EFFICACY OF CONTROLLED INTERNAL DRUG RELEASE (CIDR-B) IN SYNCHRONIZATION OF ESTRUS IN FRIESIAN X BUNAJI AND BUNAJI COWS

BY

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DEPARTMENT OF ANIMAL SCIENCE
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OCTOBER, 2014

DECLARATION

I hereby declare that the work in this thesis titled **"Efficacy of Controlled Internal Drug Release (CIDR-B) in synchronization of estrus in Friesian x Bunaji and Bunaji cows"** was carried out by me at the Department of Animal Science under the supervision of Professor P.I. Rekwot and Dr. P.P. Barje.

The information derived from literatures has been duly acknowledged in the text and a list of references provided. No part of this thesis has previously been presented for another degree or diploma in any University.

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Signature Date

CERTIFICATION

This thesis titled **"Efficacy of Controlled Internal Drug Release (CIDR-B) in synchronization of estrus in Friesian x Bunaji and Bunaji cows"** by Achi, Patrick Neyu meets the regulations governing the award of degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I want to dedicate this thesis to God Almighty, my Parents Achi Patrick Mallam (Mr), Achi Patrick Ruth (Mrs) and my siblings.

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ABSTRACT

The study was designed to evaluate the efficacy of Controlled Internal Drug Release (CIDR-B), CIDR + Prostaglandin $_{2\alpha}$ and Prostaglandin $F_{2\alpha}$ alone in synchronizing estrus, conception and pregnancy rates in Bunaji and Friesian x Bunaji cows. The study was conducted between July and October using forty seven cows (47) consisting of twenty four (24) Bunaji and twenty three (23) Friesian x Bunaji cows in a 2x3 factorial arrangement with eight (8) cows each except for treatment III which had seven (7) Friesian x Bunaji cows. Cows used for this study were rectally palpated for cyclicity, aged between 3-5 years and body condition score 2.5 and above. CIDR coated with 1.39g of progesterone was inserted through the vagina with the aid of an applicator, on day six (6) 2ml of $PGF_{2\alpha}$ (Estrumate) was administered intramuscularly before CIDR removal on day seven (7). Behavioural signs of estrus were observed after insert removal, standing to be mounted was the primary sign of estrus used in this study and cows that came into estrus were inseminated by experienced inseminators 8-12 hours after. Retention rates were 100 % for all the treatments. Estrus response rates were 62, 56 and 26 for CIDR and CIDR + $PGF_{2\alpha}$ respectively. Interval to onset of estrus was shorter for CIDR + PGF2α 3 hours and 1.75 hours than CIDR 5.0 hours and 5.0 hours in Bunaji and Friesian x Bunaji cows. Estrus duration was longer in Friesian x Bunaji; 12 hours CDIR, 13.5 hours than Bunaji 7.00 hours CIDR, 11.0 hours CIDR + $PGF_{2\alpha.}$ Number of mounts were more in Friesian x Bunaji cows; 9.00 hours CIDR, 11.25 CIDR + $PGF_{2\alpha}$ than in Bunaji 8.66 hours CIDR, 4.33 CIDR + $PGF_{2\alpha}$. More cows came into estrus between 6 am and 12 noon than 12 noon to 6 pm. Cows inseminated were rectally palpated for diagnosis of pregnancy. Conception rates were 33 % for Bunaji and 42 % Friesian x Bunaji treated with CIDR, 60 % for Bunaji and 50 % for

Friesian x Bunaji treated with CIDR + $PGF_{2\alpha}$ and 0 for Bunaji and 75 for Friesian x Bunaji treated with $PGF_{2\alpha}$. It is concluded from the study that, CIDR, CIDR + $PGF_{2\alpha}$ are capable of synchronizing estrus in Bunaji and Friesian x Bunaji cows. Prostaglandin was effective only in Friesain x Bunaji cows. Estrus response, conception and pregnancy rates could be improved using a combination of CIDR + $PGF_{2\alpha}$.

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CHAPTER ONE

1.1 INTRODUCTION

Synchronization of estrus (heat) involves manipulating the estrous cycle of female animals so they can be bred at approximately the same time. Estrus synchronization and artificial insemination (AI) are reproductive management tools that have been available to producers for over 30 years. These tools remain the most important and widely applicable reproductive biotechnologies available for cattle operations (Seidel, 1995). One measure of the reproductive efficiency of any cow-calf operation is the percent annual calf crop output. Reproductive inefficiency in cow-calf operation has been reported to reduce milk yield, number of replacement heifers and increase involuntary culling due to death, infertility and disease (Grohn and Rajala-Schultz, 2000). De Rensis (2000) reported an estimated loss of $300 million per year to the United States Dairy industry due to poor detection of estrus

There is abundant literature on the influence of season on the reproduction of domestic animals (Rekwot *et al.*, 1987). It is no longer in doubt that even in Nigeria the possibility of synchronizing estrus and ovulation in farm animals with the use of exogenous hormones exist. The broad objective of estrus synchronization programme is to be able to predict in advance the time a group of females that are ready to be bred and to obtain normal fertility at synchronized estrus.

During the last three decades, various estrus synchronization programmes have been adopted to control onset of estrus and artificial insemination in dairy cattle (Stevenson,

1997; Voh, Jr. *et al*., 2004a; b). These are dependent entirely on manipulation of the corpus luteum by prolonging the luteal phase with progestagins or inducing premature luteolysis by prostaglandins. It has been reported that manipulation of ovarian follicular development by Gonadotropin Releasing Hormone (GnRH), human Chorionic Gonadotropin (hCG) or Pregnant Mare Serum Gonadotropin (PMSG) may be necessary adjunct for synchronization of ovulation and improving fertility (De Rensis, 2000). There are several protocols available for synchronizing estrus among female animals. Traditional protocols are designed to mimic or control the corpus luteum of the ovary. New protocols have been designed to control ovulation and/or the follicular waves that occur on the ovary during the 21-days estrous cycle.

The synchronizing agents in use include progesterone compounds like Progesterone Release Intra-vaginal Device (PRID) (Sanofi, UK), Cystorelin, Ovacyst, Factrel, Fertagyl, Controlled Internal Drug Release (CIDR) (Carter Holt, New Zealand) and Synchromate B (Intervet, Holland) which extend the life span of the corpus luteum (CL) while luteolytic agents like Lutalyse (Upjohn, USA) and Estrumate (ICI, UK) which shorten the life span of the corpus luteum.

The CIDR is a T-shaped rubber device that contains progesterone. Short and long-term treatments with the CIDR have been studied for oestrous synchronization. Longer treatment periods (14-21days) resulted in a high degree of estrus synchrony but reduced fertility at the synchronized estrus (Macmillan and Peterson, 1990). But it has been suggested that hormonal injections can be administered along with the CIDR treatment to increase efficiency (Medina-Britos *et al*., 2001).

1.2 JUSTIFICATION

Reproductive performance has been declining in dairy cows with increasing number of days open and decreased conception rates over the last 25 years (Silvia, 1998). Several studies have estimated that ≤ 50% of the cows are detected in estrus (Barr, 1975; Stevenson and Britt, 1977; Heersche and Nebel, 1994). The use of milk progesterone assays indicate that between 5 and 25% of cows inseminated are not in estrus (Reimers *et al*., 1985; Nebel *et al*., 1987), with some herds having more than a 30% error rate. Average herd size continues to grow and making it even more challenging to accurately detect estrus (Xu and Burton, 1999). Increasing herd size and other factors have contributed to the difficulty with accurate estrus detection. While most producers have low estrus detection rates, leading to longer calving intervals and economic losses (Larson *et al*., 1992). Difficulties in estrus detection, silent estrus and unknown time of ovulation cause low reproductive performance in domestic animals (Zakari, 1981).

Estrus synchronization improves estrus detection rates (Ryan *et al*., 1999 and Xu and Burton, 1999). Synchronization of the estrus has the potential to shorten the calving season, increase calf uniformity, and enhance the possibilities for utilizing AI (Lamb, 2010), decrease culling rates, and improve net income to producers (Bellows *et al*., 1979). Regardless of the causes of the estrus detection problem, it has led to an increased interest in synchronization of estrus and ovulation. Estrus synchronization is a reproductive management tool that is particularly useful in artificial insemination (AI) and embryo transfer (ET) programs (Parish *et al*., 2011). It manipulates females' estrous cycles with one or more hormones to bring cattle into estrus (heat) within a short period of time

(Parish *et al*., 2011). Estrus synchronization is important for the convenient timing of the breeding of cattle in artificial insemination and embryo transfer programs.

Several methods have been used to synchronize estrus or induce heat in dairy cows; this include injection of prostaglandin (PGF2α) so as to shorten the estrous cycle and allows the cow to show heat during a restricted time period (Lamb, 2010).

Progesterone Releasing Intra-vaginal Devices (PRID; McPhee *et al.,* 1983) and Controlled Internal Drug Release-Bovine (CIDR-B) devices (Macmillan *et al.,* 1991) have been shown to successfully release progesterone at a fairly predictable rate. These two devices have been studied as methods for estrous synchronization as well as for studying follicular dynamics (Macmillan *et al.,* 1991). Macmillan and Peterson (1990) found a conception rate of 58% in heifers treated with CIDR devices for 7 days and the Prostaglandin F_2 alpha injected at device removal. The lining of the vagina absorbs the progesterone from the CIDR. Previous research has shown that increasing serum progesterone levels in dairy cows through administration of a controlled internal drug releasing device (CIDR) containing progesterone can result in increased conception and calving rates (Folman *et al*., 1990; Melendez *et al*., 2006). Buvanendran (1979), reported that several interrelated factors, such as genetic, physiologic, management and environmental factors are responsible for the less than average performance of dairy cows in Nigeria. Extreme environmental conditions such as temperature and humidity have been shown to decrease reproductive efficiency. Voh Jr. (1984) also reported that some of the factors responsible for low reproductive efficiency include irregular estrus cycles, poor signs of estrus and low fertility rates. Despite this the possibility of synchronizing estrus and ovulation in Nigerian cattle with exogenous hormone exist.

1.3 RESEARCH HYPOTHESIS

H_O: There will be no difference in estrus synchronization and conception rates when an injection of 2 ml of prostaglandin $F_2\alpha$ (Estrumate $^{(B)}$) administered on day 6 before removal of CIDR insert is compared with CIDR insert in place for 7days and 1dose of prostaglandin $F_2\alpha$ (Estrumate $^{(B)}$).

H_A: There will be difference in estrus synchronization and conception rates when an injection of 2 ml of prostaglandin $F_2\alpha$ (Estrumate $^{(B)}$) administered on day 6 before removal of CIDR insert is compared with CIDR insert in place for 7days and 1dose of prostaglandin $F_2\alpha$ (Estrumate $^{(B)}$).

1.4 OBJECTIVES OF THE STUDY

The objectives of the study were to determine:

1. the efficacy of CIDR alone, CIDR + $PGF_2\alpha$ or $PGF_2\alpha$ alone in synchronizing estrus in Bunaji and Friesian x Bunaji cows.

2. the effect of these protocols on the pregnancy and conception rates of Bunaji and Friesian x Bunaji cows.

CHAPTER TWO

LITERATURE REVIEW

2.1 Physiology of the bovine estrous cycle

2.1.1 Estrous Cycle

The bovine estrous cycle consists of a series of events which includes the growth and development of a follicle, its subsequent ovulation, and preparation of the uterus for conception, all to allow repeated attempts for pregnancy. The estrous cycle can be divided into two major parts, the follicular phase and luteal phase. The follicular phase includes the time from regression of the corpus luteum (CL) through ovulation, and can be further sub-divided into proestrus and estrus. The luteal phase includes the time from ovulation through regression of the corpus luteum and can be further sub-divided into metestrus and diestrus.

2.1.1.1 Phases of the estrus cycle

2.1.1.1.1 *Estrus phase*

Estrus is the most easily distinguished phase in the bovine reproductive cycle, as it is the time when the female is sexually receptive to the male. This behavior is caused by high concentrations of estradiol, the predominant hormone of the follicular phase. Estradiol increases about 3 days (d) prior to estrus from approximately 3.6 pg/ml during the luteal phase, to a peak concentration of 9.7 pg/ml the day before estrus (Wettemann *et al*., 1972). Estradiol concentrations have been reported to be greater in *Bos taurus* (Angus) than in

Bos indicus (Brahman) cows from days 7 to 17 of the estrous cycle (Segerson *et al*., 1984). However, Alvarez *et al*. (2000) reported no differences in basal or maximum estradiol concentrations for Angus and Brahman cows. Elevated estradiol concentrations also regulate the surge of luteinizing hormone (LH) that leads to ovulation (Walters and Schallenberger, 1984). Ovulation occurs approximately 26 hour (h) after the onset of behavioral estrus (Plasse *et al*., 1970; Pinheiro *et al*., 1998). The expression of estrus allows for copulation to occur, and provides the opportunity for pregnancy.

The primary and most reliable indicator of estrus is for a cow to allow herself to be mounted by another cow (Glencross *et al*., 1981). Secondary signs of estrus include mounting other cows, clear mucous discharge from the vulva, swelling of the vulva, restlessness, following other cows, chin resting, and lip curling. These signs can occur before, during, or after estrus and do not relate to the time of ovulation, but should instead be used as clues that a cow should be observed more closely (Nebel, 2003).

The duration of estrus varies between cattle of *Bos taurus* and *Bos indicus* breeding, and may also vary for cows undergoing a spontaneous naturally occurring estrus versus a synchronized estrus. Richardson *et al*. (2002) observed that estrus duration in *Bos taurus* heifers which underwent a synchronized and a spontaneous estrus did not differ in duration. Rae *et al*. (1999) reported that the duration of a synchronized estrus to be 8.5 hours (h) for Angus (*Bos taurus*) and 6.7 h for Brahman (*Bos indicus*) heifers, while crossbred (*Bos indicus* × *Bos taurus*) heifers had a duration of 11.9 h.

Many factors, including breed (Rae *et al*., 1999), environment (Landaeta-Hernandez *et al*., 2002), number of animals in estrus at a time (Hurnick *et al*., 1975; Landaeta-Hernandez *et al*., 2002), and estrus detection method (Rae *et al*., 1999) influence the duration of estrus. However, multiple studies indicate that *Bos indicus* heifers (Plasse *et al*., 1970) and cows (Randel, *1984*; Pinheiro *et al*., 1998) have a shorter duration of estrus compared to *Bos taurus* heifers (Richardson *et al*., 2002) and cows (Schams *et al*., 1977).

Alvarez *et al*. (2000) reported no difference in estrous cycle length of Senepol (tropical *Bos taurus*; 20.4 d), Angus (temperate *Bos taurus*; 19.5 d), or Brahman (tropical *Bos indicus*; 19.7 d) cows. However, Plasse *et al*. (1970) observed an estrous cycle length of 27.7 d in Brahman heifers evaluated over a full year, indicating that *Bos indicus* animals may have a longer estrous cycle. Length of the estrous cycle may also differ between heifers and cows, as Plasse *et al*. (1970) reported an average length of 27.7 d in Brahman heifers, compared to Llewelyn *et al*. (1987), who observed a 23 d estrous cycle in Boran cows. However, in *Bos taurus* beef cows and heifers, estrous cycle length has little variation from 21 d (Zollers *et al*., 1989; Mihm *et al*., 2000).

2.1.1.1.2. *Metestrus phase*

Metestrus is the stage following estrus from ovulation until the corpus luteum is fully functional, and generally lasts 3 to 5 d. Ovulation occurs during metestrus in cattle, approximately 24 h after the onset of estrus. In *Bos taurus* beef cows, ovulation occurs 24 to 36 h from the onset of estrus (Looper *et al*., 1998; Rorie *et al*., 1999). Plasse *et al*.

(1970) reported that ovulation occurred 25.6 h after the beginning of estrus and 18.9 h after the end of estrus in Brahman heifers. Likewise, Pinheiro *et al*. (1998) reported that ovulation occurred 26.6 h after the onset of estrus in Nelore (*Bos indicus*) cows. After the luteinizing hormone surge induces ovulation, the ovulated follicle undergoes cellular and tissue remodeling changes to form the corpus luteum. During metestrus, the predominante hormone switches from estradiol to progesterone produced by the corpus luteum. However, progesterone concentrations remain low as the corpus begins to develop and as the corpus luteum increases in size and progesterone output increases, the cow enters diestrus.

2.1.1.1.3. *Diestrus phase*

Diestrus is the longest phase of the estrous cycle, lasting 10 to 14 d, and is characterized by increased progesterone secretion. Progesterone concentrations have been shown to increase from 1 to 13 ng/mL when a corpus luteum is present in *Bos indicus* cows (Ruiz-Cortes and Olivera-Angel, 1999). In *Bos taurus* cows, progesterone concentrations increases from 4.7 to 12.0 ng/mL have been reported for cows with a corpus luteum (Henricks *et al*., 1971). Regardless of whether or not a pregnancy occurs, the corpus luteum forms into a fully functional, progesterone-producing structure. If an embryo is present in the uterus, the corpus luteum and high progesterone concentrations are maintained throughout gestation. If pregnancy does not occur, the corpus luteum remains active until d 17 to 18 of the estrous cycle, at which time it undergoes luteolysis, which is stimulated by prostaglandin $F_{2\alpha}$ secretion from the uterus and a rapid Progesterone concentrations allowing for another estrous cycle.

2.1.1.1.4. *Proestrus phase*

Proestrus occurs 3 to 4 d before estrus and is characterized by increased follicular growth. Following regression of the corpus luteum and a decline in progesterone, the predominate hormone becomes estradiol (Schams *et al*., 1998). During proestrus, a single dominant follicle emerges from a pool and is selected to grow into an ovulatory follicle which produces estradiol.

The length of the estrous cycle is approximately 21 d in *Bos taurus* breeds (Hansel *et al*., 1973).

2.2. Estrus Detection

Estrus detection is the process of determining if an animal is in estrus and is primarily conducted by visual observation. Cows display estrus following proestrus, when they have a large follicle producing adequate estradiol concentrations to induce estrus behavior. Estrus can be spontaneous and naturally occurring, or result from initiation of luteolysis by administration of prostaglandins.

Detection of estrus can be one of the most challenging aspects of an artificial insemination (AI) program.

2.2.1. Signs of estrus

The primary underlying changes in the cow that these methods of detection rely on include increased physical activity and locomotion (Farris *et al*., 1954), swelling of the vulva (Foote *et al*., 1979), and most importantly standing to be mounted by another cow.

The various signs of estrus are;

- Excited condition.
- Restlessness and nervousness.
- Bellow frequency.
- Reduced intake of feed.
- Peculiar movement of limbo sacral region will be observed.
- Licking and smelling other animals.
- Trying to mount other animals.
- Standstill when other animals try to mount. This period is known as standing heat.
- Frequent urination will be observed.
- Clear mucous discharge will be seen from the vulva.
- Swelling of the vulva will be seen.
- The tail will be in raised position.
- Milk production will be slightly decreased (in lactating animals).
- On Palpation uterus will be turgid and the cervix will be opened.

2.2.2. Methods of heat detection

2.2.2.1. *Pedometry*

Pedometry is a method of estrus detection which monitors the physical activity and locomotion based upon the number of steps a cow takes over a given period of time. Cows may be four times as active during estrus than any other phase of the estrous cycle (Kiddy, 1977).

This is monitored by a mechanically activated leg band that collects the information, which is later evaluated by a reader (Senger, 1994). Using this information, the optimal time for insemination can be predicted (Maatje *et al*, 1997). Pedometry is predominately used in dairy farms, as its application to beef operations is not practical due to the frequency of which the pedometer would need to be read.

2.2.2.2. *Measuring electrical Resistance*

Electrical resistance of vaginal fluids by use of probes is indicative of the stage of the estrous cycle the cow is in (Foote *et al*., 1979). These changes are notable due to vulvar mesenchymal tissue being 74% heavier during estrus than during diestrus because of tissue hydration (Ezov *et al*., 1990). This method of estrus detection is not commonly utilized in beef operations due to the frequency of which cows would have to be worked through a handling facility.

Therefore, estrus detection in beef operations relies primarily on the behavior of cows during estrus by allowing themselves to be mounted by other cows, which is also the most reliable indicator of estrus (Glencross *et al*., 1981). To determine whether or not a cow in estrus has been mounted by another cow, a variety of products are available to producers including paints, pressure sensitive patches, scratch-off patches, and computer transponders.

2.2.2.3. *Tail painting*

Painting of the tail head, used in conjunction with visual observation, is the least expensive of heat detection products, and has been indicated to yield similar conception rates to the more expensive alternatives (Xu *et al*., 1998). Another product, the Kamar® Heatmount Detector (Steamboat Springs, CO), is a pressure sensitive device, which releases red ink when activated and is visible from a distance on the cow. The device contains a timing mechanism which requires approximately three seconds of mounting changing from white to red. However, the Kamar® has been reported to yield false positive readings about 13.2 % of the time (Gwazdauskas *et al*., 1990).

2.2.2.4. *Estrus alert devices*

A newer product in the market is the Estrus Alert® Heat Detector (Western Point, Inc., Merrifield, MN), a scratch-off patch in the form of a sticker. This product features a silver, scratch-off surface, similar to that of a lottery ticket, which scratches away to reveal a brightly colored signal layer. Unlike the Kamar®, the Estrus Alert® allows a producer to determine if a cow has received only a few mounts compared to a large number of mounts based on the degree to which the patch is scratched off.

2.2.2.5. *Remote sensing devices*

The HeatWatch® (DDx, Inc., Denver, CO) system was the first remote sensing system to allow 24 hour monitoring of standing events associated with estrus (Nebel, 2003). This system utilizes a computer transponder tucked into a mesh patch, which is affixed to the tailhead of the cow. The transponder records data for each individual cow, including date, time, and duration of each standing event. This information is transmitted to a computer where the HeatWatch® software develops management and individual cow reports (Nebel, 2003).

2.2.2.6. *Teaser bulls*

Teaser animals can also be used to aid in the detection of estrus. These include peniledeviated, epididymectomized, vasectomized, or other bulls incapable of impregnating a female, as well as androgenized steers and cows, all of which can be fitted with chin ball markers to aid in the detection of cows in estrus (Burns and Spitzer, 1992). The chin ball marker works like a ball point pen, leaving ink marks on the dorsal surface of the cow in estrus.

2.3. Factors affecting heat detection

Breed differences are apparent in the onset of estrus, ability to detect estrus, duration of estrus, time of day estrus occurs, and the intensity of estrus. Rae *et al*. (1999) observed estrus durations for Angus (*Bos taurus*) heifers to be approximately 8.5 hours, while that of Brahman (*Bos indicus*) heifers was only 6.7 h. They also observed crossbred (*Bos indicus* × *Bos taurus*) heifers had an average estrus duration of 11.9 h. Other studies have observed estrus durations of 11 to 12 h in synchronized *Bos taurus* heifers (Richardson *et al*., 2002). Pinheiro *et al*. (1998) observed a 10.9 h estrus duration in Nelore (*Bos indicus*) cows. Rae *et al*. (1999) reported that breed had a significant effect on mounting activity during a synchronized estrus in Angus, Brahman, and Angus × Brahman, receiving an average of 19, 25, and 37 mounts, respectively.

Additionally, estrus may be more difficult to detect in *Bos indicus* cattle. Behavioral estrus in *Bos indicus* breeds may be expressed more by secondary signs of estrus, such as head butting (Galina *et al*., 1982; Orihuela *et al*., 1983). *Bos indicus* cattle may also have an increased occurrence of silent heats and absence of visual estrus (Dawuda *et al*., 1989; Lamothe-Zavaleta *et al*., 1991).

Synchronization of estrus promotes the formation of sexually-active groups, which in turn increases the mounting activities of both *Bos taurus* and *Bos indicus* cows (Hurnick *et al*., 1975; Landaeta-Hernandez *et al*., 2002) compared to a spontaneous, naturally occurring estrus.

Social status in the herd can also affect behavioral estrus. Landaeta-Hernandez *et al*. (2004) observed that dominant cows tended to receive less mounts than subordinate and

intermediate cows, due to the dominant cows avoiding being mounted by subordinate cows and by extension, dominate cows may take longer to be detected in estrus.

It has been indicated that the onset of estrus occurs more frequently during the night-time hours than daytime hours in both *Bos taurus* (Stevenson *et al*., 1996) and *Bos indicus* (Pinheiro *et al*., 1998) cows. Although Voh Jr. *et al*. (2004b) reported more Bunaji cows coming into estrus in the morning than night.

However, while Landaeta-Hernandez *et al*. (2002) observed more cows initiate estrus during night hours (60%) than day hours (40%), they also observed that more Senepol (*Bos taurus*) cows initiate estrus during the daytime hours than Brahman cows (*Bos indicus*).

Cows that are consuming feed, in a crowded pen or aisle, or housed on poor footing will demonstrate less mounting activity (Britt *et al*., 1986). Cows which have foot problems will display less mounting activity regardless of whether the problem is structural, subclinical, or clinical (Leonard *et al*., 1994). During a synchronized estrus, mounting activity is not effected by whether another cow or teaser bull is mounting the estrus cow (Pinheiro *et al*., 1998).

However, the same study indicated that cows undergoing a natural estrus received more mounts from a bull than another cow.

2.4. Follicular Development

Like many mammalian species, cattle are born with a finite amount of oogonia available during their lifetime. Oogonia are developed during mid-gestation when the primordial germ cells migrate to the gonadal ridge and are arrested in prophase I of meiosis and

stored in primordial follicles (Hirshfield, 1991). It has been hypothesized that granulosa cells modulate the transcriptional activity of the oocyte genome (De La Fuente and Eppig, 2001).

Around the time of puberty, these inhibitory factors are removed by LH stimulation, and just prior to ovulation meiotic divisions resume (Senger, 1999).

Oogonia stored in the ovary awaiting signals to grow are maintained in a quiescent stage and are known as primordial follicles. The primordial follicle is characterized by a single layer of granulosa cells surrounding the oocyte, and from this stage follicles gradually and continually leave the resting pool to begin growth (Fortune, 1994). These follicles can be in a quiescent state, with a single layer of flattened granulosa cells, or in an active state, with a single layer of flattened, and some cuboidal cells (Fair *et al*., 1997b). A quiescent, primordial follicle will have 5 to 8 flattened granulosa cells; however, when the follicle is activated, it matures to greater than 8 cells and the number of flattened cells decline rapidly as they become increasingly cuboidal (Braw-Tal, 2002). At this stage of follicular growth, the cells are still transcriptionally inactive (Fair *et al*., 1997b). The mechanism by which these follicles are gradually released and signaled to grow is unknown (Fortune, 1994).

Once the oocyte reaches an 18-cell stage, it is completely surrounded by cuboidal granulosa cells and is termed a primary follicle (Braw-Tal, 2002). As the numbers of cuboidal granulosa cells grow, they begin producing messenger ribonucleic acid (mRNA) for the protein follistatin (Braw-Tal *et al*., 1994).

Follistatin acts to inhibit activin A, thereby protect the growing follicle from the growth inhibiting effects of activin A (Braw-Tal *et al*., 1994). Additionally, the oocyte begins to

produce growth differentiation factor 9 (GDF9) and bone morphogenic protein 15 (BMP15), both of which are structurally similar to transforming growth factor-β (TGFβ) and expressed exclusively in the oocyte (McGrath *et al*., 1995; Dube *et al*., 1998) to increase granulosa proliferation (Braw- Tal, 2002). At about the same time, the surrounding granulosa cells begin to produce kit ligand to promote oocyte growth (Braw-Tal, 2002).

The oocyte continues to grow a second layer of cuboidal granulosa cells, and is termed a secondary follicle (Fair *et al*., 1997b; Braw-Tal, 2002). At this stage, the oocyte becomes transcriptionally active (Fair *et al*., 1997b), develops the zona pellucida, and forms gap junctions between the oocyte and granulosa cells (Fair *et al*., 1997a). The follicle moves from being dependent on intraovarian signals to being an autonomous unit where its growth is regulated only by follicle-produced factors (Braw-Tal, 2002). More layers of granulosa cells are added and a cavity or antrum begins to surround the oocyte, at which point it is termed a tertiary follicle. The antrum begins to accumulate follicular fluid and the size of the follicle gradually increases. The granulosa cells differentiate into inner, cumulus cells and outer, mural cells (Vanderhyden, 2002). The tertiary follicle continues to grow into a mature Graafian follicle capable of ovulation and the follicle will grow continuously until it reaches one of two fates, atresia or ovulation (Fortune, 1994).

Once follicles have developed to a graafian stage, they begin to progress through stages of recruitment, selection, and dominance. In cattle, follicles grow in a wave-like pattern to ensure that at any given time there is an eligible follicle available for ovulation (Sirois and Fortune, 1988). Typically, there are two to three follicular waves per estrous cycle, with some studies showing more two-wave cycles (Ginther *et al*., 1989), and others showing

more three wave cycles (Savio *et al*., 1988; Sirois and Fortune, 1988). In two-wave cycles, the waves begin around days 2 and 11 of the estrous cycle, whereas in cows with three-wave cycles, the waves begin around days 2, 9, and 16 of the estrous cycle (Sirois and Fortune, 1988). The number of follicular waves a cow has may be related to the level of dominance, or progesterone output, of the corpus luteum, with a three-wave cow having a more dominant corpus luteum than a two-wave cow. Townson *et al*. (2002) observed that cattle experiencing two follicular waves ovulated larger, older, and potentially less fertile follicles than cattle ovulating after three follicular waves, as shown by their subsequent pregnancy rates. This study confirmed the trend of higher pregnancy rates in three-wave cows over two-wave cows Ahmad *et al*. (1997), as well as the theory that a few days longer growth prior to ovulation compromises pregnancy rates (Mihm *et al*., 1994).

It has been shown that non-lactating Brahman cows had significantly greater numbers of small (2 to 5 mm), medium (6 to 8 mm) and large (≥ 9 mm) follicles when compared to non-lactating Angus and Senepol cows (Alvarez *et al*., 2000). However, there was no difference in the occurrence of two vs. three follicular waves or estrous cycle lengths among the breeds.

2.5. Atresia

Atresia is a form of apoptosis, or programmed cell death and is the most common fate of follicles, as only a selected number will actually achieve dominance and ovulate. While atresia can occur at any stage of follicular growth, it is not evenly distributed across follicular development. In cattle, atresia is greatest just before the final stages of follicular

dominance (Fortune, 1994). Up to this point in development, follicles can grow independently of gonadotropin stimulation (Scaramuzzi *et al*., 1993). Recruitment begins with a rise in follicle stimulating hormone, which stimulates the growth of a cohort of small follicles beyond 4 mm in diameter (Adams *et al*., 1992; Ginther *et al*., 1997). As follicle stimulating hormone declines, fewer follicles continue to grow, while others undergo atresia (Austin *et al*., 2001). A study by Austin *et al*. (2001), revealed that by about 33 h post-follicle stimulating hormone peak, there were two follicles still growing, and by 53 h a dominant follicle could be identified and measured at 8.5 mm. The dominant follicle grows between 12 to 20 mm and has enhanced estradiol and inhibin production to prevent growth of another cohort (Ginther *et al*., 1999). The dominant follicle continues to grow for an additional 3 to 4 days before it regresses from the negative feedback of progesterone on luteinizing hormone (Sunderland *et al*., 1994). The decline in estradiol from the former dominant follicle causes another follicle stimulating hormone rise and another wave begins (Sunderland *et al*., 1994). If luteolysis occurs during the time of dominance of the second dominant follicle, it will ovulate; however, if luteolysis does not occur during dominance, the follicle regresses and another follicular wave is initiated (Cooke *et al*., 1997).

2.6. Corpus Luteum Function and Luteolysis

The Corpus luteum (CL) is a heterogeneous structure on the ovary with a unique population of cells with different morphological, endocrinological, and biochemical properties (Niswender and Nett, 1988). It is comprised of endothelial cells, small luteal

cells, large luteal cells, fibroblasts, smooth muscle cells, immune cells, and pericytes (Farin *et al*., 1986; O'Shea *et al*., 1989; Hansel

et al., 1991). There are two types of steroidogenic cells in the corpus luteum, known as small luteal cells and large luteal cells, which cells produce progesterone when the animal is both cycling and pregnant (O'Shea, 1987).

The corpus luteum is formed from the granulosa and theca cells of the antral follicle (O'Shea, 1987). Preparation for the transformation of these cells to progesterone producing luteal cells is initiated prior to ovulation (McNatty and Sawers, 1975), but can occur independently of ovulation (Kesler *et al*., 1981). This transformation is termed luteinization and is characterized by the change from production of estradiol to progesterone (Juengal and Niswender, 1999). The granulosa cells of the follicle differentiate into large luteal cells (O'Shea, 1987), and are stimulated to produce progesterone primarily by growth hormone (Liebermann and Schams, 1994). Growth hormone receptors on the corpus luteum are located primarily on large luteal cells (Lucy *et al*., 1993). The theca cells of the follicle differentiate into small luteal cells (Donaldson and Hansel, 1965), and are stimulated to produce progesterone primarily by luteinizing hormone (Niswender and Nett, 1988). Luteinizing hormone receptors on the corpus luteum are primarily located in small luteal cells. The mechanism by which Luteinizing hormone stimulates progesterone production involves formation of cAMP and activation of Protein Kinase A (Schams and Berisha, 2004). It has also been suggested that large luteal cells may differentiate into small luteal cells (Fisch *et al*., 1989) and small luteal cells may differentiate into large luteal cells (Cran, 1983). Luteal structures that appear to be in an intermediate stage between the two have been identified (Priedkalns *et al*., 1968).

Small luteal cells are primarily responsible for higher magnitude, Luteinizing hormone-stimulated progesterone production, whereas large luteal cells are responsible for basal progesterone secretion (Hansel *et al*., 1991).

The actions of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), are primarily mediated by the large luteal cells, as they contain most of the prostaglandin $F_{2\alpha}$ receptors (Pate, 1994). Pulsatile secretion of luteinizing hormone is required for proper formation of the corpus luteum from days 2 to 7 of the estrous cycle and for full functionality of the corpus luteum from days 7 to 12 of the estrous cycle; however, it is not necessary for the corpus luteum to be maintained and produce normal circulating progesterone concentrations past d 12 (Peters *et al*., 1994).

2.6.1. Luteolysis

Prostaglandin $F_{2\alpha}$ is the main luteolytic agent in the bovine and initiates corpus luteum regression (Gooding *et al*., 1972; Inskeep, 1973). At the end of the estrous cycle, the corpus luteum is regressed by episodic release of prostaglandin $F_{2\alpha}$ from the uterus. The $PGF_{2\alpha}$ is transferred from the uterine vein to the ovarian artery where it acts on the corpus luteum by a unique counter-current exchange system between the uterine vein and ovarian artery (McCracken *et al*., 1972). This subsequently causes a marked decrease in progesterone concentrations between d 16 and 19 of the estrous cycle (Hansel *et al*., 1973) and leads to behavioral estrus and ovulation (Thatcher and Chenault, 1976). Prostaglandin $F_{2\alpha}$ has a very short half-life and is rapidly inactivated by oxidation in the bloodstream from a single pass through the lungs (Kindahl, 1980). For this reason, a counter-current exchange system evolved to transport $PGF_{2\alpha}$ from the uterus, by the uterine artery, to the ovary and return by the ovarian vein, thus preventing the effective amount of $PGF_{2\alpha}$ from

being lost in systemic circulation (McCracken *et al*., 1972). McCracken *et al*. (1972) also discovered that infusion of as little as 25 μg/hr of $PGF_{2\alpha}$ into the artery leading to the ovary resulted in a rapid decrease in progesterone secretion; however, the small concentrations of $PGF_{2\alpha}$ were ineffective at inducing luteolysis when administered into peripheral circulation.

It has been shown that exposure to, or inhibition of, progesterone regulates the timing of release of $PGF_{2\alpha}$, and subsequent corpus luteum regression (Schams *et al*., 1998). *In vivo* studies have shown that prior to d 12 of the estrous cycle progesterone inhibits $PGF_{2\alpha}$ release (Schams and Berisha, 2004). Additionally, progesterone exposure to the endometrium enhances synthesis of $PGF_{2\alpha}$ and has a priming effect on luteolysis (Boshier *et al*., 1981). After d 12 the luteal tissue begins to lose its sensitivity for progesterone and $PGF_{2\alpha}$ is no longer inhibited. Progesterone down-regulates its own receptors within the endometrium, thereby decreasing its own actions, and causing an increase in the actions of estradiol (Robinson *et al*., 2001). The decrease in progesterone receptors, and increase in estrogen receptors leads to an increase in oxytocin receptors (Vallet *et a*l., 1990). The increase in estradiol causes high frequency, low amplitude oxytocin release from the hypothalamic oxytocin pulse generator (McCracken *et al*., 1999) and enhances oxytocin receptors in the uterus (Meyer *et al*., 1988).

Oxytocin produced from both the posterior pituitary and endometrium activates sub-luteolytic $PGF_{2\alpha}$ concentrations from the uterus (Hooper *et al*., 1986). The release of uterine $PGF_{2\alpha}$ acts on $PGF_{2\alpha}$ receptors in luteal cells to further stimulate the release of oxytocin (Hooper *et al*., 1986).

These events culminate to cause secretion of large amounts of $PGF_{2\alpha}$ from the uterus and increases in oxytocin to induce luteolysis and continue until the $PGF_{2\alpha}$ receptor response system is desensitized and luteal oxytocin release ceases (McCracken *et al*., 1999).

Regression of the corpus luteum involves both functional and structural degradation and the progesterone producing capability of the corpus luteum is decreased rapidly at luteolysis by several mechanisms including the decrease in gene expression for steroidogenic acute regulatory protein (StAR), 3β-hydroxysteroid dehydrogenase (3β-HSD), and LH-receptor, all of which are important in maintaining luteal function (Tsai *et al*., 2001). As the CL ages, artery walls thicken and begin to deteriorate, which contributes to declining progesterone concentrations (Donaldson and Hansel, 1965). The vasoconstrictive properties of $PGF_{2\alpha}$ may also cause decreased blood flow through the CL to contribute to luteal regression (Schams and Berisha, 2004). Collectively, these processes cause the sharp decline of progesterone concentrations, allowing for an increase LH pulse amplitude and frequency in response to rising estradiol concentrations (Chenault *et al*., 1975).

2.7. Estrus Synchronization

Estrus synchronization is a powerful management tool that allows producers to have a large number of cattle in estrus during a short period of time to make breeding more practical. Through the use of hormone treatments, the estrous cycle can be manipulated in several ways. Prostaglandin $F_2\alpha$ ($PGF_{2\alpha}$) can be used to shorten the luteal phase and induce estrus in cows with a functional corpus luteum (CL). Progestins can lengthen the

luteal phase and prevent ovulation during the treatment period, which is generally 7 to 14 d. While, Gonadotropin Releasing Hormone can induce ovulation either to synchronize follicular waves, or to induce ovulation for a timed-AI protocol. Synchronization of the estrous cycle can be achieved in several ways including synchronization of estrus, synchronization of follicle wave growth, and (or) a combination of both. The combination of these two synchronization schemes can yield a tight synchrony of estrus that allows producers to either inseminate after an observed estrus or a timed-AI protocol.

In order to synchronize estrus or follicle development, exogenous administration of Gonadotropin Releasing Hormone, $PGF_{2\alpha}$, and progestins can be used either alone or in conjunction with other agents in development of effective estrous synchronization protocols. The different protocols must be matched to an individual producer's needs, facilities, and labor resources.

2.7.1. Synchronization agent

2.7.1.1. Prostaglandin $F_{2\alpha}$

Prostaglandin $F_{2\alpha}$ functions to regress the corpus luteum causing a sharp decline in progesterone production, which allows for estradiol to increase and the cow to subsequently exhibit estrus 3 to 5 d later. In order for $PGF_{2\alpha}$ to exert its luteolytic effects, the cow must have a functional corpus luteum (Rowson *et al*., 1972). Prostaglandin $F_{2\alpha}$ and its analogs are less effective at causing luteolysis during the early luteal phase of the cycle (Rowson *et al*., 1972; Lauderdale *et al*., 1974). Exogenous $PGF_{2\alpha}$ administration can

be used to initiate luteolysis without compromising the fertility of the subsequent estrus (Lauderdale *et al*., 1974).

Two major chemical classes of $PGF_{2\alpha}$ are commercially available namely; dinoprost tromethamine and cloprostenol sodium. Dinoprost tromethamine is a naturally occurring prostaglandin marketed under the names of Lutalyse®, Prostamate®, and In-Synch®, and is administered as a 5 mL (25 mg dinoprost) intramuscular injection. Cloprostenol sodium is a synthetic, structurally similar analogue of PGF2α marketed under the names of Estrumate® and Estroplan®, and is administered as a 2 mL (500 μg cloprostenol) intramuscular or subcutaneous injection. It has been used to synchronize estrus in cows for many years and has resulted in acceptable pregnancy rates (Johnson, 1978; Seguin *et al*., 1983; Jackson *et al*., 1979). The comparison of cloprostenol sodium and dinoprost tromethamine to synchronize estrus has been well documented (Young and Anderson, 1986; Salverson *et al*., 2002), but few comparisons of their effectiveness in cattle of *Bos indicus* breeding have been conducted (Hiers *et al*., 2003). Hiers *et al*. (2003) observed a 5% numerically greater timed-AI pregnancy rate with cloprostenol sodium over dinoprost tromethamine in *Bos indicus* × *Bos taurus* cows synchronized with GnRH + $PGF_{2\alpha}$ system combined with melengestrol acetate (MGA).

Stage of the estrous cycle also has a significant effect on the both the expression of estrus and the interval from $PGF_{2\alpha}$ administration to the onset of estrus. Kiracofe *et al*. (1985) observed that when $PGF_{2\alpha}$ was administered on days 1 to 4 of the estrous cycle very few displayed estrus and when inseminated had significantly decreased fertility. Tanabe and Hann (1984) observed estrus in 86.0, 90.0, and 98.0% of dairy heifers administered $PGF_{2\alpha}$

on d 7, 11, and 15 of the estrous cycle, respectively, with a significant difference in the interval to onset of estrus; day 7 heifers displaying estrus earliest (43.9 hours), day 11 heifers the latest (71.5 hours), and day 15 heifers intermediately (53.0 hours). However, the synchrony of estrus over the 24 hours interval from 32 to 56 hours was 88.4, 13.3, and 73.5% for day 7, 11, and 15, respectively. Similarly, Watts and Fuquay (1985) reported estrus responses of 43.0, 83.6 and 100% for cows administered $PGF_{2\alpha}$ on days 5-7, 8-11 and 12-15, respectively. Within the same treatment groups, they also observed an average interval to estrus of 59, 70, and 72 hours. Additionally, King *et al*. (1982) observed that the stage of cycle when $PGF_{2\alpha}$ is given influences the interval to estrus in both heifers and cows, with cows early in the estrous cycle (d 5 to 9) showing estrus earlier following $PGF_{2\alpha}$ than cows late in the estrous cycle and that the interval to estrus after $PGF_{2\alpha}$ is shorter for heifers than cows.

In New Zealand a study of large dairy herds was conducted using a single dose of $PGF_{2\alpha}$, and cows treated on either day 7 or day 15/16 of the estrous cycle demonstrated the most precise synchrony of estrus (Macmillan and Henderson, 1984). In the same study, cows which received $PGF_{2\alpha}$ on days 12 or 13 showed the most variable estrous response, and cows which received $PGF_{2\alpha}$ on days 15 or 16 were the most likely to be detected in estrus within 10 days. This, along with other studies, demonstrates the significant effects that stage of the estrous cycle has on the subsequent interval to estrus in both *Bos taurus* (Macmillan and Henderson, 1984; Stevenson *et al*., 1984) and *Bos indicus* cattle (Cornwell *et al*., 1985). Differences in the synchrony of estrus following $PGF_{2\alpha}$ administered at different stages of the estrous cycle result from different stages of

follicular development. If the cow has a dominant follicle in its growing phase at the time of corpus luteum regression by $PGF_{2\alpha}$, the interval to estrus will be shorter compared to a cow which has a regressing follicle and will, therefore, have to recruit and mature a new follicle before ovulation can occur.

Conception rates appear to be effected by stage of the estrous cycle, although the data are somewhat conflicting. Watts and Fuquay (1985) observed conception rates of 56.8, 62.1, and 78.3% for heifers administered $PGF_{2\alpha}$ on d 5 to 7, d 8 to 11, and d 12 to 15 of the estrous cycle, respectively. In contrast, other studies have observed no difference in pregnancy rates when $PGF_{2\alpha}$ was administered during the early or late luteal phase of the estrous cycle (Stevenson *et al*., 1984). Diskin *et al*. (2001) indicated that stage of cycle effects may be less pronounced in dairy heifers because they have a shorter interval to estrus with less variation than cows.

Initial work reported a considerable difference in estrus response after a $PGF_{2\alpha}$ induced estrus. In *Bos taurus* cattle, estrous responses of 70 to 95% have been reported after a single dose of $PGF_{2\alpha}$ (Tanabe and Hann, 1984; Watts and Fuquay, 1985). However, estrous responses of 46 to 62% (Landivar *et al*., 1985; Orihuela *et al*., 1983) were observed after a single dose of $PGF_{2\alpha}$ in *Bos indicus* cows. Pinheiro *et al*. (1998) reported an estrous response of only 46.4 and 33.3% in a group of Nelore (*Bos Indicus*) cows heifers, respectively, following two doses of $PGF_{2\alpha}$ 11 d apart. Additional work suggests that the decrease in estrus response maybe due to a decreased effectiveness of $PGF_{2\alpha}$ in cattle of *Bos indicus* breeding. It has been suggested that in *Bos indicus* cattle, the

decreased estrous response may be due to inhibition of estrus expression caused by incomplete regression of the corpus luteum, and therefore elevated progesterone concentrations (Pinheiro *et al*., 1998; Rekwot *et al*., 1999). Cornwell *et al*. (1985) reported estrous responses of only 50 and 67% for Brahman heifers treated with $PGF_{2\alpha}$ on days 7 and 10 of the estrous cycle, and that in heifers that did not display estrus, progesterone concentrations decreased initially, but began to increase to pre-treatment concentrations within 48 h after $PGF_{2\alpha}$. Similar findings were reported in *Bos taurus* cattle (Chenault *et al*., 1976; Copelin *et al*., 1988).

To prevent the partial luteolysis in *Bos indicus* cows, the dose of $PGF_{2\alpha}$ has been split into two consecutive doses, administered 24 h apart (Cornwell *et al*., 1985). Administration of the second dose sustains the luteolytic actions of $PGF_{2\alpha}$ and prevents the rebounding of progesterone concentrations caused by incomplete corpus luteum regression. The split-dose of $PGF_{2\alpha}$ increased estrus response, timed-AI pregnancy rate, and synchronized pregnancy rates for heifers as compared to a single dose, as a result of increased corpus luteum regression (Bridges *et al*., 2005). While corpus luteum regression was enhanced in mature cows, there was no difference in estrous response or synchronized pregnancy rates (Portillo *et al*., 2003). A split-dose of $PGF_{2\alpha}$ does not appear to be necessary in *Bos taurus* cows, as high corpus luteum regression rates (85 to 100%) are already achieved in these cows (King *et al*., 1982; Tanabe and Hann, 1984; Kiracofe *et al*., 1985).

2.7.1.2. Progestins

Exogenous progestins mimic the luteal phase of the estrous cycle and delay the expression of estrus. Progestins are commonly used to artificially extend the estrous cycle during an estrus synchronization protocol. Several types of progestins are used including natural progesterone and synthetic progestins including Norgestomet and Melengestrol acetate (MGA). The two most commonly used progestins for estrus synchronization include the Controlled Internal Drug Release (CIDR), which contains progesterone, and MGA, a feed through progesterone (that is, it is being administered to the animal through feed).

The CIDR is a T-shaped intravaginal insert which is made up of a nylon spine covered by elastic, silicone coating. The CIDR available for commercial use in the United States contains 1.38 g of progesterone and has been successfully used to synchronize estrus in both heifers and postpartum beef cows (Lucy *et al*., 2001). The typical duration of use of the CIDR is 7 days with an injection of $PGF_{2\alpha}$ either the day before CIDR removal (Lucy *et al*., 2001) or the day of CIDR removal to induce luteolysis (Larson *et al*., 2006). It is estimated that approximately 0.7 g of progesterone are used during its initial use (Savio *et al*., 1993) suggesting that there is a potential for reuse of the CIDR one more time.

Melengestrol acetate (MGA) is an orally-administered progestin, and when administered at a rate of 0.5 g/head/day it effectively suppresses estrus (Zimbelman and Smith, 1966). Treatments of progestins generally range from 7 to 14 days in length, and when administered for periods greater than 9 d, they lead to a reduction in fertility of the subsequent ovulation (Hill *et al*., 1971). This reduction in fertility is caused by the development of a persistent dominant follicle (Guthrie *et al*., 1970). Melengestrol acetate

(MGA) is most commonly used in heifers, and is administered for 14 days, followed by an injection of $PGF_{2\alpha}$ 17 (Brown *et al*., 1988) to 19 days (Lamb *et al*., 2000) after MGA withdrawal.

Ireland and Roche (1982) observed that the concentrations of exogenously administered progesterone were negatively correlated with an animal's ability to secrete luteinizing hormone. Cows receiving subluteal concentrations of progesterone have an increased frequency of luteinizing hormone pulses during the course of treatment (Roberson *et al*., 1989). This insufficient concentration of circulating progesterone allows luteinizing hormone frequency to increase, estradiol to rise, and follicles to experience a prolonged period of dominance (Kojima *et al*., 1992). This pattern of luteinizing hormone secretion prevents the wave-like growth seen in a normally cycling cow, resulting in a single follicle that grows and remains dominant with no further follicle recruitment for the duration of progestin treatment (Sirois and Fortune, 1990). Depending on the length of progestin treatment, the follicle may be aged and less fertile upon ovulation, with the period of dominance being directly correlated to the reduction in fertility (Mihm *et al*., 1994).

When low concentrations of progesterone are administered to inhibit estrus over extended (> 9 days) periods of time, it has been observed that follicles grow to a larger maximum size (Zimbelman and Smith, 1966), persist on the ovary (Trimberger and Hansel, 1955), and lead to a temporary reduction in fertility (Hill *et al*., 1971). Also, it has been reported that low doses of progesterone increased luteinizing hormone pulse frequency and prolonged pre-ovulatory secretion of estradiol (Sirois and Fortune, 1990). Hill *et al*.

(1971) reported a decreased pregnancy rates possibly caused by ovulation failure, fertilization failure, and abnormal oocytes following a 14 d MGA treatment.

Chow *et al*. (1982) also observed greater LH and estradiol concentrations during the estrus following exogenous progesterone treatment compared to non-treated heifers. Guthrie *et al*. (1970) and Kinder *et al*. (1996) concluded that the development of a persistent dominant follicle was the cause of reduced fertility following a long term exogenous progesterone treatment. In a study in which cows were set up to ovulate either normal, growing follicles or persistent follicles, Ahmad *et al*. (1995) observed larger follicles and greater estradiol concentrations in cows with persistent follicles, less oocytes/embryos were recovered from cows with persistent follicles than growing follicles, and fewer embryos from persistent follicles reached a ≥16 cell stage at 14%, compared to those from growing follicles at 86%. Uterine secretions may also be altered in animals that undergo a long-term progestin treatment (Wordinger *et al*., 1972; Gibbons *et al*., 1973).

Episodic release of LH is controlled by the circulating concentrations of progesterone (Roberson *et al*., 1989). To more closely mimic a cow's natural luteal phase, Stock and Fortune (1993) carried out a study in heifers with three treatments including a blank CIDR, one CIDR, or two CIDRs, over a period of 14 day. The blank CIDR served as a control, one CIDR mimicked a synchronization program, and two CIDRs mimicked the greater progesterone concentrations in a normal luteal phase. The single CIDR treatment resulted in ovulatory follicles that were larger in size and remained dominant for three times as long compared to controls. The two CIDR group had more follicular waves per cycle than the control and one CIDR treatments. This study demonstrated that the

progesterone provided in a normal, or one CIDR protocol was not sufficient to fully suppress LH pulses resulting in a single follicle that maintained dominance, and led to a decrease in fertility (Stock and Fortune, 1993).

One of the advantages of using progestins is that it has been shown to initiate estrus and ovulation in some anestrous cows (Fike *et al*., 1997; Lucy *et al*., 2001) and prepubertal heifers (Imwalle *et al*., 1998; Fike *et al*., 1999; Hall *et al*., 1997). This effect is achieved by stimulating LH secretion both during and after progesterone treatment, thereby accelerating follicle growth in both anestrous cows (Garcia-Winder *et al*., 1987) and prepubertal heifers (Anderson *et al.,* 1996). After a 7 days CIDR treatment, Fike *et al*. (1997) reported initiated estrus or CL formation in 70% of anestrous cows whereas Lucy *et al*. (2001) observed approximately 40% of anestrous cows to resume cycling. Similarly, Fike *et al*. (1997) observed 66.1 to 81.4% of anestrous cows and prepubertal heifers in estrus within 180 hours following MGA treatment. Hall *et al*. (1997) observed that while puberty was induced in heifers treated with Norgestomet implants for 10days, it was dependent on age, with a greater number of older heifers achieving puberty than younger heifers. Within 10 days after a 7 days MGA treatment, Imwalle *et al*. (1998) induced puberty in 8/8 MGA treated heifers, compared to only 4/9 control heifers.

2.7.1.3. Gonadotropin Releasing Hormone (GnRH)

In a normally cycling animal, when progesterone begins to decrease and estradiol from the dominant follicle rises, gonadotropin releasing hormone action is triggered and LH is released, leading to ovulation of the eligible follicle (Fortune *et al*., 1988). Administration

of an exogenous gonadotropin releasing hormone agonist (GnRH) works indirectly through the anterior pituitary to initiate ovulation.

Gonadotropin releasing hormone that can be administered exogenously as a 2 mL (100 μg) intravenous or intramuscular injection and is marketed under the names of Fertagyl®, Factrel®, and Cystorelin®. The effects of gonadotropin releasing hormone are observed within 2 to 4 hours of injection through an increase in peripheral concentrations of both luteinizing hormone and follicle stimulating hormone (Chenault *et al*., 1990; Stevenson *et al*., 1993). This induced luteinizing hormone surge causes ovulation within 24 to 32 hours after treatment (Thatcher *et al*., 1989; Macmillan and Thatcher, 1991; Pursley *et al*., 1994) of large (> 9 mm); Sirois and Fortune, 1988), and dominant ovarian follicles in their growth phase of development(Guilbault *et al*., 1993). However, Gonadotrophin will not initiate ovulation of all large follicles, particularly follicles which are regressing or in a state of atresia (Guilbault *et al*., 1993) and have a declining number of induced luteinizing receptors (Rollosson *et al*., 1994).

Following ovulation, estradiol concentrations are dramatically decreased in peripheral circulation both after a Gonadotrophin treatment induced luteinizing hormone surge (Twagiramunga *et al*., 1994b) and luteinizing hormone surge caused by removal of a large follicle (Hughes *et al*., 1987). If a follicle is, in fact, ovulated to gonadotropin releasing hormone the rapid decrease in estrogen output will result in an follicle stimulating hormone surge, causing recruitment of a new wave of follicular growth 1 to 2 d later (Ko *et al*., 1991; Adams *et al*., 1992). The follicle stimulating hormone peak provided by the gonadotropin releasing hormone injection 2 to 4 hours after treatment will stimulate the

recruitment of a new follicular wave (Chenault *et al*., 1990; Rettmer et *al*., 1992). One of the reasons that gonadotropin releasing hormone does not initiate ovulation of all large follicles is due to stage of cycle effects.

The use of gonadotropin releasing hormone agonists has been shown to synchronize follicular growth and yield precise ovulation times without compromising fertility (Twagiramungu *et al.,* 1995). It has been suggested with mixed results that, treatment with gonadotropin releasing hormone agonists may alter the subsequent corpus luteum progesterone output. There have been reports of both increased (Macmillan *et al*., 1985a, b; Stevenson *et al*., 1993) and decreased (Rodger and Stormshak, 1986) progesterone production following treatment with a gonadotropin releasing hormone agonist, while numerous other studies which showed no changes (Macmillan and Thatcher, 1991; Prescott *et al*., 1992). Studies by Twagiramungu *et al*. (1992b; 1994a) demonstrated that both the corpus luteum existing at the time of gonadotropin releasing hormone treatment, and that which was formed as a result of a gonadotropin releasing hormone -induced ovulation, respond to luteolysis by exogenous $PGF_{2\alpha}$. Therefore, gonadotropin releasing hormone -induced corpus luteum is similar to a naturally formed corpus luteum, at least with regard to luteolysis.

Since gonadotropin releasing hormone can initiate ovulation and synchronization of follicle waves, its combination with $PGF_{2\alpha}$ administered 7 d after gonadotropin releasing hormone was used in the initial development of the gonadotropin releasing hormone + $PGF_{2\alpha}$ estrous synchronization program (Thatcher *et al*., 1989; Macmillan and Thatcher, 1991; Pursley *et al*., 1994). This system allows for the turnover of any large follicle on the

ovary, the growth of a new follicular wave containing the pre-ovulatory follicle, and subsequent luteolysis to liminate the corpus luteum to bring the cow into estrus. This results in a group of cattle with highly synchronized follicular growth, and a precise estrus following $PGF_{2\alpha}$–induced luteolysis. Estrous rates of 70 to 83% (Thatcher *et al*., 1989; Coleman *et al*., 1991; Twagiramungu *et al*., 1992b, c) and pregnancy rates of 65 to 85% (Coleman *et al*., 1991; Twagiramungu *et al*., 992b, c), both over a 4 d treatment period, have been reported in beef and dairy cattle.

Another use of gonadotropin releasing hormone is to induce ovulation during the period after $PGF_{2\alpha}$ following a synchronization protocol to reduce or eliminate the need for estrous detection. This is possible, because a great degree of synchrony is achieved, with 56 to 76% of cows displaying estrus between 24 and 72 h after $PGF_{2\alpha}$ (Twagiramungu *et al*., 1992b,c). With the addition of a second Gonadotropin releasing hormone injection, given 48 hrs after $PGF_{2\alpha}$, Pursley *et al*. (1994) observed that dairy cows and heifers ovulated between 24 and 32 h after gonadotropin releasing hormone.

It also appears that breed may have an effect on response to gonadotropin releasing hormone. In a study by Griffin and Randel (1978), ovariectomized Brahman and Hereford cows were treated with gonadotropin releasing hormone and the resulting luteinizing hormone response was significantly lower for Brahman cows, indicating that they may be less responsive to gonadotropin releasing hormone -induced luteinizing hormone release.

2.8. ESTRUS SYNCHRONIZATION PROTOCOLS

2.8.1. Progesterone Insert + $PGF_{2\alpha}$

When progesterone (P_4) inserts are used in synchronizing protocols they are combined with $PGF_{2\alpha}$, gonadotropin releasing hormone, or a combination of both. Combining progesterone ($P_{4)}$ insert with $PGF_{2\alpha}$ at the end of the insert period in dairy heifers allowed for synchronized estrus and ovulations rates (Smith *et al*., 1984; Lucy *et al*., 2001). Smith *et al*. (1984) observed an increase in the interval to onset of estrus when $PGF_{2\alpha}$ was administered 24 h before removal of the insert than when the injection was concurrent with insert removal (75 ± 2 vs. 66 ± 2 h, respectively). Lucy *et al*. (2001) reported an increased proportion of dairy heifers treated with a P_4 insert and $PGF_{2\alpha}$ showing signs of estrus (84%) compared with applying only $PGF_{2\alpha}$ (57%). A positive response to the CIDR insert and $PGF_{2\alpha}$ treatment also was observed in dairy heifers with estrus-detection rates of 85.5% compared with beef heifers (89.1%), and greater pregnancy rates in dairy heifers (59%) vs. beef heifers (40.6%; Richardson *et al*., 2002). Comparable pregnancy rates also resulted when a CIDR+ $PGF_{2\alpha}$ protocol was compared with $PGF_{2\alpha}$ alone (45% vs 37%, respectively; Lucy *et al*., 2001). Earlier research using a Progesterone-Releasing Intravaginal Device (PRID) produced greater pregnancy rates (72% when $PGF_{2\alpha}$ was administered 1 d before PRID removal and 82% when injection was concurrent with PRID removal, (Smith *et al*., 1984) than those reported in later studies using the CIDR insert (Richardson *et al*., 2002; Lucy *et al*., 2001). Uses of progesterone inserts have become more commonplace because of their ability to suppress estrus with no reduction in pregnancy rate/artificial insemination (PR/AI) as reported by Rivera *et al*. (2005). Further,

they improve synchronized estrus and increase PR/AI to first AI during increased temperatures of summer (Alnimer *et al*., 2009).

2.8.2. GnRH + Progesterone Insert (7 to 14 d) + $PGF_{2\alpha}$

Previously used, autoclaved CIDRs may be best for synchronization because they increase P_4 concentrations immediately after insertion (Zuluga *et al*., 2008). The authors also found that mean P_4 in blood serum of cattle treated with an autoclaved CIDR almost doubled that of previously used CIDRs that were disinfected. Autoclaved CIDRs also produced serum concentrations 1.3 to 1.4 times greater than produced by new intravaginally placed CIDRs. Increased conception rates after applying used (47.7%) compared with new CIDRs (39.2%) were observed in heifers in addition to shorter intervals to estrus (Júnior *et al*., 2010).

Combining $PGF_{2\alpha}$ with the time of CIDR insert removal is done to initiate luteolysis in heifers for which the CL has not already regressed before $PGF_{2\alpha}$ is administered. Giving $PGF_{2\alpha}$ in a 7-d protocol shortened the onset of estrus in heifers administered the $PGF_{2\alpha}$ at CIDR removal (49.3 ± 6.2 h) compared with those injected with $PGF_{2\alpha}$ the day after insert removal (77.5 ± 9 h; Hittinger *et al*., 2004). Ambrose *et al*. (2008) also found that administering $PGF_{2\alpha}$ 24 h before CIDR removal resulted in a consistent PR/AI that was greatest when the protocol was initiated in diestrus (57%) than at other stages of the estrous cycle (34.8%).

Presynchronization of estrus can occur to allow a group of females to initiate a timed AI program at a specific and more favorable stage of the cycle to facilitate greater pregnancy

outcomes to the timed AI. Females to which a protocol is applied that do not become pregnant are sometimes resynchronized for subsequent AI. Although presynchronization did not affect dairy heifers expression of estrus, nor improved synchronization or fertility in dairy heifers (Rivera *et al*, 2003), Stevenson *et al*. (2008) observed that presynchronization with gonadotropin releasing hormone increased ovulation to the first gonadotropin releasing hormone injection at the start of the CO-Synch + CIDR protocol and led to increased ovulatory follicle size and decreased period from estrus to ovulation. The difference between these 2 studies was that the Rivera *et a*l. (2006) study did not use CIDR inserts, whereas CIDRs were used in the Stevenson *et al*. (2008) study; however, both reported an average PR/AI of approximately 50%. Busch *et al*. (2007) reported an increased synchronous estrus and greater PR/AI using the CIDR-Select protocol (heifers received a CIDR from d 0 to 14, gonadotropin releasing hormone -1 on d 23, and $PGF_{2\alpha}$ on d 30 with gonadotropin releasing hormone -2 and TAI 72 h after $PGF_{2\alpha}$ injection; 62%) compared with the CO-Synch + CIDR (47%).

Heifers were treated with CIDR insert between 14 and 20 d after AI or no insert, showed estrus by 72 h after CIDR removal (78%) and had a higher conception rate compared to (47%) the control heifers which showed estrus by 50 and 36% conception rate (Rivera *et al*., 2005).

The goal of all synchronization protocols is to have a homogenous group of females in estrus that can be submitted to AI. It is well known that virgin heifers often fail to have acceptable synchronization and pregnancy rates because of a variety of factors. Pursley *et al*. (1997) stated that a fixed TAI protocol is not an effective tool for use in heifers because of the lack of synchronization. Pregnancy outcomes in control heifers inseminated

after $PGF_{2\alpha}$-induced estrus greater than in heifers treated with Ovsynch (Pursley *et al*., 1997).

Tenhagen *et al*. (2005) determined that premature estrus and ovulation probably caused a failure of synchronization in dairy heifers. Further, Saldarriaga *et a*l. (2007) concluded that beef cattle had poor conception because 40% fail to develop a synchronized follicular wave. It has been reported that, if the synchronization protocol is started at a less opportune stage of the cycle, most females do not respond properly, resulting in less than acceptable PR/AI. Using GPG (gonadotropin releasing hormone day 0, $PGF_{2\alpha}$ day 6, gonadotropin releasing hormone and TAI day 8) in dairy heifers decreased pregnancy rate/artificial insemination where 38.3% of treated heifers conceived compared with a control of 46.5% (Rivera *et al*., 2004). Lamb *et al*. (2006) obtained acceptable PR/AI and eliminated the need for estrus detection in beef heifers with the control (CIDR d 0 to 7, $PGF_{2\alpha}$ at CIDR withdrawal, estrus detection and AI for 72 h plus AI 84 h after $PGF_{2\alpha}$ for heifers not detected in estrus) achieving 54.7% PR/AI compared with the treatment (gonadotropin releasing hormone on day 0, CIDR day 0 to 7, $PGF_{2\alpha}$ at CIDR withdrawal, gonadotropin releasing hormone and TAI 60 h later; 53.1%).

2.8.3. GnRH+CIDR (5 d) +$PGF_{2\alpha}$

Bridges *et al*. (2008) reported that in suckled beef cows were the first to show reduced interval from GnRH to $PGF_{2\alpha}$ from 7 to 5 d with AI at 60 h in the 7-d treatment and 72 h in the 5-d treatment. Reducing the CIDR insert from 7 to 5 d also required a second $PGF_{2\alpha}$ injection (given 12 h after the first $PGF_{2\alpha}$ injection) to ensure luteolysis occurred in cows that formed a new CL in response to the GnRH injection administered at the time of CIDR

insert. The PR/AI in the 7 d treatment was 59.9% and increased to 70.4% in the 5 d treatment (Bridges *et al*., 2008). In lieu of 2 injections of $PGF_{2\alpha}$ administered 12 h apart, Kasimanickam *et al*. (2009) administered $PGF_{2\alpha}$ 7 h apart and reported greater PR/AI after 2 injections (69%) than after a single injection (52%). Ahmadzadeh *et al*. (2010) observed differences between the 7- and 5-d programs in beef heifers with a tendency for greater PR/AI in the 5-d treatment (62.5%) compared with the 7-d treatment (52%).

In dairy heifers, where a similar 5-d CIDR program was administered, 1 injection of $PGF_{2\alpha}$ yielded acceptable PR/AI when compared with 2 injections of $PGF_{2\alpha}$ given 12 h apart (Rabaglino *et al*., 2010). In a study done to show the effects of gonadotropin releasing hormone at initiation of the 5-d timed AI program in dairy heifers, gonadotropin releasing hormone administration on the first day of the 5-d protocol resulted in poor ovulation incidence and no improvement in PR/AI when heifers received a single dose of $PGF_{2\alpha}$ (Lima *et al*., 2010). The authors also noted that the combination of an upfront gonadotropin releasing hormone injection given 5 d before the 2 $PGF_{2\alpha}$ injections improved PR/AI compared with no gonadotropin releasing hormone upfront and a single injection of $PGF_{2\alpha}$.

2.9. Artificial Insemination

Artificial insemination is the technique in which semen with living sperms is collected from the male and introduced into female reproductive tract at proper time with the help of instruments. This has been found to result in a normal offspring. In this process, the semen is inseminated into the female by placing a portion of it either in a collected or diluted

form into the cervix or uterus by mechanical methods at the proper time and under most hygienic conditions.

Artificial insemination is not merely a novel method of bringing about impregnation in females. Instead, it is a powerful tool mostly employed for livestock improvement. In artificial insemination the germ plasm of the bulls of superior quality can be effectively utilized with the least regard for their location in faraway places. By adoption of artificial insemination, there would be considerable reduction in both genital and non-genital diseases in the farm stock.

2.9.1. Origin

Leeuwenhoek (1678) and his assistant, Hamm, were the first persons to see sperm, which they called “animalcules.” Leeuwenhoek did not have an advanced formal education, so he did not study Latin, the scientific language of the day. Another century passed before the first successful insemination was performed by Spallanzani (1784) in a dog, which helped three pups 62 d later. Spallanzani originally trained to be a priest, but he had a great interest in natural history and pursued the latter. Another 100 yr passed before Heape (1897) and others in several countries reported that AI had been used in isolated studies with rabbits, dogs, and horses. Heape was an outstanding reproductive biologist, establishing much of the basis for the relationship between seasonality and reproduction.

2.9.2. Artificial insemination becomes a central point of research

Pioneering efforts to establish artificial insemination (AI) as a practical procedure were begun in Russia in 1899 by Ivanow (Ivanoff, 1922). By 1907 Ivanow (also transliterated

as Ivanov or Ivanoff) had studied AI in domestic farm animals, dogs, foxes, rabbits, and poultry. He developed semen extenders and trained technicians to select superior stallions and multiply their progeny through AI. Much of the AI work in Russia was taken over later by Milovanov (1938), described in a text translated into English (Milovanov, 1964). He established major projects for sheep and cattle breeding. In his own workshop Milovanov designed and made practical artificial vaginas and other items, many similar to those used today. This was an enormous improvement over the earlier method of collecting semen from sponges placed in the vagina of mount animals.

The development of AI by Ivanow also stimulated research outside of Russia. The Japanese scientist Dr. Ishikawa studied with Ivanow. He returned to Japanand began a similar program in horses in 1912 (Nishikawa, 1962). This gradually developed into AI being applied in Japan in cattle, sheep, goats, swine, and poultry. Other Japanese researchers became involved (Ito, Niwa, Sato, Yamane, etc.). Because most of the research was published in Japanese and few westerners knew Japanese, little was known about this research in the Western world until Niwa (1958) and Nishikawa (1962, 1964, and 1972) summarized the research in English. News of the extensive use of AI in Russia following the Ivanoff (1922) report became widespread in the Western world with the publication of the book on AI by Walton (1933). Walton conducted a number of experiments, including a pioneering shipment of ram semen to Poland, which 2 d later was used for successful insemination of ewes.

With Gylling-Holm, Sørensen organized the first cooperative dairy AI organization in Denmark in 1936. The program enrolled 1,070 cows the 1st yr and 59% conceived,

slightly better than natural service in the same herds. This was an important stimulus for the development of AI in dairy cattle in the United States and other Western countries.

The Danish veterinarians established the method of rectovaginal fixation of the cervix, allowing semen to be deposited deeply into the cervix or into the body of the uterus. This technique provided a tremendous advantage because fewer sperm were required for insemination of each cow. Another Danish "invention" was the straw for packaging semen (Sørensen, 1940). Later Cassou (1964) produced straws commercially that have been used worldwide. So, the French straw is a modified Danish straw. Meanwhile, the much earlier research by Spallanzani led eventually in Italy to the development of an artificial vagina for dogs by Amantea in 1914 (Perry, 1968). This work served as a model for the Russian development of artificial vaginas for bulls, stallions, and rams. Another Italian, Bonadonna (1937), continued research on AI in several species. His enthusiasm for the potential value of AI, along with Lagerlo¨f, resulted in the establishment of the highly successful International Congress on AI and Animal Reproduction held every 4 yr. The first one was held in Milan in 1948.

2.9.3. Modern advances of artificial insemination in dairy cattle

Phenomenal growth of AI occurred in the 1940s in the United States. The procedures developed in the United States became established worldwide (Salisbury *et al*., 1978). In 1936, Brownell was inseminating cows in the Cornell herd (Sipher, 1991), and other AI work was started in the late 1930s in Minnesota and Wisconsin. In 1938, an AI cooperative was established in New Jersey, modeled after the Danish system (Perry, 1968). Another one in 1938 followed in the state of New York (Sipher, 1991). The

development of the New York Artificial Breeders, Cooperative, Inc., currently Genex, Inc., in Ithaca, New York made possible the close collaboration between a farmer cooperative and researchers and extension personnel at Cornell University. This was a highly productive relationship resulting in the experimental insemination of hundreds of thousands of cows and publication of more than 100 research papers (Foote, 1998) on sire selection, testicular evaluation, semen collection, evaluation and processing; and fertility testing. The present status of AI in many countries has been summarized recently (Malafosse and Thibier, 1990; Foote, 1999).

The first documented cattle artificial insemination (AI) in Nigeria dated back to 1949. Other recorded cattle artificial insemination in the country could be traced to when the Federal Government of Nigeria under the auspices of Ahmadu Bello University, Zaria, established Artificial Insemination Unit at the National Animal Production Research Institute (NAPRI) in 1976 (Bale, 2003).

2.9.4. Advantages of artificial insemination

There are several advantages by artificial insemination over natural mating or servicing.

- There is no need of maintenance of breeding bull for a herd; hence the cost of maintenance of breeding bull is saved.
- It prevents the spread of certain diseases and sterility due to genital diseases. Eg: contagious abortion, vibriosis.
- By regular examination of semen after collection and frequent checking on fertility make early detection of interior males and better breeding efficiency is ensured.
- The progeny testing can be done at an early age.

- The semen of a desired size can be used even after the death of that particular sire.
- The semen collected can be taken to the urban areas or rural areas for insemination.
- It makes possible the mating of animals with great differences in size without injury to either of the animal.
- It is helpful to inseminate the animals that are refuse to stands or accept the male at the time of estrus.
- It helps in maintaining the accurate breeding and cawing records.
- It increases the rate of conception.
- It helps in better record keeping.
- Old, heavy and injured sires can be used.

2.9.5. Disadvantages of artificial insemination

- Requires well-trained operations and special equipment.
- Requires more time than natural services.
- Necessitates the knowledge of the structure and function of reproduction on the part of operator.
- Improper cleaning of instruments and in sanitary conditions may lead to lower fertility.
- If the bull is not properly tested, the spreading of genital diseases will be increased.
- Market for bulls will be reduced, while that for superior bull is increased.

(http://bieap.gov.in/DairyAnimalManagementTheory.pdf

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Study Location

The experiment was carried out at the Dairy Research Programme of the National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Shika-Zaria, Nigeria. Shika is located in the Northern Guinea Savanna between latitudes 11^0 12'N and between longitudes 7^0 E and 8^0 E at the elevation of 650m above sea level with an annual maximum and minimum temperature of 31.0 ± 3.2 ^{0}C and 18.0 ± 3.7 ^{0}C respectively. Shika has an average annual rainfall of 1100 mm usually lasting from May to October with a mean relative humidity of 72 % while the dry season lasts from November to April with mean daily temperatures ranging from 15 – 36 ^{0}C and mean relative humidity of between 20 – 37 % (Rekwot *et al*., 1987).

3.2. Selection and Management of Experimental Animals

The cows were grazed on free range pasture and supplemented a concentrate diet consisting of 48 % cotton seed cake, 13.7 % wheat bran, 35.3 % maize, 2 % bone meal, 1 % common salt, 88 % dry matter, 15 % crude protein and 55 % total digestible nutrients after grazing. Water and mineral licks were provided *ad-libitum*. All cows were ear-tagged to enable identification of the cows from a distance during estrus detection. The cows were dipped once a week and twice a week during the dry season, and wet season, respectively for effective control of ectoparasites.

A total of forty seven (47) animals of age between $2^1/_2$ to 5 years were examined rectally, out of which twenty three (23) Friesian x Bunaji cows and twenty four (24) Bunaji cows cycling were selected and used in the study in a 2 x 3 factorial arrangement.

Table 3.1. Distribution of cows (Bunaji and Friesian X Bunaji) treated with CIDR, CIDR + $PGF_2\alpha$ and $PGF_2\alpha$.

Treatments	**Breed**		**Total**
	Bunaji	**Friesian x Bunaji**	
CIDR	n=8	n=8	n=16
CIDR+$PGF_2\alpha$	n=8	n=8	n=16
$PGF_2\alpha$	n=8	n=7	n=15
Total	n=24	n=27	n=47

CIDR=controlled internal drug release, $PGF_2\alpha$= Prostaglandin.

3.3. Estrous synchronization

The experimental animals consisted of 23 Friesian x Bunaji cows and 24 Bunaji cows. Cows were body-condition scored using a score scale of 0–5, from the most emaciated to the fattest (Pullan, 1978) as shown below;

0=emaciated animals with no apparent subcutaneous fat

1=the spinous processes are sharp but less so than in score 0

2=the individual spinous processes are still fairly sharp to touch and the ribs can be seen individually.

3=the spinous processes can be felt but have a rounded feel and the ribs cannot be seen individually.

4=the individual spinous processes can only be felt with firm pressure and the points of the hips are covered with fat and are rounded.

5=the spinous processes cannot be felt even with hard pressure and the animal has a 'blocky' appearance.

Only cows with body condition scores of 2.5 and above will be used.

The cows were divided into three groups (Group I, II and III) consisting of 16 cows each (8 Bunaji x Friesian and 8 Bunaji cows).

Group I, CIDR alone was inserted into the vagina of the cows using a special applicator. The vulva of the cows was cleaned to avoid introducing contaminants into the vagina. The CIDR was left in place for seven days before removal, and then heat signs were observed for artificial insemination.

Group II, CIDR was inserted into the vagina of the cows using a special applicator. The vulva of the cows was cleaned to avoid introducing manure into the vagina. The CIDR were left in place for seven days. On day 6 the cows were administered 2mL $PGF_2\alpha$ (Estrumate®) intramuscularly before removals of the device on day 7 then heat signs were observed for artificial insemination.

Group III, 2mL of $PGF_2\alpha$ (Estrumate®) was administered intramuscularly on day 0 (first single shot) then heat signs were observed for artificial insemination.

The CIDR device was inserted following the guidelines as shown below:

1. Shift the animal's tail to one side and clean the vulva.
2. Make sure the tail of the CIDR insert is in the underside of the applicator to ensure the tail will be hidden from curious pen-mates.
3. Open the lips of the vulva and insert the applicator at a slightly upward angle, moving forward over the pelvic bone until it meets resistance.
4. Dispense the insert from the applicator by depressing the plunger, and then slowly withdraw the applicator.
5. To prevent removal by pen-mates, you may clip the tail, but be careful not to clip it too short because the CIDR will usually settle deeper inside the animal.
6. To withdraw the insert, give the tail a gentle but firm pull.

   Source: Manufacturers guide

3.4. Estrus Detection

Cows were monitored for mounting activities and mucus discharge. Visual detection of estrus was done in the morning and evening and by the herdsmen when grazing to improve the reliability of detecting estrus. Oestrus signs were observed in the morning at 07:00 – 09:00 hours and in the evening at 17:00 hours – 18:00 hours.

3.5. Insemination and Pregnancy Diagnosis

Cows with standing estrus (standing to be mounted) were inseminated 12 h after the onset of estrus using Frozen Friesian semen. A single experienced inseminator carried out all the inseminations using the recto-cervical method.

Animals were rectally palpated by an experienced Theriogenologist according to the procedures of Zemjanis (1970) for pregnancy diagnosis.

3.6. Data Collection

The following parameters were measured and/or calculated:

3.6.1. Interval to onset of estrus: The period between CIDR removal and/or $PGF_2\alpha$ injection and time of first mount expressed as mean ± standard error of mean (SEM).

3.6.2. Retention rate: Number of cows that retained the intra-vaginal device to the total number in each treatment group for the period of the experiment without voiding it expressed as a percentage.

3.6.3. Swollen vulva: Number of cows that showed swollen vulva expressed as a percentage.

3.6.4. Vaginal discharge rate: Number of cows with vaginal discharge on removal of the device to the total number in each treatment group expressed as a percentage.

3.6.5. Estrus response rate: The number of cows that came into estrus divided by the number of cows that were synchronized and expressed as a percentage.

3.6.6. Heat duration: The time from onset of estrus to the end of heat.

3.6.7. Pregnancy rate: The number of cows that were pregnant divided by the number of cows that were synchronized and expressed as a percentage.

3.6.8. Conception rate: The number of cows pregnant divided by the number of cows that came into estrus and expressed as a percentage.

3.7. Statistical Analysis

Data on estrus response rate, swollen vulva, vaginal discharge rate, and pregnancy and conception rates were expressed as percentages. Interval to onset of estrus, number of mounts and estrus duration were analyzed using the GLM procedure of SAS (SAS, 2002). Significant means were separated using Duncan Multiple Range Test (DMRT) Duncan, (1955).



1=Sachet containing 10 CIDRs
2=Special applicator
3=CIDR
Plate 3.1: A sample of CIDR and the applicator.

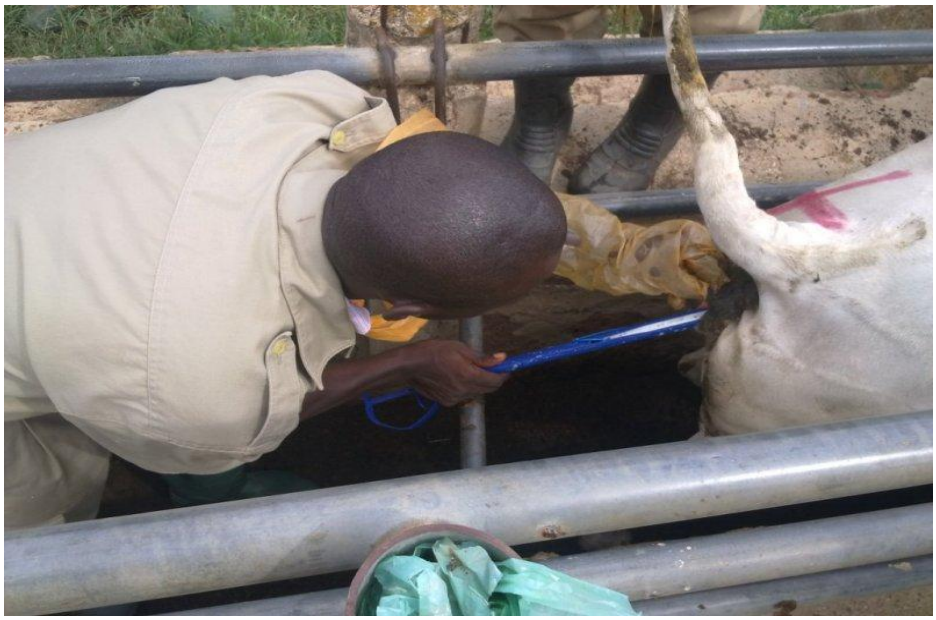


Plate 3.2: Inserting CIDR into the vagina of the cow.



Plate 3.3: Withdrawing CIDR from the Vagina of the cow with the aid of the plastic rope or string exteriorized.

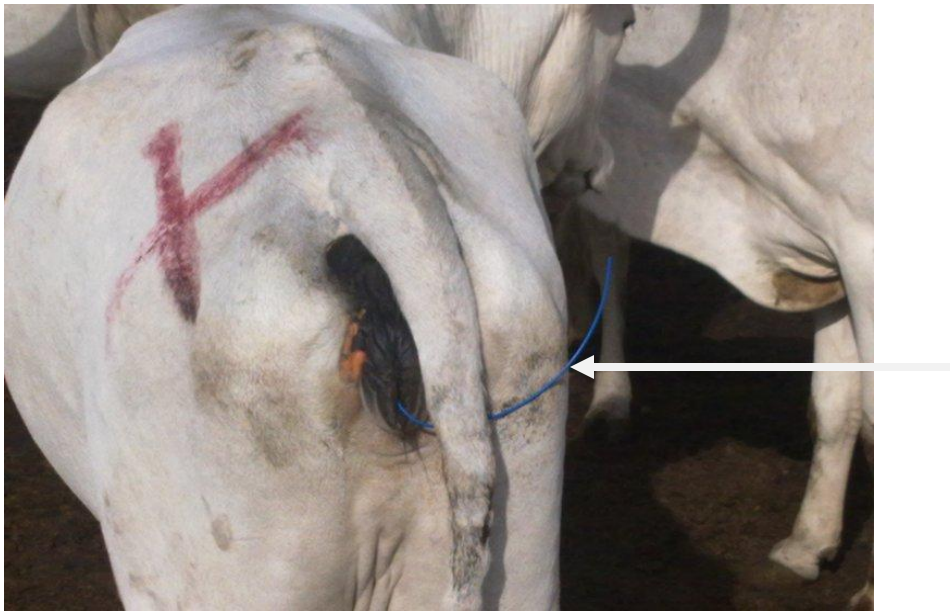


Plate 3.4: CIDR in place in the cow.



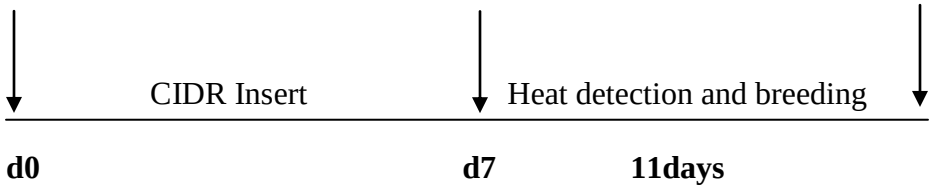
Plate 3.5: Cow in standing heat.



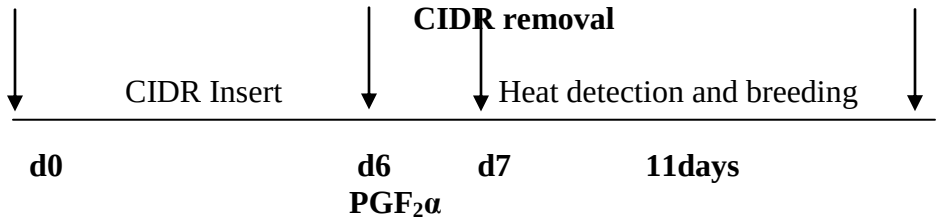
Plate 3.6: A cow in standing heat being inseminated

ESTRUS SYNCHRONIZATION PROTOCOL USED

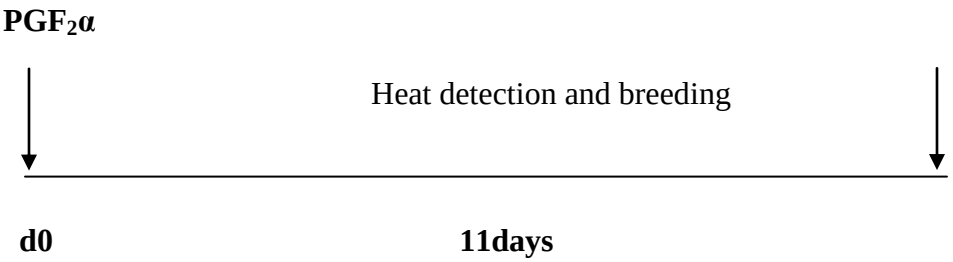
Group I: CIDR alone



Group II: CIDR + $PGE_2\alpha$



Group III: $PGF_2\alpha$ alone



CHAPTER FOUR

4.0. RESULTS

4.1. RETENTION RATE

After the seven days insertion of Controlled Internal Drug Release (CIDR) the retention rates were 8/8 (100 %) for Bunaji, 8/8 (100 %) for Friesian x Bunaji and 8/8 (100 %) for Bunaji, 7/7 (100 %) for Friesian x Bunaji cows in group I and II respectively. The retention rates for all the groups were 100 % as shown in table 4.1

4.2. ESTRUS RESPONSE RATE

Estrus response rate for treatment I were 37 % and 87 % for Bunaji and Friesian x Bunaji cows respectively. Treatment II recorded an estrus response rate of 62 % and 50 % for Bunaji and Friesian x Bunaji cows. While treatment III records were 0 % and 57 % for Bunaji and Friesian x Bunaji cows. These are all summarized in table 4.1.

4.3. INTERVAL TO ONSET OF ESTRUS

Interval to onset of estrus (mean ± s.e.m) was 5.00 ±1.0 hours and 5.00 ±1.2 hours for Bunaji and Friesian x Bunaji cows treated with CIDR respectively. Bunaji and Friesian x Bunaji cows treated with CIDR + $PGF_{2\alpha}$ had 3.00 ± 0.4 hours and 1.75 ± 0.3 hours.

4.4. ESTRUS DURATION

Estrus duration (mean ± s.e.m) was 7.00±2.9 hours and 12.00±2.8 hours for Bunaji and Friesian x Bunaji cows treated with CIDR alone, while Bunaji and Friesian x Bunaji cows treated with CIDR + $PGF_{2\alpha}$ recorded 11.00±4.2 and 13.5±.1.2 respectively.

4.5. NUMBER OF MOUNTS PER ESTRUS PERIOD

Mounts per estrus period (mean ± s.e.m) was 8.66±2.4 and 9.00±1.4 in Bunaji and Friesian x Bunaji cows treated with CIDR alone and 4.33±0.6, 11.25±1.5 in Bunaji and Friesian x Bunaji cows treated with CIDR + $PGF_{2\alpha}$.

4.6. SWOLLEN VULVA AND VAGINAL DISCHARGE RATE

The results for swollen vulva from the study were 50 % and 100 % for Bunaji and Friesian x Bunaji cow in treatment I. treatment II recorded 75 % and 62 % for Bunaji and Friesian x Bunaji cows while treatment III had 50 % and 85 % for Bunaji and Friesian x Bunaji cows respectively.

The results of vaginal discharge rate at CIDR removal from the study were 50 % and 87 % for Bunaji and Friesian x Bunaji cows in treatment I while those of treatment II were 62 % and 57 % for Bunaji and Friesian x Bunaji cows, respectively.

Table 4.1: Estrus activity of Friesian x Bunaji and Bunaji cows treated with different synchronization protocols

.

Parameter	Treatment Groups					
	CIDR		CIDR+$PGF_{2\alpha}$		$PGF_{2\alpha}$	
	BJ	FR x BJ	BJ	FR x BJ	BJ	FR x BJ
N	8	8	8	8	8	7
Retention (%)	100	100	100	100		
Estrus response (%)	37	87	62	50	0	57
Interval to onset of estrus (h)	5.00±1.0	5.00±1.2	3.00±0.4	1.75±0.3		
Estrus Duration (h)	7.00±2.9	12.00±2.8	11.00±4.2	13.50±1.2		
No of mounts	8.66±2.4	9.00±1.4	$4.33±0.6^{b}$	$11.25±1.5^{a}$		
Swollen vulva (%)	50	100	75	62	50	85
Vaginal discharge (%)	50	87	62	57		

Mean values with the different superscript within a row are significantly different ($p<0.05$)
CIDR=controlled Internal Drug Release, $PGF_{2\alpha}$=prostaglandin, Fr x BJ= Friesian x Bunaji cross, BJ= Bunaji.

4.7. NUMBER OF COWS THAT EXHIBITED STANDING ESTRUS AT DIFFERENT TIMES OF THE DAY.

From the findings of this study, cows exhibited standing estrus between 6am and 12noon s of the day for Bunaji and Friesian X Bunaji cows in as follows; 1, 5 in treatment I, 4, 1 in treatment II and 0, and 3 in treatment II respectively. While between 12noon and 6pm were 2, 2, for Bunaji and Friesian X Bunaji cows in treatment I, 1 and 3 in treatment II and 0 and 1 in treatment III respectively.

Table 4.2: Number of cows that exhibited standing estrus at different times of the day.

Time of day	Treatments								
	CIDR			CIDR + $PGF_{2\alpha}$			$PGF_{2\alpha}$		
	Bunaji	Fr x BJ	Total	Bunaji	Fr x BJ	Total	Bunaji	Fr x BJ	Total
6am-12noon	1	5	6	4	1	5	0	3	3
12noon-6pm	2	2	4	1	3	4	0	1	1

CIDR=controlled Internal Drug Release, $PGF_{2\alpha}$=prostaglandin, Fr x BJ= Friesian x Bunaji cross.

4.8. CONCEPTION AND PREGNANCY RATES

Conception rates of Bunaji and Friesian x Bunaji cows for treatment I were 33 % (1/3) and 42 % (3/7). In treatment II the conception rates for CIDR + $PGF_{2\alpha}$ were 60 % (3/5) and 50 % (2/4) for Bunaji and Friesian x Bunaji cows while those of treatment III were 0 % (0/0) and 75 % (4/7) for Bunaji and Friesian x Bunaji cows respectively.

Pregnancy rates of Bunaji and Friesian x Bunaji cows were 12 % (1/8) and 37 % (3/8) for treatment I. treatment II had pregnancy rates of 37 % (3/8) and 28.5 % (2/8) for Bunaji and Friesian x Bunaji cows while those of treatment III were 0 % (0/0) and 42 % (3/7) for Bunaji and Friesian x Bunaji cows respectively.

Table 4.3. Conception and pregnancy rates of Bunaji and Friesian x Bunaji cows treated with CIDR, CIDR + PGF2α and PGF2α.

	Treatments					
	CIDR		CIDR+ $PGF_{2\alpha}$		$PGF_{2\alpha}$	
	BJ	FR X BJ	BJ	FR X BJ	BJ	FR X BJ
Conception Rates (%)	33	42	60	50	0	75
Pregnancy Rates (%)	12	37	37	28	0	42

CIDR=controlled Internal Drug Release, $PGF_{2\alpha}$=prostaglandin, Fr x BJ= Friesian x Bunaji cross, BJ= Bunaji.

CHAPTER FIVE

5.0. DISCUSSION

5.1. RETENTION RATE

Retention rate of an intravaginal device/CIDR is a factor that can greatly affect the success of any synchronization programme. It may be affected by type of management system, experience/skill of operator during insertion, breed size, duration of progestagen insertion and nature of progestagen (Maxwel and Barnes, 1986; Rhodes and Nathaniel, 1988).

Retention rate for all the treatments in the study were 100 % this could be attributed to the T-shape nature of the intravaginal device which get attached to the vaginal walls of the cow when inserted. The 100 % retention rate observed in this study agrees with the reports of McLean (2003) for two different lengths of CIDR insert and Dare, *et al*., (2010) for PRID and PRID + PMSG during the dry season. Ryan *et al*. (1995) reported lost of over 5 % of 1, 559. Lucy *et al*. (2001) reported losing 1 % to 5 % of inserts per treatment group while Dare *et al*., (2010) reported 90 % and 85 % for PRID and PRID + PMSG during rainy season.

5.2. ESTRUS RESPONSE RATE

This study revealed a lower estrus response rate in Bunaji (37 %) than Friesian x Bunaji (87 %) cows treated with CIDR alone. This could be attributed to the poor reproductive performance (genetics) of Bunaji cows which is an indigenous breed. The total estrus response rate in treatment I (62 %) compares favourably with the report of Dare *et al*.

(2010) who reported 60 % during the rainy season but higher than 37.5 % during the dry season using in Bunaji cows treated with PRID + PMSG.

Treatment II (CIDR + $PGF_{2\alpha}$) estrus response rate showed significant difference ($p<0.05$) between Bunaji cows (62 %) and Friesian x Bunaji (50 %). The combination of progestagen (extending the life span of the CL) and prostaglandin (shortening the life span of the CL, inducing luteolysis) in synchronizing estrus seems more effective in the local breed. The rate of synchronization of estrus following this treatment has been reported as been greater than single injection of $PGF_{2\alpha}$ alone (Petterson *et al*., 1995). The total estrus response in treatment II (56 %) is lower than the report of Dare *et al*. (2010) for dry (66.7 %) and rainy season (65.0 %) respectively using PRID + PMSG. Lucy *et al*. (2001) reported estrus response rate of 45 % for anestrous cows and 48 % for prepubertal heifers which are lower than the results obtained in this study but 72 % for cyclic cows and 80 % for cyclic heifers which are higher than the result of this study treated with CIDR + $PGF_{2\alpha}$ even though cows used for this study were rectally palpated for cyclicity.

There was significant difference in estrus response of cows treated with $PGF_{2\alpha}$ (0 % for Bunaji and 57 % for Friesian x Bunaji cows). Bunaji cows with poor genetic make up for reproductive performance did not respond to the single dose of $PGF_{2\alpha}$ compared to the 57 % recorded by Friesian x Bunaji whose genetic makeup has been improved upon. This could also be attributed to partial luteolysis of the CL by the single dose of $PGF_{2\alpha}$ Though the stage of the cycle at the time of prostaglandin treatment could affect estrus response. Larson (1992) reported that 67 % of cattle showed estrus when treated between day 5 and day 17 of the estrous cycle, 77 % responded when treated day 9 through day 12, while the

greatest number of animals was seen when treated after day 12 of the estrous cycle (>91 %). The result in this study was greater than those of Lucy *et al*. (2001) for anestrous cows and prepubertal heifers but lower than those of cyclic cows and cyclic heifers respectively. The 26 % in this study is also lower than the report of Kawu (2011) in Red Sokoto does synchronized using 7.5 mg $PGF_{2\alpha}$ in dry season. The decreased estrus response rate may be due to inhibition of estrus expression caused by incomplete regression of the CL and therefore elevated progesterone concentration (Pinheiro *et al*., 1998; Rekwot *et al*., 1999) and means of estrus detection. The overall estrus response would have increased with intensive breeding to enable good estrus detection.

5.3. INTERVAL TO ONSET OF ESTRUS

The interval to onset of estrus was not significantly different ($p<0.05$) for Bunaji and Friesian x Bunaji cows treated with CIDR and CIDR + $PGF_{2\alpha}$. Bunaji and Friesian x Bunaji cows treated with CIDR took longer time to come into estrus 5±1.0 and 5±1.2 hours respectively but shorter time for Bunaji (3±0.45) and Friesian x Bunaji (1.7±0.25) cows treated with CIDR + $PGF_{2\alpha}$. This could the attributed to the breed, environment and efficacy of CIDR + PGF2α protocol to synchronize estrus. Romano (1998), Greyling and Van der Nest (2000) reported interval to onset of estrus to occur within 6-120 hours which is greater than the findings of this study.

The results from this study revealed that there was no significant difference ($p<0.05$) for both treatments. It could be observed that Friesian x Bunaji cows in both treatments recorded longer duration of estrus than Bunaji, this could be attributed to many factors, including breed (Rae *et al*., 1999), environment (Landaeta-Hernandez *et al*., 2002),

number of animals in estrus at a time (Hurnick *et al*., 1975; Landaeta-Hernandez *et al*., 2002), and estrous detection method (Rae *et al*., 1999) influence the duration of estrus; however, multiple studies indicate that *Bos indicus* heifers (Plasse *et al*., 1970) and cows (Randel, *1984*; Pinheiro *et al*., 1998) have a shorter duration of estrus compared to *Bos taurus* heifers (Richardson *et al*., 2002) and cows (Schams *et al*., 1977). The 7±2.9 hours estrus duration for Bunaji treated with CIDR in this study agrees with the report of Rae *et al*. (1999) who reported 6.7 hours for Angus (*Bos taurus*). Cows (Bunaji and Friesian x Bunaji) treated with CIDR + $PGF_{2\alpha}$ seems more effective probably due to the high progesterone levels. Friesian x Bunaji recorded 12±2.8 and 13±1.2 hours respectively corroborate with the findings of Rae *et al*. (1999) for crossbred (*Bos indicus* × *Bos taurus*) heifers (11.9 hours).

5.4. EFFECT OF PROTOCOL ON NUMBER OF MOUNTS

The primary and most reliable indicator of estrus is for a cow to allow herself to be mounted by another cow (Glencross *et al*., 1981). There was no significant difference in the number of mounts per period for Bunaji and Friesian x Bunaji cows treated with CIDR but there was significant difference between Bunaji (4.33±0.61) and Friesian x Bunaji (11.25±1.15) cows treated with CIDR + $PGF_{2\alpha}$. This could be due to the breed effect, synchronized estrus and environment. Rae *et al*. (1999) reported that breed had a significant effect on mounting activity during a synchronized estrus in Angus, Brahman, and Angus × Brahman, receiving an average of 19, 25, and 37 mounts, respectively which are higher than the findings of this study. Cows that are consuming feed in a crowded pen

or aisle, or housed on poor footing will demonstrate less mounting activity (Britt *et al*., 1986).

5.5. SWOLLEN VULVA

The result of swollen vulva in this study revealed the efficacy of CIDR, CIDR + $PGF_{2\alpha}$ and $PGF_{2\alpha}$ in synchronizing oestrus in both Bunaji and Friesian x Bunaji cows. Bunaji with low reproductive performance recorded 50 % above while Friesian x Bunaji with improved genetic makeup had better results. Secondary signs of estrus include mounting other cows, clear mucous discharge from the vulva, swelling of the vulva, restlessness, following other cows, chin resting, and lip curling. These signs can occur before, during, or after estrus and do not relate to the time of ovulation, but should instead be used as clues that a cow should be observed more closely (Nebel, 2003). Only cows that stood to be mounted were inseminated in this study.

Vaginal discharge rate at CIDR removal in this study were all 50 % and above. This could be attributed to the physiologic disturbance of the vagina by the insert. A total of 35.8 % percent discharges were recorded in heifers treated with Safe-Lube compared to 21.69 % discharges in NolvaLube treatment group as reported by McLean (2003) which are lower than those of this study recording 50% and above.

5.6. NUMBER OF COWS THAT EXHIBITED STANDING ESTRUS AT DIFFERENT TIME OF THE DAY

The results for number of cows exhibiting standing estrus at different time of the day revealed higher values between 6 am - 12 noon than 12 noon – 6 pm for all the treatments respectively. The time of day which cows display estrus can also be influenced by breed. It has been indicated that the onset of estrus occurs more frequently during the nighttime

hours than daytime hours in both *Bos taurus* (Stevenson *et al*., 1996) and *Bos indicus* (Pinheiro *et al*., 1998) cows.

However, while Landaeta-Hernandez *et al*. (2002) observed more cows initiate estrus during night hours (60%) than day hours (40%), they observed more Senepol (*Bos taurus*) cows initiate estrus during the daytime hours than Brahman cows. The result of this study agrees with the report of Voh Jr. *et al*. (2004b) who reported more Bunaji in estrus in the morning than evening treated with PRID.

5.7. CONCEPTION AND PREGNANCY RATES

This study revealed conception rates of between 33 % (Bunaji treated with CIDR) as the lowest and 75 % (Friesian x Bunaji treated $PGF_{2\alpha}$) as the highest. The low conception rate in treatment I may be attributed to the low progesterone levels which is important in pregnancy. Cows treated with CIDR + $PGF_{2\alpha}$ did better which could be due to increased levels of progesterone because of its importance in maintaining pregnancy. Conception rate of cows treated with CIDR + $PGF_{2\alpha}$ for Bunaji (60 %) and Friesian x Bunaji (50 %) in this study were lower but the 75 % for Friesian x Bunaji treated with $PGF_{2\alpha}$ agrees with the report of Dare *et al*. (2010) who reported 70.0 % (dry season) and 76.9 % (rainy season) treated with PRID + PMSG. Lucy *et al*. (2001) reported 70 % conception for cyclic cows treated with $PGF_{2\alpha}$ which corroborate with the findings of this study for Friesian x Bunaji cows treated with $PGF_{2\alpha}$. The 60 % for Bunaji cows treated with CIDR + $PGF_{2\alpha}$ in this study also agrees with reports of (Stevenson *et al*., 2000) and (Lucy *et al*., 2001) treated with 2 shots of $PGF_{2\alpha}$ and CIDR + $PGF_{2\alpha}$.

Pregnancy rates for all the treatments in this study were low. Buanji cows treated with CIDR and Friesian x Bunaji cows treated with $PGF_{2\alpha}$ recorded the lowest and highest respectively. This could be attributed to the effect on breed and/or amount of progesterone as it is a major hormone in maintaining pregnancy. Rekwot *et al*. (1999) reported pregnancy rate of 45.7 % for Zebu cattle treated with $PGF_{2\alpha}$ which is higher than the findings of this study 42 % for Firesian x Bunaji cows treated with $PGF_{2\alpha}$. All the findings of this study were lower than those reported by (Dejarnette *et al*., 2001) who reported 52 % and 60 % for select synch and select synch + MGA in suckled beef cows. The pregnancy rate of 28 % for Friesian x Bunaji cows treated with CIDR + $PGF_{2\alpha}$ in this study agrees with the findings of (Lucy *et al*., 2001) who reported 26 % for suckled beef cows treated with CIDR + $PGF_{2\alpha}$.

CHAPTER SIX

6.1. SUMMARY, CONCLUSION AND RECOMMENDATIONS

In summary, retention rate in the study was 100 % for Bunaji and Friesian x Bunaji cows respectively. Estrus response rate were 50 % and above for all the treatments except for Bunaji cows treated with CIDR (37 %). Interval to onset of estrus was not significantly different for all the treatments but Bunaji and Friesian x Bunaji cows treated with CIDR + $PGF_{2\alpha}$ took a short period of time to come into estrus. Estrus duration and number of mounts were higher and longer in Friesian x Bunaji than Bunaji cows. Swollen vulva and Vaginal Discharge rates were higher in Friesian x Bunaji cows than Bunaji cows. More cows irrespective of breed and treatment came into estrus between 6.00 am – 12 noon than 12 noon – 6 pm. Conception and pregnancy rates were better for CIDR + $PGF_{2\alpha}$ treated cows than CIDR and $PGF_{2\alpha}$ alone treated cows.

6.2. CONCLUSION

Based on the findings of this study, it can be concluded that, CIDR, CIDR + $PG_{2\alpha}$ and $PG_{2\alpha}$ are capable of inducing good estrus response, conception and pregnancy rates in Bunaji and Friesian x Bunaji cows except in Bunaji cows treated with one shot of $PGF_{2\alpha}$ alone. The combination of CIDR + $PGF_{2\alpha}$ seems more effective in synchronizing estrus, shorter time to onset of estrus and longer estrus duration in Bunaji and Friesian x Bunaji cows. One shot of $PGF_{2\alpha}$ was more effective in Friesian x Bunaji cows in terms of conception and pregnancy rates with no effect in Bunaji.

6.3 RECOMMENDATIONS

Based on the findings of this study, the following recommendations were made;

(i) CIDR + $PGF_{2\alpha}$ proved effective in synchronizing estrus in Bunaji and Friesian x Bunaji cows with good conception and pregnancy rates.

(ii) Estrus response, conception and pregnancy rates could be possibly improved with intensive breeding and by improving methods in semen placement, semen handling and AI technique by the inseminators.

(iii) Blood analysis could be done to better observe the effect of the hormone being administered to the animals to establish the progesterone profile.

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