of 31.6% and 39.6% for sexed and conventional semen, respectively. Nebraska researchers (Deutscher *et al.,* 2002) reported a 3% to 13% reduction in AI pregnancy rates when using sexed versus conventional semen in yearling beef heifers. Similarly, (Rhinehart *et al.,* 2011) co-workers reported a 4% to 38% reduction in pregnancy rates when using sexed compared to conventional semen in beef heifers. Probably lower number of sperm cells may achieve greater success in heifers rather than in cows because the virgin reproductive tract lacks the intense nutritional demands such as no lactation or postpartum stress (Dalton, 2007). Additionally, heifers are not under effect of high milk production, and usually don't have reproductive infection because they have never calved before (Silva, 2009).Using good technique and proper insemination timing may result in better conception rates to sexed semen (Silva, 2009).

6. CONCLUSION AND RECOMMENDATIONS

Based on the result, it could be concluded that heifers of both breed of cattle have better pregnancy rate than cows both to sexed semen and conventional semen. So when the intention is improving dairy replacement heifers using sexed semen and genetic improvement using high quality imported semen on a local Boran cattle, certainly heifers should be used. The large number of lactating local Boran cows didn't not respond to PGF2 comparing the counter Boran X Holstein lactating cows. Parity significantly estrus response rate in which heifers showed higher response rate than cows. Boran cattle tended showed longer interval to estrus and shorter duration of estrus compared with Boran ×H crosses under similar treatment and management conditions. Induction of estrus following by PGF2 showed significant variation on interval to onset of estrus and time of standing estrus between breeds and parity.

The following recommendations are forwarded:-

- Heifers respond very well to PGF2 which may indicates PGF2 can be used to synchronize virgin heifers'.
- The pregnancy rate to sexed semen was moderate. Hence, the use of sex sorted semen is recommendable to improve the proportions of female calves, especially when used on virgin heifers of both local (Boran) and Boran X Holstein cross. This can be used by medium and large dairy farmers to expand the numbers of milking cows.
- Pregnancy rate to non-sorted high grade imported semen was also moderate. Hence when crossbreeding to speed up genetic improvement and to expand good milk yielding dairy cattle, this semen can be used on Boran heifers which was available at lower cost.
- The cost of sex sorted semen may not be affordable to small holder dairy farmers. The semen should be used in public and government dairy farm to reduce cost of replacement heifer and hence the smallholder farmers may be benefited that way.
- Failure to detect estrus in some legible cows (>60 DIM) and few heifers need further detailed study on factor which contributes to that failure to responded.

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8. APPENDIXES

Appendix1. Photograph during hormonal injection

Appendix 2 Photograph during reproductive examination and AI materials

Appendix 3 Photography of behavioral signs observed after prostaglandin injection during study

Appendix 4 Photograph during Artificial insemination

Appendix 5 Photography of data collection of estrus signs at grazing land with herders