Improving The Reproductive Performance of the Muturu Breed of Cattle in Nigeria using Modified Ovsynch and Progesterone Synchronization Protocol

A thesis presented in partial fulfilment of the requirement for the degree of

Master of Science

in

Animal Science

Institute of Veterinary, Animal and Biomedical Sciences

Massey University

Palmerston North, New Zealand

by

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2017

DEDICATION

I dedicate this work to the almighty God for His faithfulness and His ever-present help during my period of study.

ABSTRACT

The Ovsynch protocol has been used in cattle to synchronize ovulation and facilitate fixed-time AI (FTAI) but its efficacy has not been evaluated in the Nigerian Muturu breed of *B. indicus* cattle. The effects of a modified Ovsynch protocol (modified by the addition of progesterone, eCG and modification of time for AI) upon the reproductive performance of Muturu cows were therefore studied.

The study was conducted at Abakiliki in Ebonyi state, Nigeria. Muturu cows (n=100) were selected for the study based on their previous calving history and the presence of follicle of greater than 5 mm diameter. Cows were then allotted to Untreated and Treated groups (n=50 each). Synchronization was by an Ovsynch regimen (Day 0: 100 µg GnRH, Day 7: 500 µg $PGF_{2\alpha}$ (Ovuprost), Day 10: 100 µg GnRH), augmented by a progesterone-releasing intravaginal insert (CIDR) between Days 0 and 7, and 400 IU eCG (Norvomon) on Day 7. FTAI was performed 12 and 24 h after the second GnRH injection. Untreated animals were monitored over two consecutive oestrous cycles and examined daily for the presence of oestrus over a period of 49 days, and were exposed to natural mating upon detection of oestrus in the second oestrous cycle observed. Ovarian ultrasonography to ascertain follicle size was performed at the onset and end of oestrus in the Untreated group, and on Days 0,7,10 in the Treated group. Pregnancy diagnosis (ultrasonographic) was performed 45 days after FTAI (Synchronized) and 45 days after the last observed oestrus during the breeding season (Untreated). Blood samples were collected from Treated group of animals for progesterone, LH and oestradiol concentrations assay.

All animals in the Treated group displayed oestrus after synchronization and all animals in the Untreated groups displayed oestrus during the 49-day study period. Follicle size (18 ± 0.4 mm *versus* 12± 0.2 mm), ovulation rate (100% versus 64%), duration of oestrus (54 h versus 19 h) and 45-day pregnancy rate (84% versus 36%), were all greater ($p<0.005$) in Treated than Untreated animals. Additionally, the animals in the Treated group displayed 46% multiple ovulations, compared with none in the Untreated group. Oestradiol concentrations were related to follicle size. Increase in follicle size resulted in higher concentration of oestradiol. The

presence of multiple ovulations appears to have been related to the use of eCG in the protocol. No multiple pregnancies occurred.

It is concluded that the modified Ovsynch protocol produced synchrony of oestrus, ovulation and improved follicle size, that were supported by normal endocrine patterns. It therefore appears that oestrus synchronization and FTAI can improve reproductive performance of the Muturu breed of cattle.

Key words: Muturu cows, pregnancy, follicle size, ovulation, oestrus, modified Ovsynch, Nigeria

ACKNOWLEDGEMENT

I would like to express my sincere gratitude and appreciation to my wonderful supervisors: Professor Tim Parkinson, who afforded me the opportunity to gain some knowledge from his pool of scientific knowledge and skills in cattle production, Dr Sam Peterson thanks for your thoughtfulness and patience and Associate Professor Richard Laven, thanks for your assistance with your expertise in statistical analysis, and for providing all the materials I needed in the field, making the work a reality. The previous two years have been extraordinary; guidance, advice, support and constructive criticisms and feedbacks with rare scientific and logical comments from all my supervisors, have been fundamental in accomplishing my goals at Massey University New Zealand.

Appreciation is extended to my co-supervisor from Nigeria, Dr Solomon Oloruneke who worked tirelessly with all dedication, and to my lecturers at Department of Animal Science, Ebonyi State University Abakiliki, Nigeria, Professor Osakwe I.I, head of Department of Animal Science, Mr Jude Ogunmkpebi, Professor Mrs Patience Onu, Dr Mrs Vivian Olefureh and students; Ambrose, Odinakachi, Chika and Monday, who all assisted me all through my field work. Your guidance and efforts to make this work a reality are well appreciated.

Special thanks are expressed to: Mr and Mrs Ojemba, whose inspirations and prayers kept me moving on the track, my wonderful cousins Uche Onwuama, Uche Dike, Chimma and Chizzy, your calls and encouragement are well appreciated, my wonderful friends Engineer Mrs Ifeaka Mezue, Mrs Udeh Grace, am grateful to you all. Professor and Dr Mrs Doris Adeyinka, thanks for being a wonderful family to me in New Zealand and to Grace Ehoche, thanks for being a wonderful sister.

I am grateful to the New Zealand Government for providing the scholarship through Ministry of Foreign Affairs and Trade (MFAT) to undertake my M.Sc. in New Zealand and in this great institution of learning. I appreciate all the efforts and kind gestures of Jamie Hooper, Broderick Dave, Sylvia and host of others from the Massey University International office, you all made my stay enjoyable and friendly.

Lastly, to my Dad and siblings, thanks for being there for me. To a wonderful family, Barr and lady Amaechi Okafor, I would not have attained this height without your parental guidance and efforts. Lady O, my Dearest, thank you for your daily contact all through my stay in New

Zealand, you walked with me through the thick and thin of this great journey, mum you are the best. To my Beloved, Dr Casmir, I appreciate all your love and care Together, they provided the emotional support to accomplish my goals throughout this extraordinary experience.

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LIST OF ABBREVIATIONS

AI	Artificial insemination
B	Bos
CIDR	Controlled internal drug releasing device
CL	Corpus luteum
DBG	dried brewers grain
DF	Dominant follicle
E2	Oestradiol
eCG	Equine chorionic gonadotropin
GNRH	Gonadotropin releasing hormone
GNRH-1-	First GnRH administration /injection
GNRH-2-	Second GnRH injection
FTAI	Fixed time artificial insemination
FSH	Follicle stimulating hormone
LH	Luteinizing hormone
MGA	Melengesterol acetate
OR	Ovulation rate
PR	Pregnancy rate
P/AI	Pregnancy per artificial insemination
PRID	Progesterone releasing device
PMSG	Pregnant mare serum gonadotropin
PGF	Prostaglandin
P4	Progesterone

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CHAPTER 1

1.1. INTRODUCTION

High reproductive performance in beef herds is an indispensable prerequisite to ensure optimum output and economic premium in livestock production. Cattle production is essential in the economics of the developing countries as the animals and products play vital roles in alleviating the nutritional and economic challenges of such countries. Most of the world's cattle are found in the tropical regions (Africa, Australia, and Brazil) and the *Bos indicus* predominates in these regions due to their greater adaptation to poor management condition, harsh climatic condition and diseases when compared to *Bos. taurus* (Bó *et al*., 2006).

The Muturu breed, which is a tropical breed of *B. indicus,* is a trypanotolerant breed with a good body conformation and thrives well in the southeast part of Nigeria. Current approaches aimed at improving cattle production in Nigeria hinge around rapid multiplication and expansion of trypanotolerant breeds, such as the Muturu breed especially in the eastern part of Nigeria where pasture is more abundant and available. Unfortunately, this breed has declined in number and is at risk of becoming extinct.

The *B. indicus* breeds are characterized with long post-partum anoestrus, short oestrus length and a high incidence of showing oestrus at night (Baruselli *et al*., 2004). These factors appear to be the primary reasons that impair the reproductive performance of the tropical breeds, including the Muturu, and delay adoption of artificial insemination (AI) into the breeding programme. AI would be beneficial to the beef herd and farmers, yet incorporation of AI into breeding programmes is limited by inefficient oestrus detection (Bó and Baruselli 2014).

Oestrus detection in *B. indicus* tends to be a hard task to accomplish owing to the fact that 'silent heat' or missed heat characterizes the *B. indicus*, and stressors such as rain and movement between pastures, poor management, poor nutrition and social hierarchy influence mounting and oestrus-linked activities (Galina *et al*., 1996). This is because they negatively influence the activity of the reproductive neuroendocrine axis; apparently more severely in *B. indicus* that in *B. taurus* (Galina *et al*., 1996). Farmers desire to maintain a twelve-month inter-calving interval and as such, need to employ protocols that ensure high submission rate of cows (proportion of non-pregnant cows submitted for AI). Therefore, the use of fixed-time artificial insemination (FTAI) in beef herds is geared towards eliminating the need for oestrus detection, introducing the best genetic gain into the herd and getting more cows pregnant earlier

than usual in the breeding season which will invariably lead to improved weaning weight and more-uniform calves (Baruselli *et al*., 2004; Bó *et al*., 2006; Baruselli *et al*., 2012).

Many synchronisation protocols have been developed to facilitate FTAI and achieve improved reproductive outcomes in beef herds under tropical conditions (Baruselli *et al*., 2012; Bó *et al*., 2006: Meneghetti *et al*., 2009). Some of the protocols are gonadotropin- or prostaglandin-based and may entail the use of progestin for a period of seven to nine days and the use of equine chorionic gonadotropin. In non-cycling cows, treatment with progesterone-releasing vaginal inserts such as CIDR (controlled internal drug releasing device) that release exogenous progesterone appears to (i) increase release of LH pulses and increased number of LH receptors in granulosa and theca cells of preovulatory follicles (Garcia-Winderetal.,1986), and (ii) decrease occurrence of premature luteolysis after first postpartum ovulation (Vasconcelos *et al*., 2009); and together these culminate in high pregnancy rates. The CIDR has been used to initiate cyclicity in postpartum anoestrous cows and improve their reproductive performance and this has been evident in the works of Lamb *et al*. (2001) and Schafer *et al*. (2007) which support the use of progestin as a means of presynchronization prior to $PGF_{2\alpha}$ injection in postpartum beef cows. Such protocols can have a positive impact on overcoming the challenges of long-term postpartum cyclicity and poor oestrus detection due to display of oestrus at night associated with the *B. indicus.* Busch et al. (2008), Lamb et al. (2001), Larson et al. (2006) and Schafer et al. (2007) have demonstrated improved pregnancy rates with this protocol. Previous reports on the use of PRID (progesterone releasing intravaginal device) and $PGF_{2\alpha}$ on N'dama (Voh *et al*., 2004), PRID and PMSG (pregnant mare serum gonadotropin) on Bunaji (Dare *et al*., 2010), CIDR-B and $PGF_{2\alpha}$ on Bunaji (Achi *et al*., 2016) breed of cattle (which is in the same class as Muturu) yielded higher results in terms of oestrus response, pregnancy and conception rate than that of the control group.

Optimization of follicle size may be an important consideration when treating cows in postpartum anoestrus. Cows with larger ovulatory follicles had a greater ovulation rate and this resulted in a greater number of pregnancies per insemination in beef cattle (Bó *et al*., 2006). Also, Sa Filho *et al*. (2010a) reported a positive relationship between pregnancy rate and increased ovarian follicle size. It has been established that *B. indicus* generally secrete insufficient luteinizing hormone to cause adequate final growth and maturation of the preovulatory follicle (Bó *et al*., 2003). In view of the above limitation of the *B. indicus* it becomes imperative to use equine chorionic gonadotropin to improve follicular growth and

ovulation, which culminates in high pregnancy rate. High pregnancy rate has been obtained in postpartum anoestrous cows exposed to eCG treatment (Sa Filho *et al*., 2010a, 2011; Sales *et al*., 2011; Silva Filho *et al*., 2013; Tortorella *et al*., 2013; Barreiros *et al*., 2014). Results of studies on the N'dama cattle showed higher ovulation and multiple ovulation rates in eCG-treated animals than in the control group (Okouyi and Hanzen 2016).

The use of modern biotechnological advances in breeding and reproduction is a vital tool for enhancing the reproductive efficiency of cattle production. One such technology that can be adopted is the use of oestrus synchronisation and artificial insemination, which are effective breeding and reproduction management tools for fostering genetic improvement and multiplication. There is no information on the use of these animal biotechnology tools on the Muturu breed, but current protocols that are GnRH-based with inclusion of CIDR and eCG have resulted to higher indices of oestrus, pregnancy and conception rate which all culminates in better reproductive performance, due to elimination of oestrus detection, better control of follicular wave emergence and synchrony of ovulation. Many such results have been obtained in *B. taurus* and in *B. indicus*, although not in the Muturu breed of cattle; these hormonal treatments may be expected to have similar effects on the reproductive performance of the Muturu breed, owing to the fact that Muturu cattle is a *Bos indicus* . Therefore, the objective of this study was to investigate the hypothesis that modification of Ovsynch synchronization protocol in combination with CIDR and eCG may be of benefit in improving the reproductive performance of this breed.

CHAPTER 2

LITERATURE REVIEW

2.1. Cattle production in Nigeria

2.1.1. Historic background

Nigeria has been, and is still, one of the leading countries in cattle production in sub-Saharan Africa (Winrock International, 1992). During British colonization, at a time when Great Britain was facing a shortage of beef supply due to an ever-increasing population, Nigeria was a major exporter and supplier of beef to that nation (World Bank, 1989). Although that market has long since ended, the Nigerian cattle market has continued to boom until the present day but not without challenges.

The cattle industry in Nigeria is estimated to provide 12% of agricultural gross domestic product (GDP) and about 7% of the overall GDP (Resource Inventory Management. 1992), which is an indication that the impact of cattle production on the Nigerian economy is highly significant (Mshelbwala, 2013). In 1992, the cattle industry provided more than 50% of the total meat supply in the country (Resource Inventory Management. 1992), although there were other sources of animal protein. The cattle industry also provides raw materials in the form of draught power for farming and utilization of resources that would have been laid waste, such as crop residues (Fricke, 1979).

In Nigeria today, the cattle industry does not only provide meat and milk but export of their products makes a significant contribution to the country's foreign exchange and, as well, creates employment opportunities in terms of cattle rearers, butchers, traders and transporters (Oladoku, 1976). Cattle also have a great cultural significance in Nigeria, as they play a crucial role in ceremonies such as marriage, funerals and sacrifices. In comparison to different sectors of Nigerian agriculture, beef production is second to arable crop production. The beef industry has improving prospects in the Nigerian agricultural system owing to its ready market, as consumers' demand for beef is very high due to preference and low price. This habit of consuming beef has been fed by availability of beef due to the vast area of land with grasses, water bodies (lakes, rivers), supplementary feeds like dried brewery grain (DBG), wheat offal and rice bran (which are available in all the regions of the country). Another factor that encourages beef consumption in Nigeria is the lack of religious or cultural restrictions to its

consumption. Although there are no records of number of cattle butchered in each Nigerian state daily, it is estimated that not less than five hundred animals are consumed daily in each of the thirty-seven states of the country, including the capital (Oladoku, 1976). Despite the significant role of the cattle industry in the Nigerian economy, the growth and development of this industry has not really attained great heights due to problems such as poor management practices, poor nutrition and poor reproductive performance (Ahamefule *et al*., 2007).

2.1.2. Previous practice

Nigeria is blessed with very rich ecological zones, with the climate being very hot and dry in the north and humid in the south. The majority of cattle are raised by Fulani nomads in the humid zones where there is an abundance of rainfall and, hence, good pasture availability. The Fulani are a particular ethnic group in Nigeria, who rear cattle as their major occupation. Each of these nomadic farmers can travel a distance of about 300 km with one hundred cattle in the course of a year (Adeambo, 2001). Most of the livestock are managed according to traditional rather than commercial systems. The traditional management of these animals involves the Fulani nomads, who travel with these animals as they feed, in some cases, grazing into people's homes and farms. Recently, increasing population and arable farming did not just reduce the areas available for grazing, but generated conflicts between landlords and herdsmen (Idama, 2015). The Grazing Reserve Law was enacted in 1965 by the then Northern Nigeria in order to promote cattle production and avoid such conflicts (Idama, 2015).

About 30-40 years ago, beef production was the sole occupation of the Fulani herdsmen. Ranches were not common in Nigeria; hence, these herdsmen became nomadic pastoralists due to agro-climatic limitations and in a bid to meet the nutritional requirements of their animals. The land-tenure system operating in the country was another limiting factor, as it prohibited the herdsmen from grazing any uncultivated land. The management of cattle in terms of reproduction, nutrition, husbandry practices and health, therefore, has been based on the traditional farmer's perspective for a long time. In recent years, the ever-increasing population and greater demand for beef due to its nutritional value and need for animal products, has forced the cattle industry to adopt management practices that could improve production. As a result, the government started establishing farms where they can rear cattle in semi-intensive production systems, gradually moving away from traditional Fulani nomadism. The trend moved again into research and development and many universities and individuals started self-sponsored research in animal husbandry and breeding in order to improve the indigenous

Nigerian breeds through cross breeding. This led to the establishment of the National Animal Production Research Institute (NAPRI) and the Veterinary Research Institute (VOM) in order to achieve better production in the beef industry and livestock in general.

2.1.3. Cattle breeds in Nigeria

In recent times, world-wide concern has grown over the risk of long-term loss of plant and animal genetic diversity. Hence, there is a need for conservation within the species of cattle. Nigeria has many breeds of cattle (notably , White Fulani (Bunaji), Keteku, Muturu, N'dama, Kuri, Sokoto and Adamawa Gudali (Oyedipe *et al.,* 1982)), all of which are locally adapted and which represent a genetic resource that is of current and potential future value to the cattle industry. The White Fulani is the most numerous of these breeds. Most of the breeds are found in the northern and middle belt regions of the country, but the percentage of the trpanotolerant Muturu breeds are greater in the southwest and southeast (Oyedipe *et al.*, 1982) (see Table 2.1). In the early 1960s and 70s, the Muturu breed comprised 8.3% of the total population of cattle in Nigeria, but the number declined by a third by the 1980s at which point, a special programme was initiated with the aim to increase the population of Muturu breed through importation of N'dama animals from Liberia. Moreover, Adebambo (2001) reported that N'dama had closer traits to Muturu in terms of adaptation and survival, so opportunities may exist for interbreeding between these two breeds with the aim of increasing the population of the Muturu breed. Muturu cattle have become adapted to the environment over the years through natural selection, and so have the ability to survive and reproduce in the conditions of high humidity, and heat stress, which exist in southeast Nigeria and the productivity traits of this breed can be seen in Table 2.3. This breed is well suited to the farming system practiced in that region (Table 2.4) and, because it is of great potential in this environment, it is imperative to conserve their genetic resources, improve their development and identify the genes that confer hardiness upon the breed (Adebambo 2001).

The Muturu, trypano-tolerant cattle breed, is probably one of the least known breeds of cattle in West Africa. Little has been published on its distribution, management, morphological characteristics or biological performance. Early reports showed that the Muturu cattle were once widely distributed across the continent from Liberia, across the West African sub region, to Ethiopia (Ferguson, 1967). However, due to expansion of the Zebu population and rapid urbanization, the small-bodied animal came under pressure and was found surviving in pockets of the savannahs and in the humid forest zones where it had the comparative advantage of

trypano-tolerance (Adeniji, 1985). The survival of the cattle in the humid and forest zones of Nigeria stems from the fact that the animal is still sacred in so many communities and its milk is widely used for medicinal purposes. In some states of Nigeria, the semi-feral Muturu are not tended but hunted when required for sacrifice, which is evident in Table 2.2, where Muturu population is higher in the villages than in the urban and pastoral areas.

Research effort is being expended upon the conservation and multiplication of the Muturu breed. This can best be achieved by research and development and through proper husbandry. For example, Ezekwe and Kamalu (2000), discovered that under improved husbandry and nutritional condition, bulls were able to attain puberty at the age of 11 months as against 24 months, with an average weight of 120 kg. One of the key ways in which the "unique" gene of the Muturu breed can be conserved (and to prevent its extinction) is through increased breeding efficiency, though, for example, oestrous synchronization protocols that facilitate the use of FTAI.

Table 2.1 The Muturu cattle breed as a proportion of trypanotolerant cattle per State in Nigeria. (Source: Akinwunmi and Ikpe 1985).

State	Total no of cattle	% of total trypanotolerant/State
Ogun	536	8.1
Ondo	3660	56.4
Oyo	8447	35.1
Lagos	1217	30.8
Bendel	1547	75.8
Anambra	11310	92.4
Imo	7412	96.1
Rivers	329	84.7
Cross River	2575	96.9

Table2.2 Population of cattle in Nigeria from different areas of the state. (Source:Resource Inventory Management, 1992)

Species	Pastoral	Village	Urban	Total	% SE
All cattle	11,478,145	2,358,078	49,590	13,885,813	1.6
Muturu		114,241	931	115,172	19.5
Zebu and others	11,473,800	2,248,182	48,659	13,770,641	1.6

Table2.3 N'Dama, Muturu and Zebu cattle breed production traits (Source: Adebambo, 2001).

Trait		Muturu	N'Dama x Zebu	Zebu
Age at PI calving (days)		635	684	761
Calving interval (days)		350	363	403
Weight at birth	Males (kg)	13.7	18.1	26.5
	Females (kg)	13.9	15.9	22.7
Weight at 3 months.	Males (kg)	38.9	54.6	78.0
	Females (kg)	37.5	54.3	77.5
Weight at 6 months.	Males (kg)	71.5	-	130.4
	Females (kg)	61.5	92.1	28.6
Weight at 9 months.	Males (kg)	98.1	119.3	178.2
	Females (kg)	82.1	112.4	165.0
Weight at 12 months.	Males (kg)	108.1	137.4	206.7
	Females (kg)	93.5	124.6	193.2
Cow weight 1-2 years (kg)	1-2 years (kg	109.0	181.0	242.0
	3-4 years (kg)	167.0	252.0	323.0
	5-6 years (kg)	204.0	275.0	374.0

PI=post-partum interval

Table 2.4 The opinion of various cattle farmers on the system of management of Muturu cattle. (Source: Anyanwu et al.2002)

Management system	Percentage (%)
Tethering	96.25
Free range	2.50
Intensive	1.25
BREEDING SYSTEM	
Free mating	81.25
Hand mating	18.75

2.2. The bovine oestrous cycle

The cycling cow is polyoestrus, with no clear breeding season. The average duration of the oestrous cycle is 21 days (typical range 18-24 days) (Forde *et al*., 2011). It is characterized by two phases; the luteal and follicular phases (Adams *et al*., 2008). The primary control of the oestrous cycle is through the hormones of the hypothalamo-pituitary ovarian axis, which act in synergy to bring about the physiological response needed. This hormonal control critically relies upon gonadotropin releasing hormone from the hypothalamus, follicle stimulating hormone (FSH) and luteinizing hormone from the anterior pituitary, steroids (progesterone and oestradiol) and peptides (primarily inhibin) from ovaries and $PGF_{2\alpha}$ from the uterus (Fortune, 1994). The positive and negative feedback mechanisms are the basic pathways through which these hormones exert their impact (Kanitz, 2003). It is necessary to understand the physiology of the oestrous cycle in order to manipulate it for oestrus synchronization and fixed-time AI.

2.3. Follicular phase

The follicular phase is characterized by two stages, proestrus and oestrus and it is shorter than the luteal phase. (3 vs 18 days)

2.3.1. Proestrus

This stage of the follicular phase begins with the regression of the *corpus luteum* (CL) from the preceding cycle (Fortune, 1994). Luteolysis entails functional (decrease in progesterone synthesis) and structural (cell death and decadence of luteal tissues) regression (Ginther *et al.,* 1989). The uterus secretes $PGF_{2\alpha}$, which causes regression of the CL, which, in turn, causes a reduction of circulating progesterone concentration. The regression of the CL permits an increase in LH by eliminating the negative feedback effect of progesterone on the pituitary, culminating in stabilizing follicular development and growth (Sartori and Barros, 2011). The dominant follicle at this stage grows very rapidly resulting in rising plasma concentrations of oestradiol due to proliferation of the preovulatory dominant follicle. Once it reaches a threshold value, oestradiol provokes a positive feedback effect on the pituitary eliciting an LH surge which induces ovulation (Ginther, 2000; Sartorelli *et al.,* 2005). The dominant follicle completes development and cows return to oestrus. In the presence of a conceptus, luteolysis is prevented due to production of interferon-τ (IFNT) by the conceptus (Mann *et al*., 1998), which prevents the oxytocin-induced release of $PGF_{2\alpha}$ from the uterus. This hormonal interplay results in a GnRH-surge, provoking oestrus behaviour only when the animal is not pregnant.

2.3.2. Oestrus

This stage is characterized by standing oestrus or behavioural oestrus, during which the cow is receptive to mounting by the male (or other females). Oestrus is initiated by rising concentrations of oestrogen from the dominant follicle, which thereafter completes its maturation and ovulates (Ginther, 2000). Peak concentrations of LH peak occur 3-10 h after onset of standing oestrus, with ovulation occurring 21-30 h after LH has reached its peak concentration (Adams *et al*., 2008), and the luteal phase of the oestrous cycle follows.

2.4. Luteal phase

The luteal phase is divided into metoestrus and dioestrus. Metoestrus, the process of formation of the CL from a regressed ovulated follicle (corpus haemorragicum), starts immediately after ovulation and is initiated by the LH surge (Forde *et al*., 2011). Indeed, all of the events of metoestrus are regulated by the LH surge, and LH has little or no role in the regulation of the CL until the dioestrus stage is reached.

The luteal phase is longer than the follicular phase, typically lasting 17-18 days. Most of the variation in the length of the oestrous cycle is due to variation in the length of the luteal phase (Kanitz, 2003). The luteal phase is characterized by increased progesterone concentration due to its synthesis by the CL. Raised progesterone concentration creates a favourable uterine environment for establishment and maintenance of pregnancy (Fortune, 1994). The progesterone synthesized by the CL inhibits LH release by the anterior pituitary and prevents ovulation by inhibiting follicular development and maturation (Gibbons *et al*., 1997).

The LH surge causes a rapid loss of aromatase activity in the pre-ovulatory follicle, resulting in a declining plasma oestrogen concentration that terminates the period of behavioural oestrus (Diskin *et al*., 2002), Thereafter, the CL forms over a period of three-four days, as the degenerated ovulated follicle passes through the corpus haemorragicum phase and turns into a functional CL (Adams *et al*., 2008).

By the dioestrus stage, the CL has largely ceased its growth, progesterone concentration is maximal, and it only requires the presence of low-level LH secretion for its maintenance. In the non-pregnant animal, peak progesterone concentrations occur somewhere between days 12 and 16 (Kanitz, 2003). After day 18, luteal regression is initiated and circulating progesterone concentrations decline. The decrease in circulating progesterone concentration eliminates the

negative feedback effect of progesterone upon LH secretion by the anterior pituitary. Hence, LH pulse frequency increases during luteal regression (Forde *et al*., 2011).

Also, there is variation in the amplitude and frequency of LH during the luteal phase. Such variation appears to be defined by the stage of the luteal phase whether in early or mid-luteal phase (Adams *et al*., 2008). The early luteal phase features lower amplitude and greater frequency (20-30 pulses/24 hours of LH) while the mid-luteal phase is of lower frequency and greater amplitude (6-8 pulses/24 hours) (Rahe *et al*., 1980). In both cases, there is insufficient amplitude and frequency for the final maturation and subsequent ovulation of the dominant follicle (Rahe *et al*., 1980). The dominant follicle produced during the luteal phase of the oestrous cycle undergoes atresia, whereupon oestradiol and inhibin synthesis become reduced eliminating the negative feedback block upon FSH secretion, which therefore increases and a new follicle emerges.

2.5. Ovarian dynamics

The use of technology such as ultrasonography, hormonal assays of gonadotropins and steroids, examination of ovaries from slaughtered animals and use of laparoscopy (see Sirois and Fortune, 1988; Singh *et al*., 2003) to investigate and examine the events taking place on the ovaries during the oestrous cycles have provided a clear basis for understanding follicular dynamics. Cattle are generally monovulatory, so most ultrasound examinations of ovaries depict a single dominant follicle but Sartorelli *et al*. (2005) observed a few cases of co-dominance in Zebu.

Follicles are blister-like structures that grow on the ovaries and each contains an unfertilized egg that will be released to the oviduct if the follicle ovulates (Kanitz, 2003). Follicular waves are a key characteristic of follicular development. Sirois and Fortune (1988) reported that follicle development and regression appear in a wave-like pattern throughout the oestrous cycle of cattle. It is from these waves that a dominant follicle emerges which inhibits the growth of other smaller follicles. The intrinsic life span of each follicular wave is 7-10 days (Diskin *et al*., 2002) as it goes through the various phases of development. The follicle wave is made up of three phases of development; recruitment of follicles, selection and dominance, followed by either atresia or ovulation of the dominant follicle, and the dominant follicle is only seen at a certain time in each wave (Adams *et al*., 2008). The pituitary hormone, FSH, is regarded as the key controller of these waves such that, during dioestrus, follicle waves appear in response to elevations in FSH concentrations, and are terminated either by the negative feedback effects of

inhibin (and, maybe, oestrogens) upon FSH, or the absence of sufficient LH to support the latter stages of follicle growth (i.e., due to the negative feedback effect of progesterone upon LH) (Ginther *et al*., 1989; Adams *et al*., 1992). Previous studies have established that most oestrous cycles contain two or three follicular waves growing antral follicles (Ginther *et al*., 1989, 1996; Figueiredo *et* al., 1997; Alvarez *et al*., 2000; Gimenes *et al*., 2008), although cycles containing one to four waves have also been reported (Ginther *et al*., 1989).

During the recruitment phase, there exists five to twenty cohorts of follicles, which are greater than 5 mm in diameter, associated with temporary elevations of plasma FSH concentrations (Ginther *et al*., 1996). FSH receptors located in their granulosa cells by day 3 of the follicle wave and these growing antral follicles are reliant on FSH for their growth. This enables the FSH to accelerate follicle growth and proliferation of cells resulting in the growth of the follicles up to 4-7 mm in size (Ginther *et al*., 1996).

During the period of follicle wave emergence, a number of antral follicles are present. Surprisingly, there are more follicles present at the time of emergence in *B. indicus* than in *B. taurus* cattle: Bastos *et al*. (2011) observed an average ovarian antral follicle count of 42 and 19 in Nellore and Holstein cows, respectively. Carvalho *et al*. (2008) reported 83±2 compared to 21±4 small follicles at wave emergence in *B. indicus* than *B. taurus* heifers, respectively. Likewise, Alvarez *et al*. (2000) observed 39±4 follicles in lactating Braham compared to Angus (21±4) cows. Alvarez *et al*. (2000) reported that this significant difference in number of follicle present in *B. indicus* and *B. taurus* could probably be due to variation in Insulin-Like Growth Factor (IGF-1) concentration. Likewise, Batista *et al*. (2014) suggested that this difference may be due to greater circulating anti-mullerian hormone in *B. indicus* compared to *B. taurus*. Animals with high plasma anti-mullerian hormone concentration had higher antral follicle count because anti-mullerian hormone decreased follicle atresia, thereby resulting in greater antral follicle population (Batista *et al*., 2014). This greater number of antral follicles observed in *B. indicus* helps to explain the success of *in-vitro* embryo production in Zebu.

Follicular wave emergence differs between two- and three-wave oestrous cycles. Emergence of the first follicular wave emergence occurs on, or close to, day 0 (i.e. the day after ovulation), and the second emerges on days 9 or 10 in two-wave cycles or days 8 or 9 in three-wave cycles, while the third emerges on days 15-16 (Ginther *et al*., 1989; Sartori and Barros, 2011). Occurrence of two-four follicular waves has been reported in *B. taurus* (Holstein) cattle and

two-three follicular waves cycle in Nellore and Gir cows (Viana *et al*., 2000), whilst Braham cows generally have three-wave cycles (Zeitoun *et al.,* 1996). The length of oestrous cycles tends to differ between two and three wave cycles: typically, 20 days and 23 days respectively (Sartori and Barros 2011). Nellore cattle have an average cycle length of 20.7 days and 22.7 days for cycles with two or three follicular waves, respectively (Figueiredo *et al*., 1997).

Prior to follicle deviation, the dominant follicle and the largest of the subordinate follicles can be determined on the basis of their large diameter compared to that of other follicles within the ovary. Close to the onset of diameter deviation, the largest follicle establishes as the dominant follicle probably prior to the next follicle attaining similar diameter (Diskin. *et al*., 2002). At the time of follicle deviation, follicle size is usually greater in the developing dominant follicle (8.5-9.00 mm diameter) whilst the largest subdominant follicle is about 1 mm smaller (7.5-8.0 mm diameter) (Adams *et al*., 2008). Follicle sizes at time of deviation in Nellore cattle are significantly smaller than those in *B. taurus* cattle (5-6 mm diameter) for the largest growing follicle (Ginther *et al*., 1996, 2003). Sartori *et al*. (2004) reported a follicle size of 8.3 – 9.8 mm in Holstein cattle and 7.0 -7.7 mm in non-lactating Nellore cows. The dominant follicle acquires preovulatory capacity as it develops the capability to respond to LH. Sartori *et al*. (2001) reported that lack of induction of LH receptors on the granulosa cell of other follicles is the reason behind the regression of subordinate follicles (even though it may have a large diameter). The dominant follicle is also distinguishable from the subordinate follicle even if it has a similar follicle diameter. The dominant follicle is associated with increased oestradiol production (Ginther *et al*., 2000), capacity to sustain low intrafollicular concentration of insulin-like growth factor binding proteins -2, -4 and -5 (IGFBPs) and follistatin (Austin *et al*., 2001) as well as an elevation in free intrafollicular concentration of IGF-1 (Ginther *et al.,* 2001). The above features of the dominant follicle are the inverse of the subordinate follicle which after some time will become static in growth, with reduced size and less sensitivity to gonadotropin binding sites (Diskin *et al*., 2002)

Selection of a dominant follicle occurs from antral follicles, which are greater than about 4 mm in size and, once a dominant follicle is preferred, other follicles become atretic (Bó *et al*., 2003). During this selection phase, some physiological events confer on the dominant follicle the capacity to inhibit the growth of other follicles. When the dominant follicle is growing, at some point FSH begins to decline, probably due to the negative feedback products from the growing follicles (primarily oestradiol and inhibin) (Ginther *et al*., 1989). In each follicle wave, as the dominant follicle emerges, there are LH receptors present on the granulosa cells of the

dominant follicle which makes it become LH-reliant rather than FSH-dependent, while the other follicles still need FSH to grow become atretic (Ginther *et al*., 1996). The expression of Messenger RNA (mRNA) for LH receptors by the dominant follicle is the key to its continuous growth under LH stimulation (Diskin *et al*., 2002) and in cases where the LH pulse frequency is extended, the resultant effect may be a persistent follicle. The growing phase of the dominant follicle lasts for 6 days and then stops. Once the follicle has become reliant upon LH, its existence depends upon adequate LH concentrations: thus, where LH is suppressed by the negative feedback effects of luteal progesterone, the dominant follicle then degenerates, plasma FSH concentrations increase again and establish the onset of a new follicle wave (Adams *et al.,* 2008).

The diameter of the dominant follicle differs between *B. indicus* and *B. taurus* cattle, with typical values of 10-12 mm in Nellore and 16-20 mm in Holstein cattle (Savio *et al.,* 1988). Ovulation occurs in *B. taurus* from follicles whose diameter ranges between 12-22 mm (Carvalho *et al.,* 2008), whilst the range in Nellore cattle is 10-13 mm (Figueiredo et al*.,* 1997; Sartorelli *et al*., 2005). There tends to be lower growth rate of follicles between the period of selection and ovulation in *B. indicus* compared with that in *B. taurus* cows: *B. indicus* has follicular growth rate of 0.9 mm/day (Figueiredo *et al.,* 1997; Carvalho *et al*., 2008) compared to 1.1-2.0 mm/day in *B. taurus* (Forde *et al*., 2011). There is a relationship between size of *corpora lutea* and ovulatory follicle size; *B. indicus* cattle have small follicles, which are reflected in the volume of their *corpora lutea* as well. Reports of luteal volume of 11248 mm^3 (27.8 mm diameter) for lactating Holstein cows (Sartori *et al*.,2004) and 1987 mm^3 -1999 mm^3 (15.6-21.5 mm) diameter for *B. indicus* Nellore cows (Figueiredo *et al*., 1997; Machado *et al*., 2008) have been established.

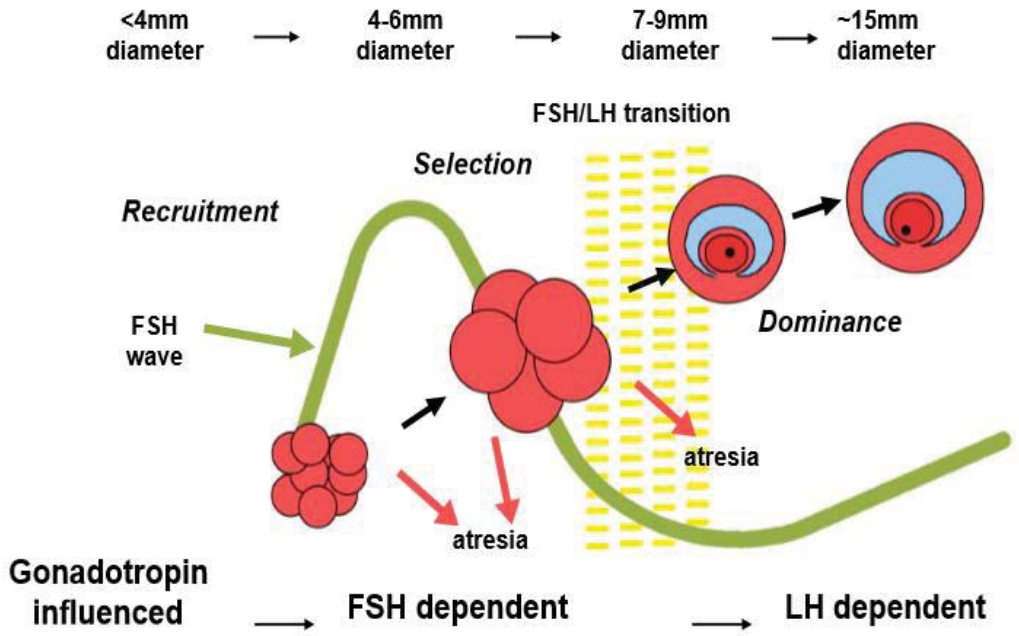

Figure 2.1 The role of gonadotropins in follicular selection and dominance in cattle. Note the transition period when follicles become LH dependent. Adapted from Webb et al. (2007).

In summary, ovarian follicle growth is categorized into gonadotropin dependent and gonadotropin independent stage. The gonadotropin dependent stage as illustrated in Figure 2.1; this entails the emergence of follicle cohorts of about 5-20 follicles of less than 5 mm in response to the hormonal environment provided by high FSH concentration. The follicles are FSH-dependent owing to the fact that there are FSH receptors (FSH-R) on the granulosa cells of the follicles (Ginther *et al.*, 1996) which facilitate promotion of follicular growth and proliferation.

The dominant follicle (DF) emerges from this cohort of follicles with increased diameter and a corresponding increase in follicular fluid (oestradiol and inhibin) (Hillier, 1994). This hormonal increase leads to suppression of FSH concentration from the anterior pituitary gland via a negative feedback reducing FSH to basal concentration (Hillier, 1994)). As the DF grows, there is a transient switch from FSH dependence to LH reliance. The DF becomes more responsive to LH and such switch is propagated through the presence of LH receptors (LH-R) on the granulosa cells. There also tends to be a transient increase in circulating LH concentration that occur at or around the time of follicle selection (Ginther *et al.*, 2000) which allows the DF to grow and continue oestradiol production while being in an environment with lower FSH concentration.

Recent work has led to the hypothesis that kisspeptin/neurokinin B/dynorphin(KNDy) neurons in the arcuate nucleus (ARC) play a key role in gonadotropin-releasing hormone (GnRH) pulse generation and gonadal steroid feedback,(Tanco *et al*., 2016) with kisspeptin driving GnRH release and neurokinin B and dynorphin acting as pulse start and stop signals, respectively. The relative immunoreactivity of kisspeptin, dynorphin, and RFRP-3and their possible connectivity to GnRH neurons in the hypothalamic of perioestrus and dioestrus bovine were examined. While GnRH and RFRP-3 immunoreactivity were unchanged, kisspeptin and dynorphin immunoreactivity levels varied in relation to plasma progesterone concentrations and oestrous status. Animals with higher plasma progesterone concentrations in dioestrus had lower kisspeptin and increased dynorphin immunoreactivity in the ARC (Tanco *et al*., 2016)

In view of the different phases of development of each follicular wave as seen in Figure 2.1, hormonal treatments can be used to adjust the effect of FSH and LH on a follicular wave. Therefore, positive manipulation of follicular waves to enhance hormonal concentration of the follicle and competence of the oocyte produced after ovulation results in enhanced breeding outcomes.

2.6. Oestrus behaviour

By the time of the onset of oestrus, the preovulatory follicle has grown large and is producing copious quantities of oestradiol, to the point at which oestradiol concentrations are sufficient to elicit behavioural oestrus and provoke the preovulatory circulating LH surge (Pinheiro *et al*., 1998). Studies in *B. indicus* cows have shown that the interval between onset of oestrus and ovulation is 26.8±0.8 h and 28.0±0.9 h for $PGF_{2\alpha}$-induced and natural oestrus, respectively (Pinheiro *et al*., 1998). Mizuta (2003) reported that the duration of oestrus in Nellore (12.9±2.9 h) cattle is shorter than that in Angus (16.3±4.8 h) cows, although the interval between the onset of oestrus and ovulation is similar in *B. indicus* and *B. taurus* (Nellore 27.1±3.3 h and Angus 26.1±6.3 h). Duration of oestrus appears to differ between *B. indicus* and *B. taurus*, with Lopez *et al. (*2004) reporting 30 minutes-27 h for *B. taurus* while Bó *et al*. (2003) reported 1-20 h for *B. indicus*.

There is not a clear consensus whether peak oestrogen concentrations vary between *B. indicus* and *B. taurus.* Segerson *et al*. (1984) reported higher concentrations of oestradiol 17-β and progesterone in Angus than those in Braham cattle, but in contrast, Alvarez *et al*. (2000) did

not observe any such difference. These contradictions may be accrued to breed or sensitivity of the assay.

2.7. Relationship between follicular dynamics and reproductive outcomes

Follicle size may have a relationship with oestrus expression. Cerri *et al.* (2004) reported that lactating dairy cows ovulating smaller follicles following Ovsynch protocols did not express oestrus. Vasconcelos *et al*. (2009) were of the opinion that decreased concentrations of oestradiol in circulation appeared to be behind the decline in oestrus expression. Small follicles are correlated with lower plasma oestradiol concentration, leading to poor oestrus response, because oestrus response is solely reliant on circulating concentration of oestradiol at proestrus (Vasconcelos *et al.,* 2001). When cows have small follicles at proestrus, the consequences on the reproductive outcome are not beneficial. Several studies have reported the negative influence of small follicles during the proestrus period, including; decreased ovulation rate (Vasconcelos *et al*., 2001), small *corpora lutea* and consequent low synthesis of progesterone (Vasconcelos *et al.,* 2001; 2009); inadequate preovulatory oestradiol secretion (Vasconcelos *et al*., 2001; Sa Filho *et al*., 2009), compromised uterine and oviductal environments (Geisert *et al*., 1988; King *et al*., 1994) and a greater likelihood of premature luteolysis (Vasconcelos *et al.,* 2001) and a low pregnancy rate (Perry *et al*., 2007)

Oestrus occurrences during oestrus synchronization programmes have been linked to positive ovarian and follicular responses that culminate in improved conception and pregnancy rates in both *B. indicus* and *B. taurus* cattle (Perry *et al*., 2005, 2007; Meneghetti *et al*., 2009; Peres *et al*., 2009; Sa Filho *et al*., 2011). Follicle size also influences oestrus response: the presence of large follicles at the time of progesterone device removal and timed AI has been linked to higher oestrus occurrence, which led to better ovulatory capacity and pregnancy rate. Sa Filho *et al*. (2011), reported that, in using a protocol that includes any of progesterone, oestradiol, eCG, GnRH or any combination, there was a positive influence upon the occurrence of oestrus and size of *corpora lutea* and, hence, higher serum concentration of progesterone. There may be a reduction in progesterone concentration during subsequent dioestrus (Sa Filho *et al*., 2010c). There is considerable evidence that low plasma concentration of oestradiol will lead to early luteolysis in the next cycle (Mann and Lamming 2001). Similarly, Peres *et al*. (2009) reported the positive effect of behavioural oestrus on conception rate. This may be due to the fact that there is a high concentration of oestradiol in circulation during proestrus (Perry *et al*., 2007). Absence of oestrus prior to FTAI, has been linked with

low concentration of oestradiol at proestrus, and as such may reduce fertility, since this result in a decrease in functionality of the next CL, and incidence of cows with early luteolysis.

Follicle size influences progesterone concentration in subsequent dioestrus by causing an increase in CL size (Murdoch and Van Kirk, 1998; Peres *et al*., 2009). It has been reported that when ovine follicles are stimulated to ovulate prematurely, there were few granulosa cells and a small CL was formed that synthesized less progesterone (Murdoch and Van Kirk, 1998). Peres *et al*. (2009) reported lower plasma concentrations of progesterone in cows that had ovulations occurring from small follicles. Smith *et al*. (1994) and McNatty *et al.* (1979) were of the opinion that such incidence may be due to: (a) decreased numbers and size of granulosa cells, or (b) decline in the number of LH receptors on granulosa and theca cells to produce sufficient progesterone after luteinisation. Studies have shown that sufficient progesterone concentration in circulation during dioestrus is fundamental for inducing endometrial secretion (Geisert *et al.,* 1988) along with (Mann and Lamming, 1999), embryonic development and maternal recognition and maintenance of pregnancy.

The significant role of LH pulsatility in follicle growth and development cannot be ignored. Many studies have shown that enhanced LH pulse frequency stimulates final follicle maturation and improves preovulatory oestradiol production (which, in turn, helps to stimulate the responsiveness of the pituitary to GnRH stimulus). An adequate LH pulse frequency influences follicular growth after divergence, which enhances chances of oestrus and ovulation (Gimenes *et al*., 2008). Wiltbank *et al*. (2002) reported that when there is an inadequate LH pulse frequency, there is a suppression of the DF attaining preovulatory size, as well as suppression of oestradiol production which, together, culminate in the absence of ovulation because of a lack of adequate oestradiol to induce the LH surge. When a dominant follicle is exposed to an improved LH pulse frequency, final growth and maturation are ensured and this will bring about increased oestradiol production and ovulation (Vasconcelos *et al*., 2009).

The size of the dominant follicle at the end of an oestrus synchronisation protocol is, therefore, a contributory factor to the success of reproductive outcomes. Several studies have shown that the size of the DF influences ovulation and pregnancy rate (Vasconcelos *et al*., 2001; Perry *et al*., 2005, 2007; Meneghetti *et al*., 2009; Sa Filho *et al*., 2009, 2010c; Tortorella *et al*., 2013). Consequently, much attention has been given to devising protocols and treatments that bring about synchrony of oestrus and ovulation, which directly induce follicle development and enhance LH pulses to result in improved pregnancy rates.

All of the above results indicate the importance of the interaction among hormones and follicular growth and development, final ovulatory follicle size, conception and pregnancy rate.

Although there is no available information on the follicular dynamics in the Muturu breed, it would be expected from the foregoing that it would be generally similar to that of other *B. indicus* cattle in terms of the relationship between follicular development, activities of the reproductive hormones and reproduction outcomes measured in terms of pregnancy and conception rates. Understanding the follicular dynamics is critical to determine the hormonal treatments that will be able to regulate the CL, the time of follicular-wave emergence (Pursley *et al*., 1995) in synchronization protocols to maximize reproductive outcomes, determines the effectiveness of an oestrus synchronization protocol, particularly where hormonal treatments have been developed that eliminate the need for oestrus detection in B. *indicus* (Fernandes *et al*., 2001; Baruselli *et al*., 2004).

2.8. Anoestrus

Anoestrus is a state of lack of sexual receptivity in the female animal or when a female animal does not exhibit normal oestrous cycles (Peter *et al*., 2009). This occurs in prepubertal heifers and in cows after parturition and during anoestrus the female animal has no chance of becoming pregnant. In a review by Peter *et al*. (2009), anoestrus was classified into four types *viz*; 1) occurrence of follicle growth up to the point of emergence but no deviation, hence absence of a dominant follicle; 2) follicle growth and deviation occur and are then succeeded by atresia or regression; 3) follicle growth, deviation and emergence of DF occur but ovulation does not occur; and 4) ovulation occurs but CL with extended luteal function is established due to absence of luteal regression.

Resumption of oestrous cycles after calving is key to achieving high reproductive potential. Prolonged postpartum anoestrus commonly occurs in pasture-based systems, due to insufficient intake of nutrients to meet energy demands for growth and lactation (Wiltbank, 1970; Randel, 1990; Short *et al.,* 1994; Rhodes *et al.,* 2002). *B. indicus* cows are characterized by long postpartum intervals, especially under pasture-based systems (Baruselli *et al*., 2004). Hence, extended calving intervals are usually observed in *B. indicus* linked to prolonged resumption of oestrus expression and ovulation, which culminates in poor conception and pregnancy rate.

2.9. Physiology of the Postpartum Period

During the last trimester of pregnancy, there is a strong negative feedback effect from progesterone and oestrogens which leads to a decline in the concentrations of the gonadotropins (Peter *et al*., 2009). Increase in plasma concentration of FSH after calving gives rise to the resumption of ovarian activity, and growth of the DF starts at 10-14 days postpartum (Rhodes *et al*., 2002). Two things are likely to happen with the first DF: either it ovulates, or it becomes atretic and becomes replaced by a further DF. When oestradiol synthesis by the follicle is sufficient to induce a preovulatory surge of LH and FSH, then the DF ovulates. However, where follicular growth is inadequate, or where there is inadequate development of oestradiol receptors in the hypothalamic regions that regulate the LH surge, or where there is inadequate pituitary storage of LH, ovulation fails (Ramirez-Godinez *et al*., 1982; Williams, 2005).

The first postpartum ovulation is characterized by a lack of oestrus behaviour and a short-lived luteal phase (Webb *et al*., 1980). The low plasma progesterone concentrations of the first luteal phase are a result of a combination of poor pre-ovulatory follicle growth, inadequate LH secretion and premature appearance of endometrial oxytocin receptors (Ramirez-Godinez *et al*., 1982). This creates opportunity for premature onset of the positive feedback interaction between $PGF_{2\alpha}$ and oxytocin.

The need for a normal life span of the luteal structures after first postpartum ovulation has indicated the use of progestin as a source of exogenous progesterone to allow the re-establishment of progesterone-dependent aspects of the restoration of oestrous cycles in the postpartum period. Progestin treatment in anoestrous cows enhances LH concentration which acts as a catalyst to growth of the follicle and oestradiol synthesis, thereby culminating in ovulation (Rhodes *et al*., 2002). Taken together, these form a prerequisite for establishment of behavioural oestrus, hence the use of progestin and other hormonal treatments that will allow for such.

2.10. Trends in oestrous synchronization protocols

Improving the reproductive efficiency in a cattle herd goes a long way to increase profitability and net returns from that herd. The goals of having a 12-month calving interval, enhanced weaning weight, uniform calf, and incorporation of genetic gains into the beef herd are achieved when the reproductive potential of the animals are properly harnessed (Bó *et al.,* 2006). Achieving genetic gain is rather slow in cattle, due to their long gestation period (Belloso *et al*., 2002); hence, in such circumstances, the manipulation of the oestrous cycle

becomes a strategy to improve reproductive goals. Synchronization of oestrus is a strategy that can be used to control the oestrous cycle in such a manner that most of the females in the herd are induced to be on heat at a predetermined time (Odde, 1990). Several studies have shown the effectiveness of AI and synchronization protocols in achieving improved reproductive performance in beef herds (see Bó and Baruselli, 2014). Synchronization protocols employed to achieve enhanced reproductive performance use several hormones to attain the targets of the synchronization protocols. Such hormones include $PGF_{2\alpha}$, progesterone and oestrogen, GnRH eCG.

2.10.1. *Progestins*

The commercial progestin has the capacity to mimic the effects of natural progesterone as it can be used to extend the luteal phase (Martinez *et al*., 2002a) or to act as an 'artificial' luteal phase. Exogenous progestin delays ovulation, prevents oestrus and suppresses LH release in cattle (Adams *et al*., 1992). When used to extend the luteal phase, instead of the animal ovulating after natural regression of the CL, there is continued growth of the follicle and then, at withdrawal of the progesterone device, progesterone concentrations decrease, allowing for occurrence of oestrus and ovulation (Martinez *et al*., 2002b).

The use of progestin in synchronization protocols can be achieved in three basic ways; through feed as melengestrol acetate (MGA), subcutaneous implants in the ear (Norgestomet) and intravaginal inserts (PRID, CIDR) (Day, 2002; Martinez *et al*., 2002a). The use of progestin treatments dates back to the 1960s (as reported by Odde, (1990)). Progestin brings a high percentage of females into oestrus at a fixed time (Macmillan and Peterson, 1993). Several studies have been done over the years to ascertain the efficacy of progestin when used alone (Hansel *et al*., 1961; Zimbelman and Smith 1966) or in combination with oestrogens (Thimonier *et al*., 1975; Sales *et al*., 2012), or PGF (Smith *et al*., 1984; Voh *et al*.,1987; Lamb *et al*., 2001).The use of progestin ranged from daily injection (Wiltbank *et al*., 1965), to use of 6-methyl-17-acetocyl progesterone (MAP) orally (Hansel *et al*., 1966), to use of MGA(Zimbelman and Smith, 1966). The use of MGA orally was of higher potency than MAP as it was able to suppress ovulation and oestrus in nearly all animals (Zimbelman and Smith, 1966). The period or duration of treatment with progestin appears to be crucial in affecting outcome of the synchronization protocol (Revah and Butler, 1996). Critically, however, many studies (e.g. Hansel *et al*., 1966) showed that long-term exposure of the animals to progestin treatment resulted in a low conception rate. Also Zimbelman and Smith (1966) and Zimbelman

et al. (1970) reported that 1st service conception rate in MGA-treated animals was 14% lower than that of the control group.

The circulating concentration of progesterone during the oestrous cycle is an important factor to consider during treatment periods. This is because it regulates LH pulsatility, final growth of the DF (Savio *et al*., 1993) and ovulation response (Sirois and Fortune, 1988). Progestin can therefore be used to manipulate LH pulse frequency (Garcia-Winder *et al*. (1986): as, for example, in terms of the greater GnRH-induced LH release in cows 24 h after norgestomet removal (Smith *et al.,* 1983). Conversely, where progesterone concentrations are sub-luteal (e.g, 2.14 ng/ml: Roberson *et al*., 1989) then LH pulsatility increases, enabling the growing DF to attain a higher diameter, culminating in larger CL and high synthesis of progesterone elevating the concentration of progesterone. Perry *et al.* (2007) reported that sufficient progesterone production, oocyte capacity, oviductal and uterine environment forms the basis for maternal recognition and maintenance of pregnancy. There are three key steps in regulating the oviductal and uterine environment to be conducive to pregnancy: progesterone exposure prior to proestrus; high oestradiol concentration at proestrus and oestrus; and maintenance of sufficient progesterone concentration during metoestrus (Wilmut *et al*., 1986)

The mechanism responsible for reduced fertility following very long progestogen treatment was first demonstrated by Lamond *et al. (*1971) to be due to modified follicular growth and an increment in the number of atretic follicles. Thus, when progestin is administered for intervals that are longer than the life span of the CL, the resulting high synchrony of oestrus at progestin withdrawal is achieved, but the subsequent oestrus is associated with low fertility (Odde, 1990). In order to resolve the decreased fertility observed in long-term treatment, Wiltbank and Kasson (1968) reported no negative effect of short-term progestogen on conception rate, hence short-term progestogen administration has a better effect on fertility than does long-term use. The benefit of short-term progesterone treatment appears to be that pituitary stores of LH increase, hence oestradiol synthesis and maturation of follicles are improved in the pre-ovulatory period (Martinez *et al*., 2002a).

2.10.2. Progesterone device (CIDR)

The development of vaginal inserts such as the CIDR (Macmillan and Peterson 1993; Day, 2002) gave rise to new protocols eliminating the difficulties associated with feeding of MGA and reduced frequency of animal handling. The CIDR is a t-shaped device as seen in Figure

2.2. Use of CIDRs in synchronization protocols tends to have enhanced reproductive outcomes compared with other methods of administering progestin.

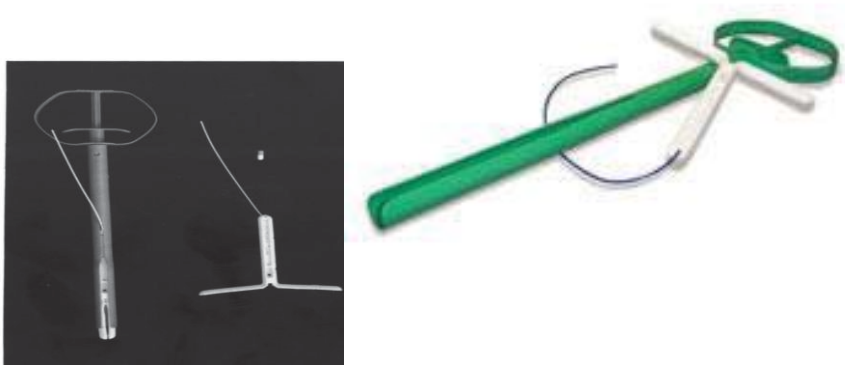

Figure 2.2 The CIDR device before and after placement into the applicator used for its intravaginal insertion. (Source: Macmillan and Peterson 1993)

The hypothesis was that inclusion of CIDR in synchronization protocols would suppress the premature onset of oestrus prior to giving $PGF_{2\alpha}$ and, thereby, lead to improved fertility. Inclusion of CIDR resulted in higher pregnancy rate than traditional methods (as evident in the results of Lamb *et al*. (2001), Larson *et al*. (2004a, b), Larson *et al*. (2006) and Stevenson *et al*. (2003). This could be due to the ability of CIDR to induce ovulation in a high proportion of anoestrous cows and/or decrease occurrence of oestrus before FTAI (Martinez *et al*., 2002b). Results obtained in terms of conception and pregnancy rate from *B. taurus* appear to be better than those in *B. indicus*, but that may be due to stress and management practices especially since the *B. indicus* are in a tropical environment (Baruselli *et al*., 2004).

2.10.3. *$PGF_{2\alpha}$ -based protocols*

$PGF_{2\alpha}$ is the prostaglandin that is primarily responsible for luteolysis and prostaglandins, and consist of 20-carbon saturated hydroxyl fatty acids that are derived from arachidonic acid (McCracken *et al*., 1999; Islam, 2011). The use of prostaglandin in oestrous synchronization protocols dates back to the 1970s (Odde, 1990).

The ability to regulate the life span of the *CL* to a great extent determines the effectiveness of synchronization protocols controlling the oestrous cycle. A luteolytic agent such as $PGF_{2\alpha}$ or its analogues can be employed to cause regression of the CL and thereby stimulate oestrus. These pharmaceutical agents have been extensively used in synchronization protocols. The main rationale behind the use of $PGF_{2\alpha}$ in oestrus synchronization is that it brings about luteal

regression, hence eliminating the negative feedback effect of progesterone upon LH, which then permits the ovulation of the next dominant follicle (Beal *et al*., 1988). The basic pathway through which $PGF_{2\alpha}$ induces luteolysis of the CL is by uncoupling the LH receptor from its associated adenylate cyclase, causing loss of activity of key steroidogenic enzymes (McCracken *et al*., 1999). Additionally, it may cause a decrease in the blood flowing to the CL, hence causing the gland to lack substrates for hormone synthesis (Niswender *et al*., 2000).

Initial protocols employed were focused on altering the oestrous cycle by causing regression of CL with an injection of $PGF_{2\alpha}$ (Lauderale *et al*., 1974). The trend moved from single injection to multiple PGF injections, to obviate the need for oestrus detection and/or allow for FTAI protocols.

The stage of oestrous at the onset of treatment influences the outcome. Macmillan and Henderson (1984) reported that animals at dioestrus stage of the cycle at time of PGF treatment produced more variable results than animals with advancing dioestrus stage of oestrous cycle.. Prostaglandin $F_{2\alpha}$ is ineffective in causing luteolysis before day 5-7 of the oestrous cycle. Thus, Stevenson *et al*. (1984) demonstrated that when cattle are given $PGF_{2\alpha}$ during days 5-9 of the oestrous cycle, there is decreased response compared to the response of those animals given the injection at the latter stages of the cycle. The $PGF_{2\alpha}$ -based protocol relies entirely on eliminating an active CL, so only works in cycling animals, so it is not effective in inducing cyclicity in anoestrous cows. Additionally, there is a significant variability in the interval between $PGF_{2\alpha}$ administration and oestrus/ovulation (Voh *et al*., 1987; Cavalieri *et al*., 1997), depending upon the stage of the follicular wave at the onset of treatment (Anyam, 2006). Both of these factors limit its use. To some extent, the variability of interval to ovulation can be mitigated by oestrus detection in order to obtain high pregnancy rates, but this does not facilitate the use of FTAI in beef herds. Taken together, these factors mean that the oestrus response of *B. indicus* is low (60%) compared to that in *B. taurus* (90%: Galina and Arthur, 1990). Hence, synchronization regimens based upon $PGF_{2\alpha}$ alone appear not to be ideal for *B. indicus* due to reduced and inconsistent oestrus response and the risk of animals being anoestrous.

The above results indicate that using $PGF_{2\alpha}$ alone in synchronization protocols lacks the capacity to completely regulate the oestrous cycle. This therefore, emphasizes the necessity to use treatments that can regulate luteal as well as follicular development, eliminating need for

oestrus detection and being capable of inducing oestrus and ovulation, in order to achieve improved pregnancy rate to FTAI.

2.10.4.PGF and progestin

The essence of inclusion of progestin in $PGF_{2\alpha}$-based protocols was to suppress onset of oestrus after spontaneous or $PGF_{2\alpha}$-induced luteolysis (Lucy *et al.*, 2001). Hansel and Beal (1979) combined an intravaginal progesterone with an injection of $PGF_{2\alpha}$ two days before implant removal and this programme gave improved control of the CL. Moreover, such regimens offered better conception rates (Patterson *et al*., 1995; Lucy *et al*., 2001) than did synchronization using $PGF_{2\alpha}$ alone (Beal *et al*., 1984; Smith *et al*., 1984; Voh *et al*., 1987).

The use of progestin with $PGF_{2\alpha}$ requires identification of the most suitable time for giving the $PGF_{2\alpha}$. Early studies established that $PGF_{2\alpha}$ is best given late, rather than early, in the period of progestogen administration (e.g. Heersche *et al*., 1979). Other work by Hansel and co-workers in the 1960s and 1970s discussed in earlier sections on progestin, established that short duration (7-9 days) of progestogen administration with $PGF_{2\alpha}$ given either at, or two days before, the end of that period as being an effective means of synchronization which required minimal handling of animals.

2.10.5. $PGF_{2\alpha}$ and GnRH

Combinations of GnRH and PGF treatments have been successfully used in synchronisation protocols for AI in beef and dairy cattle (Twagiramungu *et al*., 1995; Pursley *et al*., 1997; Thatcher *et al*., 2001). The next major step in the development of synchronisation protocols was the combination of $PGF_{2\alpha}$ with GnRH in the so-called 'Ovsynch' protocol.

The need for protocols that stimulate follicle wave emergence and synchronize ovulation which facilitates FTAI, necessitated the need to develop new protocols. GnRH+$PGF_{2\alpha}$-based oestrus synchronization protocols (Ovsynch, Cosynch, Select-synch and Hybrid synch) can control follicle waves and then induce ovulation and or luteinisation of the dominant follicle (see Velladurai *et al*., 2015). The Ovsynch protocol (Pursley *et al*., 1995) consists of a GnRH injection on day 0, which, within two days, induces emergence of a new follicular wave after induction of ovulation/luteinisation of the dominant follicle. Prostaglandin $F_{2\alpha}$ is then given six or seven days later (Pursley *et a*l., 1995; Twagiramungu *et al*., 1995) to cause regression of the CL. Finally, a second dose of GnRH is given to synchronize ovulation to allow FTAI without oestrus detection 16 h later.

Due to the focus on regulation of CL and follicular wave emergence, a combination of GnRH and $PGF_{2\alpha}$ appears to be better than progestin and $PGF_{2\alpha}$ alone. Geary *et al.* (1998) reported higher conception rate with Ovsynch in beef cattle compared to the use of Synchromate B (SMB) in cycling *B. taurus*. The Ovsynch protocol has also been used to control follicular development and synchronise ovulation in *B. indicus* cattle (Barros *et al*., 2000).However, Ovsynch is not particularly effective in anoestrous cows. Fernandes *et al*. (2001) reported lower pregnancy rates for non-cycling cows (20%) than for cycling (47%) cows, so animals must be cycling to achieve higher pregnancy rates. Likewise, Fernandes *et al*., (2001) reported lower pregnancy rates in postpartum anoestrous cows treated with Ovsynch (14%) versus 46% in cycling cows. Pregnancy rates in beef cattle with Ovsynch have been very low (15%) compared to 53% obtained using progesterone+oestradiol (see Baruselli *et al*., 2004). Likewise, Barros *et al. (*2000) achieved better ovulatory response using a gonadotropin-prostagalndin-gonadotropin (GPG) plus oestradiol regimen gave better outcomes than using Ovsynch

It is important to note that Ovsynch appears to be a less efficient means of oestrus synchronization in *B. indicus* than in *B. taurus* cattle. Comparing pregnancy rates in GnRH-based protocols, 56% was obtained in *B. taurus* (Lamb *et al*., 2001; Larson *et al*., 2006) while less than 33.5% was obtained in *B. indicus* (Fernandes *et al*., 2001; Williams *et al*., 2002 Saldarriaga *et al*., 2007; Vasconcelos *et al*., 2009).

The ovulation that occurs in response to the first GnRH relies on the presence of a DF with ovulatory potency (i.e., which follicles acquire when they attain greater than 8.5 mm diameter (Wiltbank *et al*., 2011)) at time of treatment. A new follicular wave usually occurs within two days of GnRH treatment (Bó et al.,2002). Martinez *et al*. (1999) reported that only if the first GnRH treatment results in ovulation does a synchronized follicle wave emergence occur. Interestingly, therefore, Vasconcelos *et al.* (1999) showed that the higher the proportion of animals that ovulate in response to the first GnRH injection, the better the final pregnancy rate. Likewise, a poor response to first GnRH treatment resulted in low pregnancy rate to AI in beef heifers (Martinez *et al*., 2002b). The ovulation response to the first GnRH is less in anoestrous cows than in cycling females (Vasconcelos *et al*., 2009; Fernandes *et al*., 2001), which probably underpins the poorer reproductive outcomes in such animals compared to cycling cows.

Various studies reported that the stage of oestrous cycle of the cows at which the treatment is initiated (Martinez *et al*., 1999, Vasconcelos *et al.,* 1999) affects the synchronization outcome. It has become evident that the ideal time for initiation of Ovsynch protocol is on day 5-8 of the oestrous cycle (Wiltbank *et al*., 2011), with the result that much recent activity has been devoted to developing pre-synchronization systems that attempt to increase the proportion of cows in the ideal stage of oestrous cycle on the day of the first GnRH administration (Moreira *et al*., 2000; Souza *et al*., 2008; Kasimanckam *et al*., 2009)

Outcomes of GnRH+$PGF_{2\alpha}$ protocols are determined by the degree of induced luteolysis. Complete luteolysis more reliably leads to oestrus and ovulation of selected DF; whereas where there is partial luteolysis, there can be failure of oestrus and development of a persistent DF. When sub-luteal progesterone concentrations are present for an extended period, it brings about an abnormal elevation of LH pulse frequency and formation of persistent follicles. The first dose of GnRH increases the number of cows with a functional CL; studies by Pursley *et al*. (1995) and Fernandes *et al*. (2001) reported an increase in the percentage of functional CLs from 61.2% at the time of GnRH treatment to 86.2% seven days later. The second GnRH treatment improves ovulation rate. Pursley *et al.* (1995) and Barros *et al*. (2000) reported improved ovulation synchrony in cows that received a second dose of GnRH compared to those that only received the first dose.

2.10.6. *Protocols based upon GnRH, Prostaglandin $F_{2\alpha}$ and progestogens*

Progestogens are used in GnRH/$PGF_{2\alpha}$-based protocols either to manage the timing of ovulation or for presynchronisation. GPG protocols entail the use of GnRH treatment followed by PGF injection seven days later and a second GnRH treatment two days later, and FTAI either concurrently with the second GnRH treatment (Cosynch) or 16-48 hours later (Ovsynch). Achieving greater reproductive outcome in FTAI protocols begins with synchrony of recruitment of a new follicular wave, and so using drugs to achieve ovulation stimulation in a higher proportion of cows at the onset of treatment is the basis of achieving better reproductive outcomes to oestrus synchronisation.

The Ovsynch protocols have been used mainly in dairy cattle (Peters *et al.*, 2003) and have not been yielding acceptable pregnancy rates in anoestrous cows. The use of Ovsynch protocol in beef herds that are anoestrous resulted in decreased pregnancy rates: for example, Baruselli *et al*. (2004) found only 15% of cows responded to Ovsynch compared to 53% that was obtained with oestradiol and progestin treatment. Although GnRH when used in Ovsynch protocols may

be able to stimulate ovulation in non-cycling cows, there may be a resultant decrease in the subsequent conception rate, hence, there is a need to have a greater proportion of cycling cows in the herd. Therefore, incorporating progestin into GPG protocols may be a better option to achieve improved reproductive performance in anoestrous beef cows (see Bo and Baruselli, 2014). The inclusion of progestin in protocols appears to offer enhanced pregnancy rates that are achieved in protocols that do not include progestogen: for example, Lamb *et al*. (2001), Larson *et al*. (2006) and Busch *et al.* (2008) reported higher pregnancy rates when progestin was included in the protocol than using Ovsynch alone.

2.10.7. Equine chorionic gonadotropin (eCG)

There may be a benefit from using exogenous gonadotropin to achieve improved ovarian response. This is especially important where deeply anoestrous cows predominate (Bó *et al*., 2006), such as in a tropical environment. The anoestrous postpartum cow lacks adequate pulsatile release of LH, hence, limiting the success of the traditional FTAI protocols (Baruselli *et al*., 2004).

The benefits of using eCG are probably due to optimising follicle size when treating cows in postpartum anoestrus. Cows with larger ovulatory follicles are associated with greater ovulation rate and greater number of pregnancies per AI in beef cattle (Bó *et al*., 2006; Souza *et al*., 2009). Also, Sa Filho *et al*. (2010c) reported a positive relationship between pregnancy rate and increased ovarian follicle size. There may, therefore, be benefits to developing synchronization protocols that have the ability to augment the growth and ovulation of the follicle, particularly where this also offers the opportunity to use assisted reproductive technologies without oestrus detection. One such protocol is the inclusion of eCG in synchronization protocols.

Synchronization protocols involving eCG have been used in *B. indicus and B. taurus* cows with postpartum anoestrus, resulting in high pregnancy rates (Bó *et al*., 2003; Baruselli *et al*., 2004). Huguenine *et al*. (2013) reported a higher pregnancy rate for cows treated with eCG at progestin withdrawal, compared to those that were not treated (54 .5% vs 26.8%). Nasser *et al.* (2004) and Duffy *et al.* (2004) reported higher pregnancy rates for anoestrous cows treated with eCG and oestradiol compared to those without eCG. The use of eCG to replace oestradiol valerate appears to offer higher pregnancy rates in both heifers (Kerr *et al.,* 1991) and postpartum cows (Bó *et al*., 2003). In particular, eCG has been found to be beneficial in postpartum anoestrous cows, as it has the capacity to improve ovulation rate (Sa Filho *et al*

2010a), improve reproductive performance of animals with low body condition score (BCS) (Bó *et al*., 2002,2006; Sales *et al*., 2011). Synchrony of oestrus, ovulation and preovulatory LH surge were greater than in non-lactating *B. indicus* cows treated with eCG than those not treated with eCG at implant removal (Nasser *et al*., 2004).

The use of eCG in synchronization protocols has been found to have positive influence on the concentration of circulating progesterone during the treatment period. Sa Filho *et al*. (2010b) reported that treatment with eCG induced a greater concentration of circulating progesterone in the ensuing oestrous cycle, which could be attributed to a larger diameter of the CL (Bo *et al*., 2002; Dias *et al*., 2009). Pincinato *et al.* (2012) reported a beneficial effect of eCG when included in Cosynch protocol in *B. indicus* cattle.

The inclusion of eCG has been found to have beneficial effect in oestrus response, reduction in interval from treatment to ovulation, improved follicle growth and reduced time to onset of oestrus in African N'dama cattle, as reported by Drion and Hanzen (2015) and Okouyi and Hanzen (2016). Treatments with eCG under adverse environmental conditions may, therefore, offer better reproductive outcomes than the Ovsynch (Barros *et al*., 2000) or Cosynch programs. The use of eCG has been shown to be an effective tool in combating the problem of poor follicular growth and development associated with anoestrous cows, hence can be used as an alternative to improve ovarian and follicular function, resulting in better conception rates even in anoestrous beef cows or those with low BCS (Bó *et al.,* 2006; Sa Filho *et al.,* 2009; Sales *et al*., 2011).

The enhanced reproductive outcomes associated with inclusion of eCG in synchronization protocols could be due to the ability of eCG to invoke growth of the DF, effect the final growth of the pre-ovulatory follicle that consequently increases ovulation rate in postpartum anoestrous cows (Bo and Baruselli, 2014). Also Baruselli *et al.* (2003) reported that when cows were given eCG treatment two days prior to removal of a 9-day progesterone insert, eCG was more efficient and capable to induce growth of the DF than the control group without eCG.. Therefore, eCG can be used to support adequate pulsatile LH needed for better follicular development and ovulation, hence improve pregnancy rate. The capacity of eCG to improve pregnancy rate could be due to; its long half-life length (Murphy and Martinuk 1991); ability to bind to FSH and LH receptors (Murphy and Martinuk, 1991); ability to induce progesterone secretion by the early CL (Baruselli *et al*., 2004), or: provision of a luteotrophic effect which

allows for a conducive maternal endocrine environment during early dioestrus (Sa Filho *et al*., 2009).

2.11 Summary and research hypothesis

There is no information on the use of GPG synchronization protocols in the Muturu breed. Having reviewed trends in the synchronization protocols, this thesis investigates the hypothesis that Modified Ovsynch protocol in combination with CIDR and eCG (hormonal treatments) and artificial insemination (FTAI) can increase the pregnancy rate achieved in cattle compared to an extended period of insemination to observed oestrus. In examining this hypothesis, the thesis will also examine the relationship between follicular dynamics, hormonal concentration (oestradiol, progesterone and luteinizing hormone) and pregnancy rate per insemination.

CHAPTER 3

MATERIALS AND METHOD

The experiment was conducted in three regions of Ebonyi State, which is located in the South Eastern geopolitical zone of Nigeria. The temperature and rainfall pattern in this region are summarized in Figure 3.1.

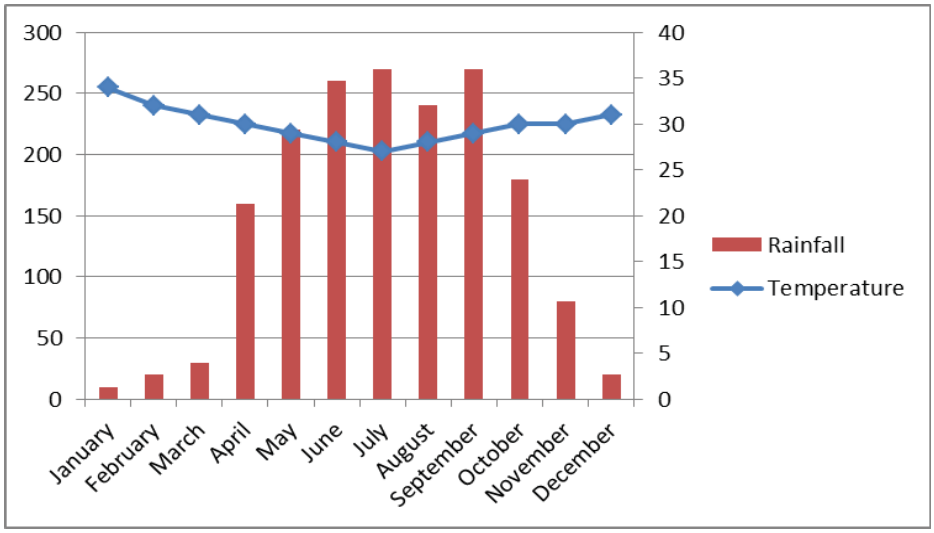

Figure 3.1 Temperature and rainfall distribution across the year in Ebonyi State

(Source; Ebonyi state university metrological statio)

This study was carried out between February and May 2016. Muturu cattle from three locations were used in the study: 1) Abulaleke community in Ezza North: 2) Oshegbe community in Ezzamgbo and : 3) Ebonyi State University Research Farm in Abakaliki. The Abulaleke and Oshegbe communities provided the cows used in the Untreated group while cattle on the University Research Farm were used for the Synchronised group. These three regions identified with red shades can be seen on the map in Figure 3.2.

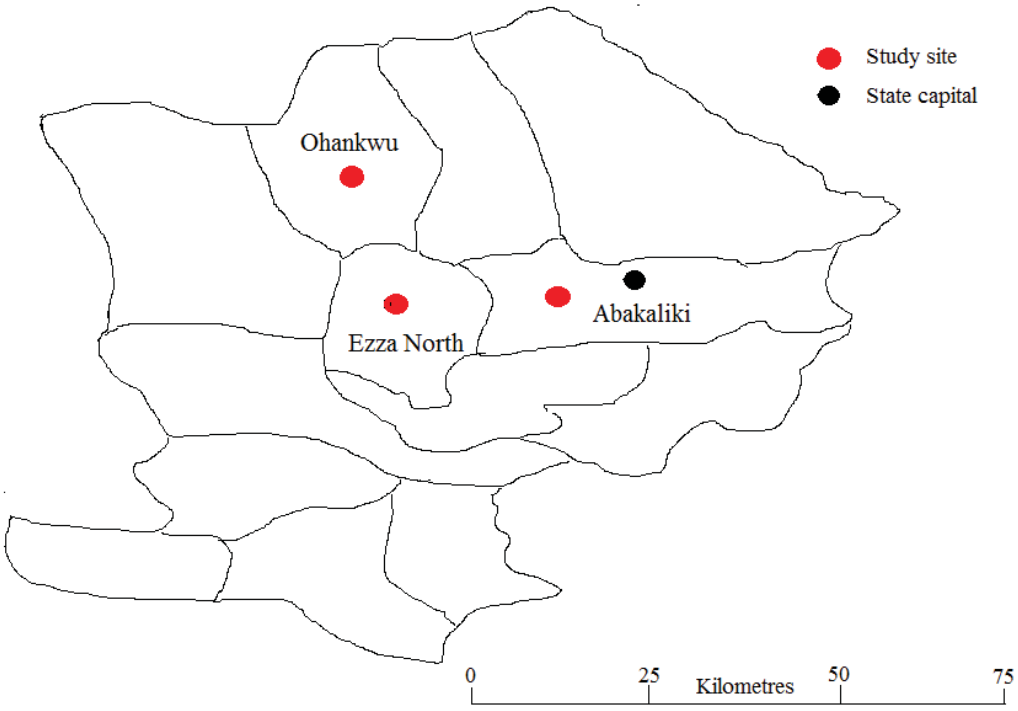

Figure 3.2 Map of Ebonyi state showing the three regions used for the study. (source :google map)

3.1. Cattle selection

The two locations selected for the Untreated groups were based on 1) Muturu cattle being were common and 2) farmers being willing to let their cattle be used for the study. As an incentive for the farmers, all cattle presented for the study were treated with an acaricide, (Cypermethrin pour-on, CIPLA, India) to treat ticks. At both sites, all available cows with previous calving history were examined using transrectal ultrasonography with a 7.5 Mhz transrectal probe (iScan Dramniski, Poland) to confirm absence of pregnancy and the presence of ovarian follicles of greater than 5 mm in diameter. Pregnant cows or cows with follicles less than 5 mm were excluded from the study. Recruitment continued until 25 animals were selected per site.

On the University Research Farm, Muturu cattle came from two sources: 1) the University farm and 2) cattle presented by and purchased from local farmers in the Oshegbe community. The recruitment process was the same as that for the control cows; all selected cows had to be non-pregnant and have at least one follicle greater than 5 mm in size. This recruitment process continued until 50 animals were obtained. Once selected, all animals at all three sites were tagged with plastic ear tags for identification. The selection procedures for the Treated and Untreated groups are summarized as a flow chart in Figure 3.3

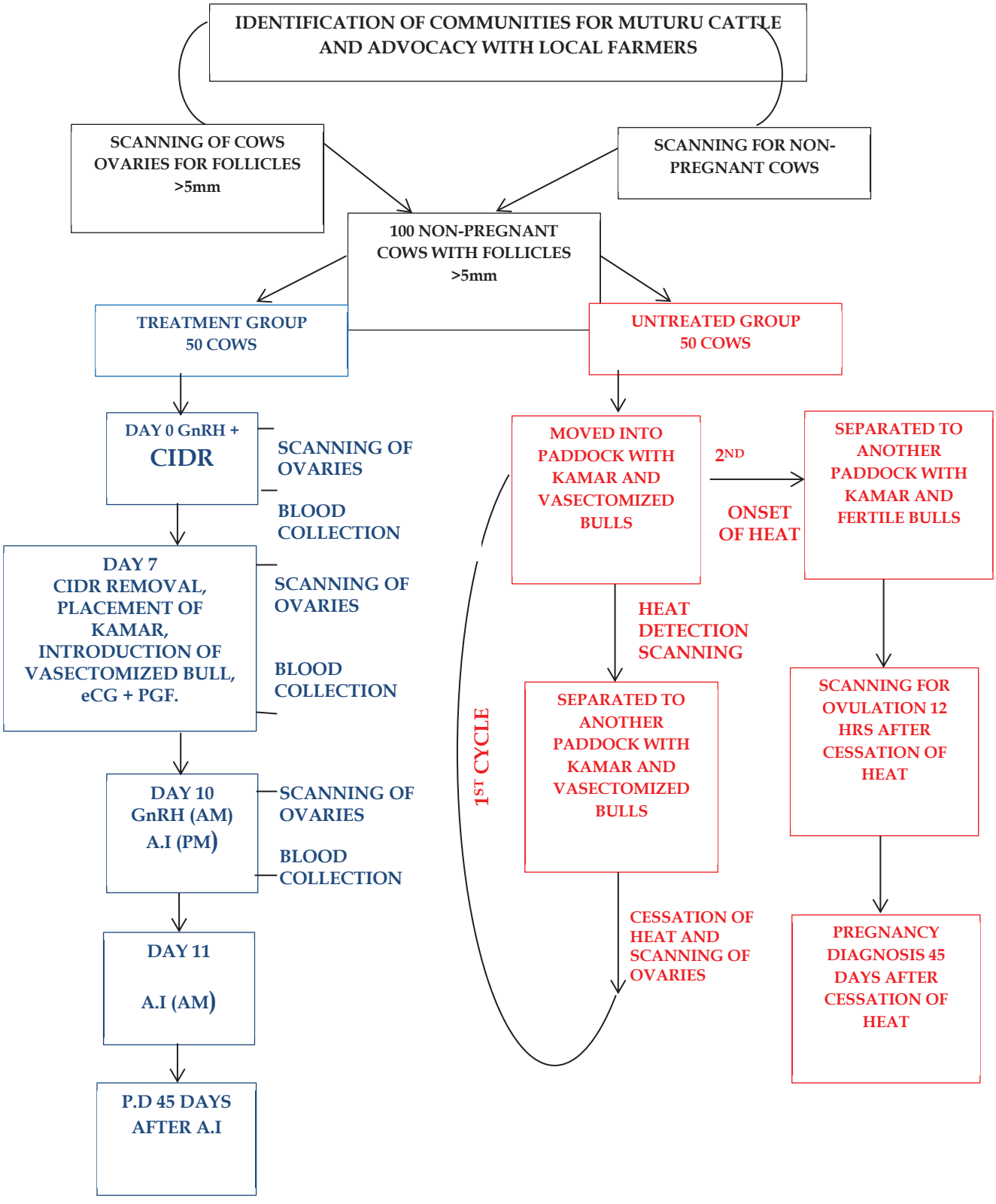

Figure 3.3. Flow chart showing the study design of the experiment conducted with Muturu cattle breed at University research farm and Oshegbe and Abulaleke community for both Treated group and Untreated group respectively

3.2. Management of the Untreated group

In both Abulaleke and Oshegbe, selected cattle were kept together as one group and were kept tethered in a confined area with access to pasture (principally *Chloris gayana, Cydon dactylon* and *Pennisetum* species*)* and water as is typical for Muturu cattle in this state (Anyanwu et al., 2002). The Muturu cattle breed can be seen in Figure 3.4,

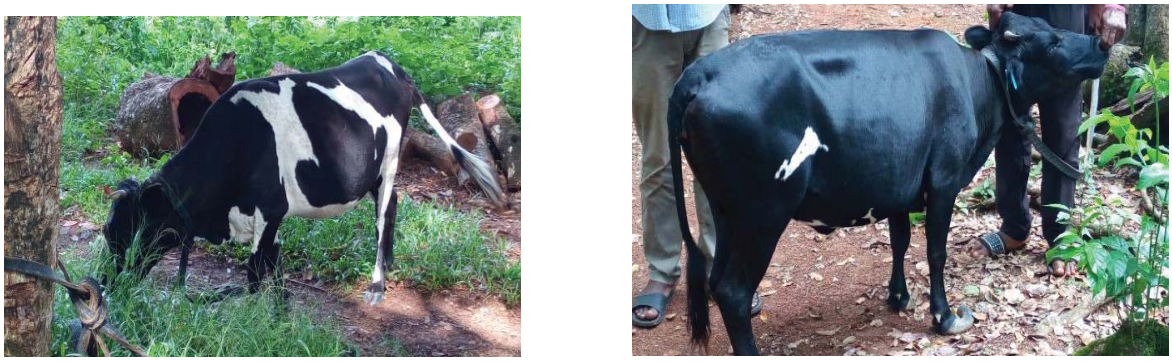

Figure 3.4. The Muturu cattle breed.

At the start of the study, Kamar heatmount detectors (LIC New Zealand) were placed onto the rostral tail head of all cows and two vasectomized bulls were introduced into the group (two per site). Cattle were observed for standing oestrus twice daily at 8:00-10:00 am and 4:00-6:00 pm. Cows detected as being in oestrus (Kamar turned red or receptivity to mounting observed) were separated from the herd into a different paddock along with one of the two vasectomized bulls, and a new Kamar detector fitted. Cessation of oestrus was recorded as the first observation period after oestrus detection when neither Kamar detector colour change nor mounting receptivity was observed. This process continued until all animals in the Untreated group had been observed in oestrus and all moved into the separate paddock.

Once oestrus activity had ceased in an individual cow, a further Kamar was attached and the same observation process undertaken for another oestrous cycle. However, for the second oestrus, animals identified as being on heat were transferred to a separate paddock with a fertile bull. A maximum ratio of 10 cows to one bull was allowed. The bulls used all had previous farmer-reported successful mating histories; bulls were kept with the cows until 15 days after cessation of second oestrus. The cows were then released back to their owners and managed by them, with bulls being allowed to run with them as per standard practices.

3.3. Ultrasound examination

Transrectal ultrasonography (iScan, Dramniski) was undertaken to evaluate follicular growth and occurrence of ovulation and presence/absence of pregnancy as outlined in Table 3.1

Table 3.1 Timing of ultrasound examination of follicle and pregnancy and records made in the Untreated group of cows.

	Time of scanning	Records made
First	Onset of oestrus (1st and 2nd cycle)	Dominant follicle size
Second	Cessation of oestrus (1st and 2nd cycle)	Dominant follicle size
Third	12 h after cessation of oestrus (2nd cycle only)	Ovulation: presence/absence of previously recorded dominant follicle
Fourth	45 days after cessation of oestrus with respect to individual cow	Detection of viable fetus (heart beat)

3.4. Management of the Treated group

All the cows selected for the Treated group were kept indoors as one group in cattle shed. They were allowed access to pasture not tethered (mainly *Chloris gayana, Cydon dactylon* and *Pennisetum* specie.) twice daily: 8:00 am - 11:00 am and 4:00 pm - 6:00 pm. No other feed or supplementary minerals were provided.

3.4.1. Synchronization protocol

The synchronization protocol used is illustrated in Figure 3.5. On day 0, cows received 100 µg gonadorelin i/m (Ovurelin, Bomac Laboratories New Zealand) along with the insertion of a progesterone-releasing device containing 1.56 g of progesterone (CIDR, Zoetis Animal Health, New Zealand). On Day 7 (am), the CIDR device was removed and cows received 500 µg cloprostenol i/m (Ovuprost, Bomac Laboratories, New Zealand) and 400 IU eCG i/m (Norvomon, Syntex, Australia). A second dose of 100 µg gonadorelin was given on day 10 (am) and FTAI undertaken that afternoon and on day 11 (am)

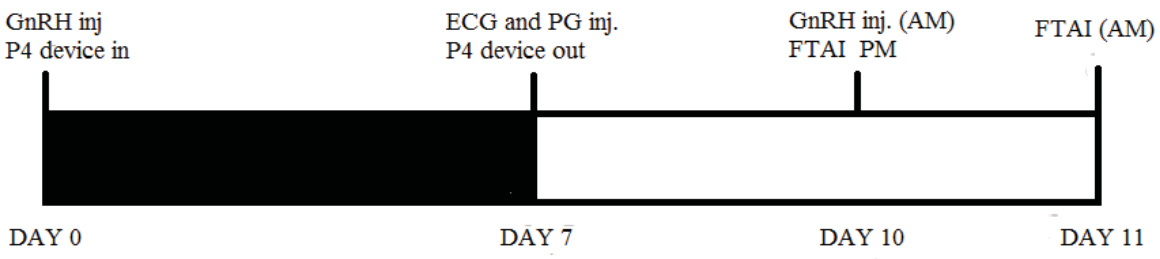

Figure 3.5 Synchronization protocol used for the Treated group. CIDR(controlled internal drug releasing device), Intravaginal progesterone device; GnRH(gonadotropin releasing hormone) gonadorelin; PGF,(prostaglandin) cloprostenol; eCG, equine chorionic gonadotropin; FTAI is fixed-time artificial insemination.

One Jersey x White Fulani bull provided semen for the AI and was used based on previous successful breeding history. Twenty bottles containing 200 ml of semen were purchased from NAPRI, Zaria Kaduna State Nigeria. They were transported on an ice pack overnight from Zaria to Abakiliki, the day prior to the start of insemination. On arrival, semen sample was taken from each viju bottle to confirm the motility of the sperm by viewing it under a light microscope; the semen was then refrigerated at 4 ºC until used. At insemination time, the required number of bottles was taken to the field on an ice pack. Each bottle was thawed prior to use by hand friction and 2 ml per animal collected via a pipette fitted with an adapter. All animals were inseminated within 10 minutes of the semen being thawed (warmed to ambient temperature).

3.4.2. Heat detection

Five vasectomized bulls were introduced into the herd on Day 7. Kamar heatmount detectors were placed on the rostral tail of each cow to aid oestrus detection. Twelve hours after eCG treatment (~6:00 pm on Day 7), oestrus recording started. Animals were continuously observed (24 h a day) until Day 11. Oestrus onset was defined as first observed mount (by cow or vasectomised bull); oestrus cessation was defined as last observed mount with oestrus duration as the time between these two events.

3.4.3. Ultrasonography

Both ovaries of each animal were examined and scanned (with iScan) on Day 0, Day 7, and Day 10 to ascertain the diameter of the DF and on Day 12 to confirm ovulation (based on the disappearance of the previous DF).

3.4.4. Pregnancy diagnosis

Pregnancy diagnosis was undertaken 45 days after FTAI.

3.4.5. Blood sample collection

Blood samples (5 ml) were collected from the jugular vein of each animal, on Days 0, 7 and 10. Blood samples were collected into plain tubes, refrigerated on ice at 4 ºC during the first 2 h and then centrifuged (300*g*) for 20 min.). Serum duplicates were stored at -20 °C until they were assayed. Serum oestradiol 17β (E_2), progesterone and LH concentrations were estimated using commercially available ELISA (Enzyme linked-immuno assay) kits ((E2 AccuBind ELISA, Progesterone AccuBind ELISA and LH Extended Range AccuBind ELISA, respectively, Monobind, Lake Forest, USA) (Bhat *et al.,* 2015). The sensitivity of the assay was 0.003mIU/Ml, 0.105 ng/ml and 8.2 pg/ML for progesterone, oestradiol and luteinizing hormone respectively. The coefficient of variation for inter and intra –assays were 7.5% and 3.8% for progesterone, 3.2% and 8.5% for oestradiol and 7.8% and 3.9% for luteinizing hormone.

3.5. Statistical analysis

All data obtained were subjected to statistical analysis using statistical product and service solutions (SPSS), statistical package. The descriptive statistics was used to analyse results to obtain the mean, median, mode and frequencies. Results obtained were given as averages plus or minus the standard deviation (M ± SD). Data were processed using a general linear model (repeated measures analysis) to measure the effect of treatment on the variables measured and if there is an interaction between the variables and treatment. Logistic regression model was used to analyse if any of the variables measured could be a predictor of pregnancy. Paired t-test was used to compare group means and bivariate correlation was used to establish relationships between variables.

CHAPTER 4

RESULTS

The results of the Untreated group (group without hormonal treatment) are described below under the following headings: indices of oestrus, and follicular dynamics.

4.1 Untreated group

4.1.1. Indices of oestrus

The findings on length of oestrus and interoestrous interval for the Untreated group are summarised in Table 4.1.

Table 4.1. Duration of oestrus, number of observation periods and interoestrous interval for the 50 Untreated control cows kept at Abulaleke and Oshegbe.

	First observed oestrous cycle		Second observed oestrous cycle		Interoestrous interval (days)
	Number of observation periods	Duration (h)	Number of observation periods	Duration (h)	
Mean (±95% CI)	N/A	23.7 (21.9-25.4)	N/A	19.4 (17.4-21.3)	19 (18.8-19.2)
Median	2	24	2	24	19
Minimum	1	8	1	8	18
Maximum	3	40	3	40	20
95% PI		11.5-35.9		5.5-33.4	17.8-20.2

Abbreviation : CI= confidence interval, PI= prediction interval, N/A=not applicable.

Number of observation periods: animals were observed for oestrus during the morning hours 8:00 am -10:00 am and in the evening 4:00 pm-6:00 pm; an animal observed on heat at 8:00am and which showed oestrus signs last at 8:00am the following morning, was observed on heat during three observation periods.

Overall, mean duration of oestrus observed was shorter in the second observed oestrous cycle than in the first (19.4 vs 23.7 h, respectively; $p<0.001$). The difference in the mean length was

that in the first observed oestrous cycle, only 8/50 cows had an oestrus length of less than 24 h, whereas in the second cycle 22/50 did so. In both oestrous cycles, the length of the observed oestrus ranged from 8 to 40 h, with 24 h being the mode (37/50 came on heat during the evening in the second observed oestrous cycle while 27/50 came on heat in the morning during the first observed oestrous cycle).

Cattle that had a short duration of oestrus relative to the others in the group during the first period of observation were also likely to have a relatively short duration of oestrus in the second period of observation ($\tau = 0.353$; $p=0.012$).

When pregnancy status at the end of the breeding season was included in the statistical model using repeated measures analysis, there was no significant association between pregnancy and length of oestrus or interaction between pregnancy and oestrus observation periods on length of oestrus ($P =0.80$ and 0.93 respectively).

In the Untreated group, the overall mean interoestrous interval was 19 days; based on this dataset we would expect that 95% of Muturu cattle would have an interoestrous interval between 17.8 and 20.2 days. In contrast to oestrus length, there was a significant association ($p=0.003$) between interoestrous interval and pregnancy status at the end of the breeding season. Cattle that were recorded as not pregnant at the end of the study had a longer interoestrous interval than those which were pregnant (19.2 vs 18.7 days, respectively; $p=0.03$). Compared to cattle that had an interval of 19 days, cattle that had an interoestrous interval of 18 days were 4.5 times more likely to get pregnant ($p=0.012$). Cattle that had a 20-day interval were less likely to get pregnant than cows with an interval of 19 days, but this effect was not significant (relative risk =0.36; $p=0.252$). Interoestrous interval was negatively correlated with duration of oestrus ($\tau = -0.25$ ($p=0.058$) and -0.32 ($p=0.013$) for the first and second observed oestrous cycles, respectively. When all the variables (duration of oestrus, hours observed and interoestrous interval) were included in a logistic regression model, only interoestrous interval was significantly associated with the odds of pregnancy ($p<0.05$).

4.1.2. Follicular dynamics

The results for DF size at onset and cessation of oestrus are summarised for cows in the Untreated group in Table 4.2. The mean DF size increased during oestrus ($p<0.001$). Follicle size at onset of oestrus was strongly correlated with follicle size at the end of oestrus ($r=0.85$; $p<0.001$).

When pregnancy status at the end of the breeding season was included in the statistical model, there was no significant association between subsequent pregnancy status and follicle size at either time of oestrus onset or cessation (p=0.26), nor was there any interaction between time (onset and cessation of oestrus) and subsequent pregnancy status on follicle size (p=0.72).

Follicle size at the start and end of oestrus was negatively correlated with interoestrus interval (τ =- 0.26 (p=0.029) and -0.21 (p=0.08), respectively), and follicle size at the start of oestrus was moderately correlated with duration of oestrus (τ=0.234, p=0.047), but follicle size at the end was not correlated with duration of oestrus (τ=0.138, p=0.241).

Table 4.2. Dominant follicle size at onset and end of oestrus for 50 Untreated Muturu cows kept at Abulaleke and Oshegbe.

	Dominant follicle size (mm)	
	At onset of oestrus	At end of oestrus
Mean	11.0	14..0
(± 95% CI)	(10.9-12.1)	(14.1-15.7)
Median	11	14
Minimum	8	10
Maximum	16	22
95% PI	7.3 -15.5	9.3-20.6

Abbreviations: CI= confidence interval, PI=prediction interval.

4.2. TREATED GROUP

The results of the Treated group are described under the following headings: indices of oestrus, follicle dynamics and circulating steroid hormone concentrations.

4.2.1. Indices of oestrus

The descriptive statistics for time between the injection of eCG, and the onset of the induced oestrus and the duration of that oestrus are shown in Table 4.3. All 50 Treated cattle were observed on heat, with all starting within a 6-h period from 29 to 35 h after the injection of eCG. The mean for time onset of oestrus and duration of oestrus were 32 h and 54 h, respectively. Cattle that came on heat sooner tended to have an induced oestrus of a longer duration (τ =0.238, p=0.031). Cattle that did not become pregnant at the end of the breeding period had similar oestrus duration compared to cows that were pregnant, although this was not statistically different (mean=54.13 h vs 54.10 h p=0.976). Cattle that were pregnant at the end of the breeding period came into heat 0.4 h earlier (mean=31.4 h vs 31.8 h) on average than cattle that were not pregnant (p<0.001). However, oestrus duration was not associated with subsequent pregnancy status (τ =-0.22; p=0.91).

Table 4.3. Timing of the onset and duration of the induced oestrus of the 50 Treated Muturu cows synchronised using the progesterone/GPG programme at the Ebonyi State University Research Farm.

	Onset of oestrus (h)	Duration of oestrus (h)
Mean	31.9	54.1
(±95% CI)	(31.5-32.3)	(53.4-54.8)
Median	32	54
Minimum	29	50
Maximum	35	59
95% PI	28.9-34.9	49.1-59.1

Abbreviations: CI=confidence interval, PI= prediction interval, onset of oestrus relative to eCG treatment.

4.2.2. Follicular dynamics

The results of the ultrasound examination of the ovaries are summarised in Table 4.4. The mean follicle size increased between Day 0 and Day 7 ($p<0.001$) and then decreased between Day 7 and Day 10 ($p<0.001$), and there was no difference in follicle size between Day 0 and Day 10 ($p=0.261$). Also, there was no association with subsequent pregnancy status ($p=0.38$) nor interaction between final pregnancy status and time (Day 0, 7, and 10) ($p=0.23$; see Figure 4.1).

There was a high positive correlation between mean follicle size on Day 0 and Day 7 ($\tau=0.773$, $p<0.001$), while values for Day 7 and Day 10 ($\tau= -0.338$, $p=0.016$) and Day 0 and Day 10 ($\tau= -0.321$, $p=0.023$), were negatively correlated.

Table 4.4 Dominant follicle size on Day 0, 7 and 10 following start of treatment of 50 Treated Muturu cows synchronised using the progesterone/GPG programme at the Ebonyi State University Research Farm.

	Dominant follicle size (mm)		
	Day 0	Day 7	Day 10
Mean	12.0	18.6	12.8
(±95% CI)	(11.3-12.7)	(17.7-19.3)	(11.8-13.8)
Median	12	18.5	12
Minimum	8	12	8
Maximum	18	26	21
95% PI	7.0-17.0	13.1-23.9	5.9-19.7

Abbreviations: CL=confidence interval, PI= prediction interval

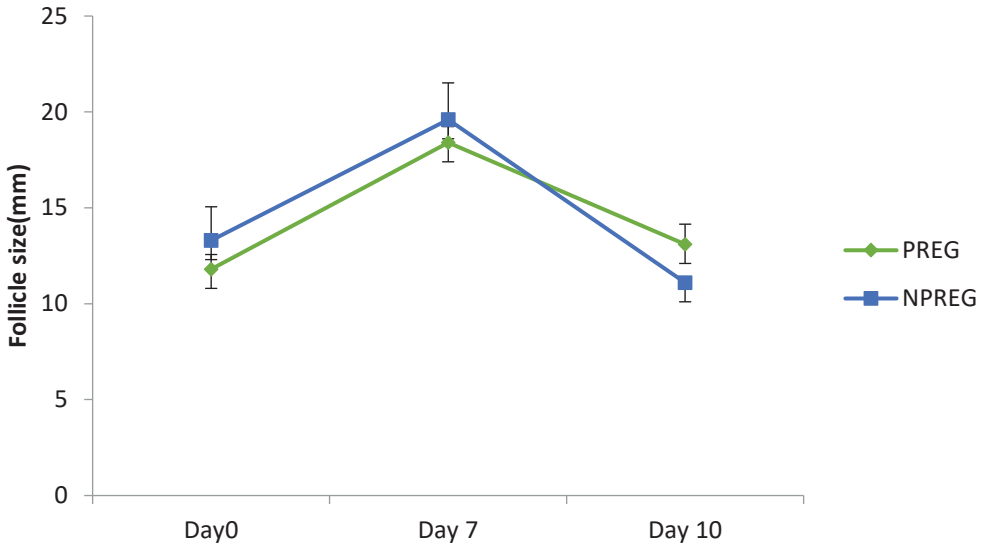

Figure 4.1 Change in dominant follicle size with time from beginning of treatment (Day 0= CIDR+GnRH, Day 7= CIDR removal and $PGF_{2\alpha}$ +eCG, Day10= GnRH (am)+FTAI (pm)) for pregnant and non-pregnant animals at the Ebonyi State University Research Farm treated with progesterone/GPG synchronization protocol. Error bars indicate 95% confidence interval, Abbreviations: PREG=pregnant animals, NPREG=non-pregnant animals, CIDR=controlled internal drug releasing device, PGF= prostaglandin ,GnRH=gonadotropin releasing hormone, eCG=equine chorionic gonadotropin ,FTAI=fixed time artificial insemination.

4.3. Circulating steroid hormone concentrations

The results of an assay of hormones in blood samples of the animals in the Treated group are described under the following headings: oestradiol, luteinizing hormone and progesterone.

4.3.1. Oestradiol

Oestradiol concentrations are summarised in Table 4.5. Oestradiol concentration increased significantly ($p<0.001$) following the start of treatment and there was an association with subsequent pregnancy status ($p=0.004$), but there was no difference between pregnant and non-pregnant cows in oestradiol concentration in the ten days following treatment ($p=0.632$, see Figure 4.2).

No strong evidence of correlation among plasma concentrations of oestradiol in each of the three days was found, however (oestradiol concentrations on Day 7 and Day 10 may be moderately correlated ($r=-0.143$ to 0.036; $p=0.322$).

Follicle size on Day 7 was moderately correlated with oestradiol concentration on Day 7 ($r=0.428$, $p=0.002$) but not with that on Day 0 ($r= 0.148$, $p=0.304$).

Table 4.5. Concentration of circulating plasma oestradiol on Day 0, 7 and 10 following start of treatment of 50 Treated Muturu cows synchronised using the progesterone/GPG programme at the Ebonyi State University Research Farm

	Oestradiol Concentration (pg/ml)		
	Day 0	Day 7	Day 10
Mean (±95% CI)	72 (65-80)	93 (86-100)	222 (212-233)
Median	71	93.5	220
Minimum	42	47	162
Maximum	195	212	354
95% PI	22-123	41-145	148-297

Abbreviations: CI= confidence interval, PI= prediction interval.

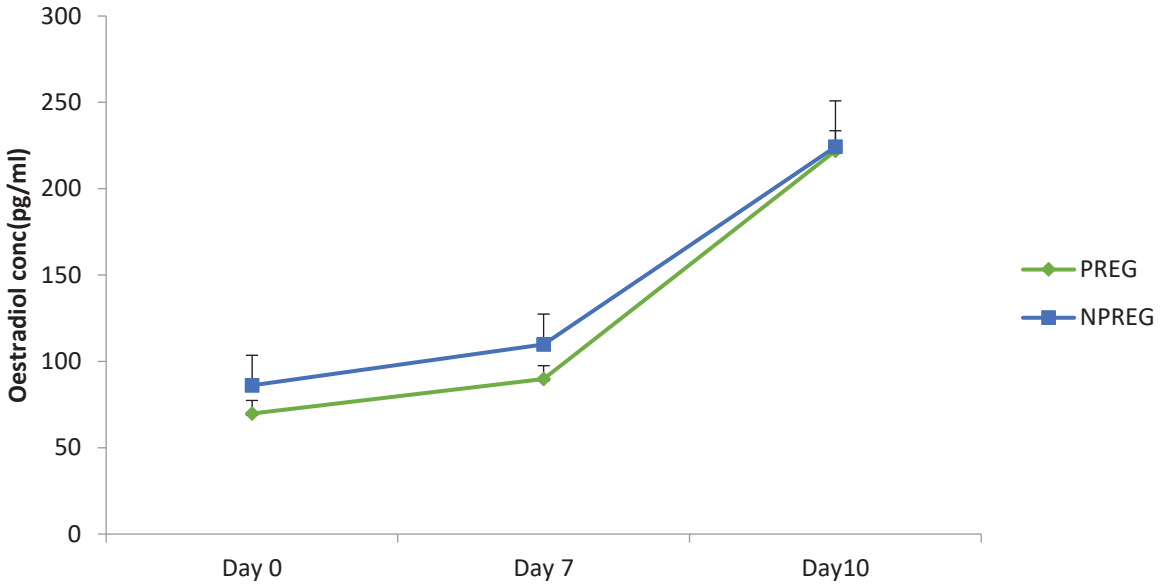

Figure 4.2. Changes in circulating plasma oestradiol concentration with time from start of treatment (Day 0=CIDR+GnRH, Day 7=CIDR removal and $PGF_{2\alpha}$ +eCG, Day10=GnRH (am)+FTAI (pm)) for pregnant and non-pregnant animals in the Ebonyi State University Research Farm treated with progesterone/GPG synchronization protocol. Abbreviations: PREG=pregnant animals, NPREG=non-pregnant animals ,CIDR=controlled internal drug releasing device, PGF= prostaglandin ,GnRH=gonadotropin releasing hormone, eCG=equine chorionic gonadotropin ,FTAI=fixed time artificial insemination. Error bars indicate 95% confidence interval.

4.3.2. Luteinizing hormone

The plasma LH concentrations are summarised in Table 4.6. Overall, the mean concentration of LH increased significantly ($p<0.001$) following the start of treatment but there was no association with subsequent pregnancy status ($p=0.765$) nor interaction between final pregnancy status and LH concentration at the three times of treatment (Day 0, 7, or 10) ($p=0.916$, see Figure 4.3).

There was no evidence of strong correlations between plasma LH concentrations on Day 0 and Day 7 ($r=0.18$; $p=0.903$) or between Day 0 and Day 10 LH concentrations ($r=0.214$, $p=0.136$), but there was evidence of a moderately strong correlation between plasma LH concentrations on Day 7 and Day 10 ($r=0.403$, $p=0.004$).

Follicle size on Day 7 did not appear to be correlated with LH concentration on Day 0 ($r=0.139$, $p=0.337$) nor with that on Day 10 ($r=0.063$, $p=0.662$).

Table 4.6. Concentration of circulating plasma luteinizing hormone (LH) on Day 0, 7 and 10 following start of treatment of 50 Treated Muturu cows synchronised using the progesterone/GPG programme at the Ebonyi State University Research Farm.

	Luteinizing Hormone Concentration (mIU/ml)		
	Day 0	Day 7	Day 10
Mean (±95% CI)	3.5 (3.2-3.9)	5.1 (4.4-5.9)	15.1 (13.8-16.3)
Median	3.1	4.7	14.0
Minimum	1.5	1.4	10.0
Maximum	7.5	11.0	31.0
95% PI	1.0-6.1	0-10.6	6.4-23.8

Abbreviations : CI=confidence interval, PI= prediction interval.

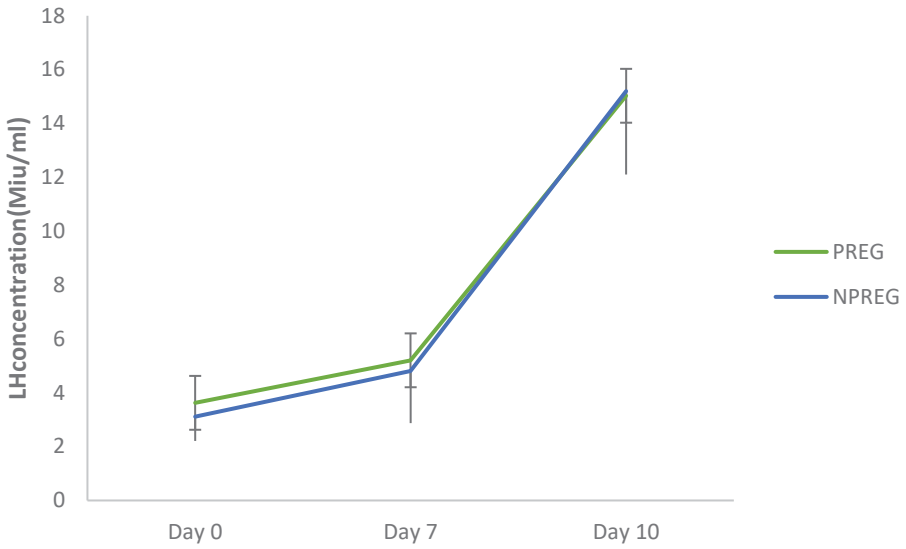

Figure 4.3. Changes in circulating luteinizing hormone concentration with time from start of treatment (Day 0=CIDR+GnRH, Day 7= CIDR removal and $PGF_{2\alpha}$ +eCG, Day10=GnRH (am)+FTAI (pm)) for pregnant and non-pregnant animals in the Ebonyi State University Research Farm treated with progesterone/GPG synchronization protocol. Abbreviations; ,CIDR=controlled internal drug releasing device, PGF= prostaglandin ,GnRH=gonadotropin releasing hormone, eCG=equine chorionic gonadotropin ,FTAI=fixed time artificial insemination, LH=Luteinizing hormone, PREG=pregnant animals, NPR=non-pregnant animals, Error bars indicate 95% confidence interval.

4.3.3. Progesterone

Plasma progesterone concentration values measurements are summarised in Table 4.7. Mean progesterone concentration decreased ($p<0.001$) between Day 0 and Day 7 and between Day 7 and Day 10 ($p<0.001$). Also, the mean concentration of progesterone was lowest on Day 10 ($p<0.001$). There were no association of progesterone concentration with subsequent pregnancy status ($p=0.525$) nor any interaction between final pregnancy status and time (Day 0, 7, or10) ($p=0.719$, see Figure 4.4).

There was a strong correlation between plasma progesterone concentration on Day 0 and Day 10 ($r=0.83$, $p=0.565$) and a moderate correlation between Day 0 and Day 7 ($r=0.279$, $p=0.050$) and between Day 7 and Day 10 ($r=0.141$, $p=0.330$).

There was no strong evidence of correlation between follicle size on Day 7 and plasma progesterone concentration on Day 0 ($r=-0.090$, $p=0.536$) and Day 7 ($r=-0.098$, $p=0.500$).

Table 4.7. Concentration of circulating plasma progesterone on Day 0, 7 and 10 following treatment of 50 Treated Muturu cows synchronised using the progesterone/GPG programme at the Ebonyi State University Research Farm.

	Progesterone concentration (ng/ml)		
	Day 0	Day 7	Day 10
Mean (±95% CI)	7.95 (7.24-8.66)	4.19 (3.88-4.50)	1.9 (1.71-2.09)
Median	7.80	4.50	2.00
Minimum	3.50	2.00	0.80
Maximum	15.00	6.10	3.50
95% PI	2.87-13.03	1.95-6.43	0.54-3.26

Abbreviations;CI=confidence interval, PI= prediction interval.

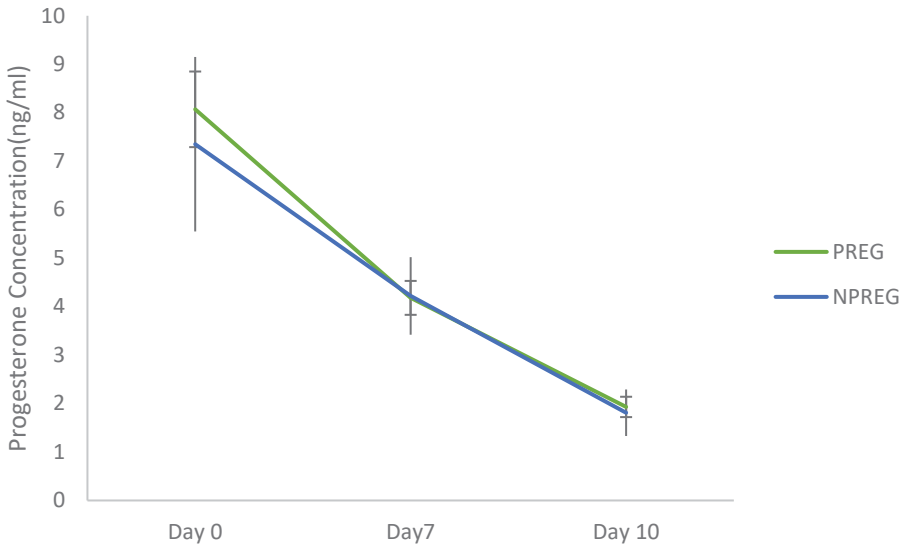

Figure 4.4. Changes in circulating plasma progesterone concentration with time from start of treatment (Day 0=CIDR+GnRH, Day 7-=CIDR removal and $PGF_{2\alpha}$ +eCG, Day10=GnRH (am)+FTAI (pm)) for pregnant and non-pregnant animals in the Ebonyi State University Research Farm treated with progesterone/GPG synchronization protocol. Abbreviations: P4=progesterone,PREG=pregnant animals, NPREG=non-pregnant animals, CIDR=controlled internal drug releasing device, PGF= prostaglandin , GnRH=gonadotropin releasing hormone, eCG=equine chorionic gonadotropin ,FTAI=fixed time artificial insemination. Error bars indicate 95% confidence interval.

In the Treated group, when the peak value of each variable including oestrus duration, follicle size, oestradiol concentration, progesterone concentration, and LH concentration were included as explanatory variables in the logistic regression, there was no significant association with subsequent pregnancy status (P=0.196).

4.4. Ovulation rate and pregnancy rate

Compared effects of Untreated and Treated groups of cows on indices of oestrus, pregnancy and ovulation rate are seen in Table 4.8. In the Untreated group, 64% of the animals ovulated and 36% of the animals were pregnant. Of the 32 animals that ovulated, only 17 animals became pregnant while 15 ovulated but did not conceive, and 1 animal apparently did not ovulate but became pregnant. In the Treatment group, ovulation rate and pregnancy rate were 100% and 84%, respectively. The animals in the Treatment group had 46% multiple ovulations while the animals in the Untreated group had none. Scanning on Day 12 showed the disappearance of mature DF seen on previous scanning.

Table 4.8 Compared reproductive performance of the Untreated and Treated Muturu cows.

Variable	Untreated	Treated	p value
Duration of oestrus	19.4 ± 0.2 h	54.1± 0.35 h	p<0.001
Ovulation rate	32/50 (64%)	50/50 (100%)	p<0.001
Multiple ovulation	0	23/50 (46%)	p<0.001
Pregnancy rate	18/50 36%	42/50 (84%)	p<0.001
Pregnancy rate/ovulation	17/50	42/50	p<0.001

Ovulation rate = number of cows that ovulated/number of cows synchronised, multiple ovulation = number of animals with multiple ovulation / number of animals synchronized, pregnancy rate= number of cows that were pregnant/number of cows inseminated, pregnancy rate/ovulation =animals that were pregnant /number of animals that ovulated.

CHAPTER 5

DISCUSSION

The present study was undertaken to determine whether using a modified Ovsynch plus progesterone synchronization protocol could improve the reproductive performance of the Muturu breed of cattle in Nigeria. The rationale behind the synchronization protocol was that it would achieve an effective synchronisation of the follicular wave, oestrus and ovulation, and that eCG would help to stimulate growth and final maturation of the preovulatory follicle.

This study has shown that when combined with good management, a modified Ovsynch and progesterone synchronization programme can result in significantly better fertility in Muturu cows than oestrus detection alone. Synchronization increased the proportion of cattle ovulating from 64% to 100% and led to a marked improvement in pregnancy rate (84% to FTAI after a single synchronization regimen compared to 36% over a 49-day period of insemination to detected oestrus in the Untreated group).

This appears to be the first study which identifies a beneficial effect of modified Ovsynch +CIDR on reproductive outcomes in the Muturu breed of cattle. The challenge with the Muturu breed is scarce information on their basic reproductive physiology and performance. In view of this challenge, the discussion on the reproductive performance of the Muturu cattle in the present study is based on knowledge generated from other *B. indicus* cattle and a few temperate breeds.

5.1. Limitations to Reproductive Efficiency

The success of synchronization in this study shows that the currently-achieved reproductive performance of Muturu cattle is not optimal. The most likely factor driving this poor performance is poor nutrition leading to prolonged postpartum anoestrus and a delayed return to cyclicity (Rhodes *et al*., 2003). The cows used in this study have a long postpartum interval and, in such circumstance, there may be inadequate pulsatile LH to accelerate the final stages of ovarian follicular development and ovulation (Bó *et al*., 2003). In such cases there may be a poor ovulation rate and the oocytes that ovulate may be physiologically immature and infertile.

When cows are kept and managed on pasture, there is a risk of postpartum anoestrus, which is commonly linked with the presence of unweaned calves and/or poor nutrition (Short *et al*., 1994). Primparous cows in pasture-based systems typically have long postpartum anoestrous

periods (Randel, 1990) and there is much evidence for the deleterious effects of poor nutrition upon the resumption of ovarian cycles in both *B. taurus* and *B. indicus* beef cows (Galina and Arthur 1990; Rhodes *et al*., 2003). The cows used in the present study were either primparous or multiparous and reared under semi-intensive systems of management, where most farmers allow their calves to run with their dams over a long period of time. This tends to impair their reproductive performance.

Most beef suckler operations rely upon a high reproductive rate, with a target of an inter-calving interval of one year. In this situation, the significance of postpartum anoestrus is that re-conception is delayed. Consequently, a long postpartum anoestrus interval results in low income for the cattle-farm enterprise if cows do not conceive during the breeding season (Ribeiro *et al*., 2012). This also leads to a low replacement rate. Most of the cows used in the present study typically had an extended postpartum anoestrus interval. Adopting a synchronization protocol, such as that used in this study, was able to induce ovulation and facilitate oestrus expression in cows, thereby mitigating the negative effect of postpartum anoestrus, despite the cows being managed under a semi-intensive system.

Artificial insemination based upon oestrus detection in anoestrous cows may not always be the best option to increase pregnancy rate in these cows, since suboptimal detection rates will result in low service rates (Pinheiro *et al*., 1998; Sartori and Barros 2011; Fricke *et al*., 2014), and AI programmes based on oestrus detection often fail to produce satisfactory pregnancy rates (Ahuja *et al*., 2005; Ribeiro *et al*., 2012; Esterman *et al*., 2016). The use of FTAI avoids the need for oestrus detection, potentially resulting in a higher pregnancy rate and reduced days to pregnancy (Ahuja *et al*., 2005; Silva Filho *et al*., 2013), at least, providing the synchronization of oestrus is reasonably efficient. One example of a positive outcome of FTAI in beef cows was that reported by Ahuja *et al*. (2005), pregnancy rate to FTAI (24.5%) was higher than that to oestrus detection (0%). Furthermore, the use of FTAI in cattle breeding programme offers the farmer some additional benefits, which include incorporation of genetic gains into the herd, and more cows being inseminated on the same day, culminating in an enhanced reproductive efficiency (Bo and Baruselli, 2014).

5.2. Untreated group

In the present study, 100% oestrus rate was recorded, as all the cows in the Untreated group came on heat during the study period. Previous experiments (Landivar *et al*., 1985; Dare *et al*., 2010; Achi *et al*., 2016; Okouyi and Hanzen, 2016) with *B. indicus* reported lower oestrus rates

compared to that obtained in the present study. Indeed, it is well established that the oestrus period in *B. indicus* is characterized by silent heats, difficult oestrus detection, shorter and less intense oestrus, and a preponderance of oestrus periods occurring at night (Orihuela *et al*., 1983; Galina *et al*., 1996; Figueiredo *et al*.,1997; Pinheiro *et al*., 1998; Mizuta 2003). The use of Kamar heatmount detectors aided in heat detection and oestrus observation during the two consecutive oestrous cycles monitored for the Untreated group in the present study, so it is likely that the 100% response to oestrus obtained may be due to continuous monitoring and detection method employed. Previous studies with Bunaji cattle (which is a similar breed in Nigeria) used tail paint and employed visual observation, which may not have been efficient, so achieved a poorer oestrus detection rate. Although Zakari *et al*. (1981) was of the view that there may be an effect of season on oestrus behaviour of Zebu cows in Nigeria and observed lower oestrus behaviour during the hot season, this study was carried out during the hottest part of the year and the oestrus rate obtained was not a reflection of seasonal effect.

Results from the present study indicate that the majority of the Muturu cows in the Untreated group came on heat during the morning hours in the first oestrous cycle observed, while more animals came on heat during the evening hours in the second oestrous cycle observed. This affirms the previous report by Ezekwe and Okwun (1997) on Muturu cattle, and is in contrast to other reports that *B. indicus* show oestrus mainly in the night (Orihuela *et al*., 1983). In the Untreated group, an interoestrous interval of 19 days was recorded between two oestrous cycles. This is similar to the work of Ezekwe and Okwun (1997), who reported an interval of 20 days in Muturu cattle, and Zakari *et al*. (1981) in Nigeria reported 22 days and 24 days for the White Fulani and Sokoto Gudali, respectively. Results from the present study show that there was a relationship between interoestrous interval and pregnancy at the end of the breeding season, such that cattle that were not pregnant at the end of the study had longer interoestrous intervals compared to those that were pregnant. The reason for the impact of a long interoestrous interval on pregnancy rate is not clear: most probably, poorer dominant follicles were present in the cows that had long intervals and did not conceive. The duration of oestrus in the Untreated group was higher in comparison to the 11 h reported by Ezekwe and Okwun (1997). The duration of oestrus might also be a reflection of the number of periods the animals were observed on heat, as animals were not monitored for 24 hours, hence, observation periods were used to obtain the duration of oestrus.

The sizes of follicles in the Untreated group were 11 mm and 14 mm, respectively; at the onset and cessation of oestrus. There is no direct comparison in the literature for the follicular size obtained in this breed. However, the results are comparable to 10-13 mm reported by Figueiredo *et al*. (1997) and Sartorelli *et al*. (2005) in other *B. indicus* breeds. There was a strong correlation observed between the sizes of the preovulatory follicle at the onset of oestrus and at the end of the oestrus period. Such increase in follicle size may be attributed to a breed effect, and /or sufficient gonadotropic support in terms of LH to induce follicle growth.

The ovulation rate obtained in the Untreated group was 64%. In the Untreated group, a significant proportion of the animals ovulated but less became pregnant, suggesting that there may be cases of cows ovulating ova that are physiological immature and infertile. It has been established that *B. indicus* lacks adequate LH to cause final growth and maturation of the preovulatory follicles (Bo *et al*., 2003), so the endocrine environment for oocyte competence and physiological maturity and fertilization may not have been sufficiently met. Ovulation of follicles of greater than 11.5 mm resulted in reduced fertility possibly due to development of a smaller CL and decreased circulating concentration of progesterone. (Vasconcelos *et al*., 2001), and such was similar to the results obtained in the present study. The acquisition of ovulatory capacity is dependent, at least in part, on follicular size. Full ovulatory capacity has been reported to be obtained when the DF reaches a diameter of 10 mm in *B. indicus* and 12 mm in *B. taurus* cattle (Sartori *et al*., 2001; Gimenes *et al*., 2008). However, Tortorella *et al*. (2013) obtained a low ovulation rate despite large follicle diameters. Therefore, it can be inferred that follicle size is not the only determinant of the capacity of the dominant preovulatory follicle to ovulate. In the present study, it seems that ovulation rate can be attributable to many factors other than follicle size, which may infer that it was the endocrine environment rather than follicle size that had the key influence on the oocyte competence and fertilization.

A pregnancy rate (PR) of 36% was obtained in the Untreated group. Probably factors other than follicle size may have contributed to the PR obtained in the present study. It was unexpected that with a mean follicle diameter of 12 mm, recorded in the Untreated group, PR was still low. Perhaps this is attributable to a lack of an adequate endocrine environment needed for oocyte competence and physiological maturity. *B. indicus* cattle are associated with difficulties in oestrus detection, so inseminating at observed oestrus may be a futile venture as oestrus detection may be compromised irrespective of whether the cattle are cycling. Thus, inseminating *B. indicus* at detected oestrus typically results in low PR. Dare *et al*. (2010) achieved a PR of 34%, which is comparable to that obtained in Untreated cows in the present

study. Again, it could be argued that the endocrine environment needed for pregnancy establishment may not have been adequate, resulting in poor uterine environment. One example to support such a notion is the work of Sartori and Barros (2011), who showed that *B. indicus* cattle are characterized by small CL and, as such, may be incapable of synthesizing sufficient progesterone to support pregnancy establishment.

Previous experiments by Mussard *et al*. (2003) and Perry *et al*. (2005) reported that GnRH-induced ovulations of follicles of less than 11 mm resulted in decreased PR and increased embryonic mortality. In the Untreated group, a significant proportion of the animals ovulated, but the proportion of ovulations that resulted in pregnancies was lower than in the Treated group, suggesting that at least some of the Untreated cows ovulated oocytes that were indeed physiologically immature, subfertile and/or subject to subsequent embryo mortality. Vasconcelos *et al*. (2001) showed that ovulation of follicles less than 11.5 mm resulted in reduced fertility (possibly due to development of smaller CL and decreased circulating concentration of progesterone), but some of the follicles observed in the present study were greater than 11.5 mm and were still associated with reduced fertility. Taken together, these data indicate that endocrine environment as well as follicle size influence oocyte competence and fertilization.

The reproductive outcomes measured in terms of pregnancy and ovulation rate were low and such reproductive performance lacks the capacity to conserve this breed through breeding programmes, resulting in the breed becoming at risk of extinction. However, as a trypanotolerant breed, it must be conserved. Conservation of this rare gene may only be achieved through multiplication of this breed, including through the use of hormonal treatments (Baruselli *et al*., 2004) that enhance reproductive efficiency. For such a breed, recent hormonal treatments, which are designed to control both luteal and follicular function, synchronization of follicle waves, oestrus and ovulation without the need for detection of oestrus, permit exciting possibilities for the improvement of its reproductive efficiency. Therefore, there is a need to employ hormonal treatments that can enhance the reproductive potential of this rare breed.

5.3. Treated group

The key result from this study was an improved PR in the Treated group (85% to FTAI after a single synchronization regimen) compared to the Untreated group (36% over a 49-days period

of insemination to detected oestrus). This was accompanied by improved ovulation rate (Treated: 100% versus Untreated: 64%), a longer duration of oestrus (Treated: 54.1 h versus Untreated: 19 h), greater follicle size in the Treated group (18 mm after a single synchronization regimen) compared to the Untreated group (11 mm over a 6-week period) and adequate hormone (oestradiol, progesterone and LH) concentrations in the Treated group. These results demonstrate the efficacy of this synchronization protocol in improving the reproductive performance of Muturu cows when measured in terms of follicle size, favourable endocrine environment, ovulation rate and PR. The hypothesis that hormonal treatment can improve the reproductive performance of the Muturu breed was therefore supported by the results obtained.

5.3.1. The modified Ovsynch protocol

The success of reproduction depends on the coordination between behavioural changes and physiological events that ensure the production and emission of mature gametes and their subsequent fertilization. To this end, in the past 20 years, GPG protocols had been employed in synchronization of beef and dairy cows (Geary *et al*., 1998; Barros *et al*., 2000; Lamb *et al*., 2001; Larson *et al*., 2006; Busch *et al*., 2008). The present study is the first to report the use of modified Ovsynch protocol in the Muturu breed of cattle, and one of only very few reports of the use of such a protocol in *B. indicus* cattle in West Africa.

The first principle in achieving reproductive efficiency with FTAI protocols is that the treatment must be able to induce atresia or ovulation of the DF. The first administration of GnRH on Day 0 of the Ovsynch protocol was intended to induce ovulation of the DF, which, in turn, induces an endogenous surge of FSH and, hence, initiates recruitment of a new follicular wave. The administration of the second GnRH injection is to stimulate synchrony of ovulation. These effects have been well-validated in previous experiments to induce ovulation or atresia of the DF, as well as synchronizing the follicle wave (Twagiramungu *et al*., 1994, 1995; Pursley *et al*., 1995; Geary *et al*., 1998; Barros *et al*., 2000; Fernandes *et al*., 2001) and, in the present study, inclusion of GnRH injection in the protocol produced positive results evident in the high degree of synchrony ovulation rate obtained.

Cows that are in negative energy balance or are being suckled have, through the direct effects of hypoglycaemia or the indirect effects of high prolactin concentrations (Short *et al*., 1994), inadequate pulsatile LH to accelerate final stages of ovarian follicular development and ovulation (Bo *et al*., 2003). In such circumstance, there is benefit in incorporating progesterone in the protocol owing to the fact that the essence of using a progesterone device is to induce

resumption of oestrous cycles in anoestrous cows, through augmenting the synthesis of gonadotropins within the anterior pituitary. Therefore, the use of exogenous progestin to enhance reproductive outcomes in FTAI protocols, owing to the potency of progestin to reduce the period of anoestrus and initiate resumption of cyclicity has been established in many studies (e.g. Geary *et al*., 1998; Lamb *et al*., 2001; Rhodes *et al*., 2003; Baruselli *et al*., 2004; Larson *et al*., 2006). Inclusion of a progesterone device in synchronization protocols has been found to improve the oestrus response (Lucy *et al*., 2001; Flores *et al*., 2006) and induce formation of CL with normal life-spans (Fike *et al*., 1997).

Incorporation of eCG in the protocol used in the present study was based on previous experiments which reported a positive effect of this hormone on reproductive outcome of *B. indicus* cows when administered at time of progesterone device removal (Cavalieri *et al*., 1997; Marques *et al*., 2003; Baruselli *et al*., 2003; Sa Filho *et al*., 2004; Ayres *et al*., 2008; Pinheiro *et al*., 2009). Also, results from studies reported by Marques *et al*. (2003), Baruselli *et al*. (2004) and Sa Filho *et al*. (2004) indicated that administration of eCG at time of device withdrawal enhanced the interval from device removal to ovulation. Schams *et al.* (1978) reported that the ability of eCG to stimulate follicular growth and induce ovulation is due to its unique nature (dual LH and FSH activities and persistence in the circulating blood due to its long half-life). The above results from previous studies provided the baseline justification for inclusion of this hormone in the protocol used in the present study.

5.3.2 *Oestrus*

Understanding the temporal relationship between oestrus onset and associated endocrine changes is critical for ensuring successful breeding. Oestrus occurs when the growth of an oestrogenic DF occurs in coordination with regression of the CL (Senger, 2003). A synchronization protocol must therefore ensure that both processes occur. The synchronization protocol employed in the present study was able to achieve this.

In the present study, 100% response to oestrus was recorded in the Treated group. Previous experiments (Diop *et al*., 1998 cited in Okouyi and Hanzen 2016; Rekwot *et al*., 1999; Voh *et al*., 2004; Dare *et al*., 2010; Achi *et al*., 2016; Okouyi and Hanzen, 2016) with *B. indicus* have reported poorer oestrus responses than that obtained in the present study. The 100% response to oestrus obtained may well be due to the continuous monitoring and detection method that was employed in the study, i.e. the use of Kamar heatmount detectors aided in heat detection and oestrus observation during the treatment period in the Treated group. The higher number

of Treated animals observed in oestrus in the study could be attributable to the ability of eCG to induce oestrus and this affirms previous results from Okouyi and Hanzen (2016), Cavalieri *et al*. (1997), and Dare *et al*. (2010), who reported enhanced response to oestrus and synchrony of oestrus in an eCG Treated group compared to a group without eCG.

Results obtained in the present study shows that none of the animals in the treatment group were observed in oestrus prior to PGF treatment, which is contrary to incidence of premature oestrus reported by Ahuja *et al*. (2005) and that with a Cosynch protocol reported by Geary *et a*l. (2001). In the present study, the onset of oestrus in the Synchronised group occurred at an average of 32 h after CIDR removal/eCG treatment. This compares to previous experiments which reported average intervals between CIDR removal an oestrus of 48 h (Okouyi and Hanzen 2016), 36 h (Diop *et a*l. 1998 cited in Okouyi and Hanzen, 2016), 48-72 h (Voh *et al*., 2004) and 58-96 h (Orihuela *et al*., 1983; Landivar *et al*., 1985; Mukargwassa-Mugerwa *et al*., 1989; Pinhiero *et al*., 1998). The shorter time interval to onset of oestrus from time of device removal and eCG injection in the present experiment may be associated with the potency of eCG treatment used in the study, although the effects of incorporating progesterone into Ovsynch protocols has probably yet to be fully explored. More interestingly, the early onset of oestrus in the present compared to earlier studies, suggests a more rapid increase in follicular size; at least, inasmuch as achieving threshold oestrogen concentrations is a representative of follicular growth (Perry *et al*., 2005).

Furthermore, the results show that cows that became pregnant had an earlier onset of oestrus after CIDR withdrawal than did those that did not become pregnant, which may also have contributed to the high pregnancy rate: it was reported by Esterman *et al*. (2016) that animals which came into oestrus 36 h after CIDR removal had higher PR (75% vs 65%) than those which came into oestrus 48 h after CIDR removal. It can therefore be concluded that the high proportion of animals that expressed oestrus during the treatment period was pivotal to obtaining high PR. Animals that display oestrus prior to FTAI have greater PR (Perry *et al*., 2007; Echternkamp and Thallman 2011) than those which do not. It has been reported that cows that display oestrus have higher oestradiol concentrations, a larger preovulatory LH surge, increased myometrial tone and greater vaginal mucosal secretions (Senger, 2003). In the present study, the high plasma concentration of oestradiol and LH obtained after onset of oestrus suggest that this was also the case.

The duration of oestrus in the Treated group (54 h) was much longer than that in the Untreated group (19 h) and in many similar reports in the literature. For example, Bunaji (11 hours) (Voh, *et al*., 1987, 2004; Achi *et al.*, 2016) and in N'dama (9 hours) (Okouyi and Hanzen 2016) cattle. Moreover, animals in the Synchronised group were still being mounted and still had clear vaginal discharge on Day 10 at the time of FTAI (i.e. which was expected to be after the end of behavioural oestrus). This can probably be attributed to the hormonal regimen that was used in the present study. Combarnous (1988) reported that the overall activity of eCG relies on various factors that include the hormone's half-life (28 h: Schams *et al*., 1978), its persistence in the circulating blood due to its heavy glycosylation and siliac acid content (Murphy and Martinuk 1991), its possession of both LH- and FSH-like activity, and its efficiency in triggering oestradiol synthesis by follicular cells. Additionally, 46% of the Synchronised cows had multiple ovulations, with the second follicle still present on Day 10. In other words, in the animals that had multiple ovulations, it is likely that they would have continuously attained oestradiol concentration above the threshold which triggers oestrus behaviour. It is interesting to speculate whether it was the dose of eCG *per se* or responsiveness of the Muturu breed to the eCG that affected the duration of oestrus. However, there are no previously published data for the Muturu breed on the duration of oestrus in cows treated with this protocol. This is the first study to use this protocol on this breed, and in addition to examine the oestrus duration, and further research is required to ascertain if there are other factors contributing to the long duration of oestrus observed in this breed apart from hormonal treatment.

The high proportion of animals observed in oestrus in the present study is presumably due to the influence of both the progesterone device and PGF treatment. Bunaji cows (*B. indicus)* had a higher response to synchrony of oestrus when treated with CIDR+PGF than PGF alone (Voh *et al*., 2004; Achi *et al*., 2016). Indeed, the synergistic role of progesterone and prostaglandin in inducing oestrus in anoestrous cows is well recognised; the CIDR increases the duration of elevated progesterone concentrations, whilst exogenous PGF reliably terminates the life-span of an endogenous CL. Incomplete regression of CL and increased concentration of plasma progesterone during the follicular phase inhibit oestrus (Pinheiro *et al*., 1998; Rekwot *et al*., 1999), but this was not the case in the present study. The use of PGF injection on Day 7 in the present study caused complete regression of luteal tissue developed due to the Day 0 GnRH injection. This is in contrast to some reports in which CL fails to completely regress (Cornwell *et al*., 1985; Twagiramungu *et al*., 1994). If there was an incomplete regression of the GnRH-

induced CL, progesterone would continue to supress oestrus even in the presence of an ovulatory follicle. Such a scenario would have decreased the number of animals detected in oestrus.

The cycling status of the animal at the onset of the experiment may be a contributory factor to the high oestrus rate obtained in the present study. All cows at the onset of the treatment had follicles present, which indicates selection and recruitment of follicles, but not necessarily the presence of oestrous cycles. It may be that use of progesterone and GnRH at the onset of the treatment may have induced cyclicity (Geary *et al*., 1998; Rhodes *et al*., 2003; Baruselli *et al*., 2004) in these animals. Stevenson *et al*. (2000) reported lower numbers of animals expressing oestrus when less than 50% were cycling at the onset of the experiment. Probably, the hormonal treatments reduced the negative effect of cycling status on the response to oestrus, thereby enhancing the percentage of animals expressing oestrus in the present study.

The higher concentration of oestradiol obtained in the present study may be a contributory element to the long duration of oestrus observed in the study. Reames *et al*. (2011) reported that duration of oestrus differed (17 vs 8 h) for cows maintained on high (12 pg/ml) and low (6 pg/ml) circulating concentrations of oestradiol, respectively, although Sood *et al*. (2015) obtained similar oestrus duration for cows with higher oestradiol concentration than cows in the control group. Contrary to these two studies, previous reports suggested that once the oestradiol concentration threshold that triggers oestrus is attained, the subsequent concentration does not influence oestrus duration (Glencross *et al*., 1981; Allrich 1994).

In summary, therefore, it is clear that a prerequisite for optimal timing of FTAI is the cows' ability to exhibit behavioural oestrus culminating in successful ovulation and pregnancy. The synchronization protocol used in the present study was able to induce and synchronize oestrus, which harnessed a better endocrine environment which resulted in higher ovulation and pregnancy rates in the Treated group.

5.3.3. Follicular dynamics

Reproductive efficiency and follicular dynamics are highly inter-related, because of the significance of the growth, development and maturation of ovarian preovulatory follicles in determining reproductive outcomes.

There was a significant effect of time of treatment on follicle size obtained in the Synchronised group, i.e. inasmuch as each component of the hormonal treatments affected the next stage of

follicle growth. The hormonal treatment used in the study improved ovulatory follicle size (18 mm) such that it can be comparable to that obtained in *B. taurus* (14-18 mm: Sartori *et al*., 2016). Similar increases in follicle size on the day of CIDR removal and eCG administration have been reported in a number of earlier studies (e.g. Baruselli *et al*., 2004; Marana *et al*., 2005; Sa Filho *et al*., 2009, 2010b, c; Santos *et al*., 2010; Sales *et al*., 2015). Moreover, the control of the progesterone concentrations (whether endogenous from the CL or exogenous from the CIDR) is likely to have regulated the rate of growth of the preovulatory follicle better in the Treated than the Untreated group; a finding also reported by Barreios *et al*. (2014) in Nellore cows (*B. indicus*). The protocol used in this study was therefore the major factor contributing to the improved follicular dynamics recorded.Thus, the protocol provided sufficient time for deviation of the dominant follicle in the follicular wave and, hence, sufficient time to express LH receptors in growing follicles.

In this context, it should be re-emphasised that the inclusion of eCG in the synchronization protocol in the present study increased the chances of achieving optimal size and physiological maturity of the preovulatory follicle, with subsequent beneficial effects upon fertility. Preovulatory follicle size is of utmost importance in achieving high reproductive efficiency, because it influences oocyte maturity (Pincinato *et al*., 2012), CL volume and progesterone concentrations (Sa Filho *et al.*, 2010b), oestradiol concentration (Senger 2003; Sartori *et al*., 2016) and, hence, high pregnancy rate (Bo *et al*., 2003; Baruselli *et al*., 2004; Huguenine *et al*., 2013).

Scanning for ovulation was done after GnRH-2 and after cessation of oestrus for Treated and Untreated animals, respectively, with ovulations rates of 100% vs 64% for Treated and Untreated groups, respectively. The present results are rather more demarcated between groups than are most of the reports of similar trials in similar cattle: Okouyi and Hanzen (2016) obtained 88% for treated and 73% for control N'dama cows treated with eCG; Vasconcelos *et al*. (1999, 2001), reported 87 and 91% ovulation rates, Pursley *et al*. (1995) reported 100% ovulation rate within 32 h after PGF treatment; and, in beef cattle, Souza *et al*. (2009) found no effect of eCG on ovulation rate as similar results were obtained in both groups (eCG 79.6 vs 81% no eCG);

5.3.4. Endocrine relationships between the modified Ovsynch protocol and follicular activity

It has been established that *B. indicus* generally secretes insufficient LH to cause adequate final growth and maturation of the preovulatory follicle (Bó *et al*., 2003). The high ovulation rate obtained for the Treatment group in the present study could be explained in terms of the ability of eCG to induce ovulation due to its positive effect on the ovarian preovulatory follicle. Previous experiments (Baruselli *et al*., 2004; Sa Filho *et al*., 2004, 2005, 2010a; Bó *et al*., 2006; Small *et al*., 2009; Pincinato *et al*., 2012) would substantiate this notion. Moreover, the 46% multiple ovulation in the Treated group (*vs* none in the Untreated group) can probably also be attributed to the FSH-like activity of the eCG. In other words, eCG may have stimulated the multiple ovulations because it has both LH and FSH actions in non-equine species (Murphy and Martinuk, 1991). This is the first study to report multiple ovulations in the Muturu breed.

Induction of ovulation in FTAI protocols can be achieved with drugs that induce ovulation of potential preovulatory follicles. Hence, GnRH was included in the present study, according to established GPG/Ovsynch protocols, as it induces a preovulatory surge of LH (as also reported by Twagiramungu *et al*., 1994, 1995; Pursely *et al*., 1995). The first GnRH administration is given to induce ovulation or atresia of the DF in order to initiate a new follicular wave. The second GnRH administration controls the synchrony of final ovulation.

The better the response to the first GnRH injection, the better the synchrony of the final ovulation and the higher the probability of a successful reproductive outcome (Geary *et al*., 2000; Atkins *et al*., 2010; Bridges *et al*., 2014). Waqas *et al*. (2016) reported that buffalo which responded to the first GnRH injection achieved significantly greater ovulatory follicle diameter in the successive follicular wave than did those buffalo that did not respond to the GnRH. Bridges *et al*. (2014) reported that when cows respond to the first GnRH, a positive relationship between that response and oestrogen and progesterone concentrations was established, and that enhanced concentration of these hormones is associated with improved ovulation and PR (Perry *et al*., 2005; Vasconcelos *et al*., 2001). On the other hand, if GnRH fails to cause ovulation of the preovulatory DF, a persistent DF may occur bringing about reduced ovulation rate at the end of the protocol (Bridges *et al*., 2014). However, when the DF present at the time of the first GnRH does ovulate, the treatment appears to either provoke the recruitment of follicles from a new follicular wave through FSH release within 2-4 h after administration (Pursely *et al*., 1997; Ayres *et al*., 2008), or indirectly facilitates elevated FSH concentration 1-2 days after removal of the first DF (Ayres *et al*., 2008; Atkins *et al*., 2010). The size and

narrow range of diameters of follicles that were extant on Day 7 of the present study argues that there was an effective response to the first GnRH in terms of abrogating the existing DF and recruiting a new follicular wave. Exactly when divergence of that wave occurred was not determined in the present study.

The importance of the final GnRH injection in controlling and synchronising ovulation has been debated, and some studies consider that it is of marginal value. On the other hand, the final GnRH consistently induces an LH surge (Perry *et al*., 2007) which improves oestradiol production before ovulation and, hence, it is argued that this GnRH administration improves synchrony of ovulation in *B. taurus* (Pursely *et al*., 1995) and *B. indicus* (Barros *et al*., 2000) allowing the effective use of FTAI.

Different GnRH-based protocols have, however, produced conflicting results. In the work of Martinez *et al.* (1999) and Perry and Perry (2009), even where a DF of greater than 10 mm diameter was present, ovulation rate was low; therefore factors other than follicle size appear to influence whether or not ovulation occurs in response to GnRH administration. Progesterone appears to be of importance in this regard. In a study of ovulatory responses by Nellore heifers to the first GnRH in a 5-day Cosynch + CIDR protocol (Biehl *et al*., 2013), ovulation rate was 85.7% and 25.8% in animals with low and high progesterone concentration, respectively. Likewise, in *B. taurus*, Melo *et al*. (2016) have also shown a negative correlation between circulating progesterone concentrations and the ovulatory response to GnRH, and others (Colazo *et al*., 2008; Perry and Perry, 2009; Giordano *et al*., 2012) have shown a negative relationship between circulating progesterone and the magnitude of the GnRH-induced surge.

Decreasing the circulating concentration of progesterone prior to beginning of proestrus and development of the ovulatory follicle therefore enhances chances of ovulation (Dadarwal *et al*., 2013). The modified Ovsynch protocol exploits this phenomenon, by progesterone withdrawal and PGF injection on Day 7 of the regimen. PGF2α induces luteolysis, so when complete luteolysis occurs (progesterone less than 1.0 ng /ml) there is a greater probability of the occurrence of oestrus and subsequent ovulation of selected DF (Pursely *et al*., 1997). Interestingly, in the present study, plasma progesterone concentrations of greater than 2.0 ng/ml were present on Day 7, and 100% ovulation and 100% response to oestrus were obtained. It is most likely that this apparent discrepancy was attributed to the limit of sensitivity of the ELISA assay, rather than to an aberrant response of the animals to the presence of progesterone.

5.3.4. Pregnancy outcomes

The PR obtained in the Treated group differed significantly to the Untreated group (84% vs 36%). The PR reported for the Treated group in the present study appears to be the highest yet recorded for *B .indicus* following a modified Ovsynch protocol. There could be several factors accounting for the different PR obtained in the present study between the Treated and Untreated groups beyond treatment *per se*. These may include prior oestrous cycling status, management, insemination timing, breed and location.

It is known that the cycling status of the animal (i.e. presence or absence of oestrous cycles) at the onset of a synchronisation regimen may be a contributory factor to both the oestrus response and PR. Stevenson *et al*. (2000), for example, reported lower number of animals expressing oestrus when less than 50% were cycling at the onset of the experiment. In the present study, the presence/absence of prior oestrous cycles of the animals were not ascertained, although selection into the study was based on presence of follicles of greater than 5 mm. Nonetheless, the 100% ovulation response in the Treated group (compared to 64% in the Untreated group), suggest that there was a significant augmentation of ovulation rate in response to the modified Ovsynch regimen. Fernandes *et al*. (2001) suggested that use of a GPG protocol was able to synchronize ovulation in cycling Nellore cows, contributing to a higher pregnancy rate after FTAI (45%) than in non-cycling cows (20%).

Results obtained from the present study indicated a relationship between preovulatory follicle size and pregnancy. Ovulatory follicle diameter influences PR in beef (Lamb *et al*., 2001; Perry *et al*., 2007) and dairy (Vasconcelos *et al*., 2001) cattle in GPG/FTAI programs. The high PR obtained in the Treated group in the present study could be due to increased size of the preovulatory follicle (Perry *et al*., 2007; Sa Filho *et al*., 2010c).

B. indicus cattle are associated with difficulties of oestrus detection, so inseminating at observed oestrus may be a futile venture as oestrus detection may be compromised irrespective of whether the cows are anoestrous or cycling. Adopting FTAI is a common means of circumventing this difficulty and, in the present study, it seems to have been a major contributor to the high PR obtained in the Synchronised group. Ayres *et al*. (2008) suggested that AI should be undertaken close to ovulation in order to achieve sperm access to the oocyte, but not so late that the oocyte becomes aged before sperm arrival. The modified Ovsynch protocol used in the present study, in which FTAI was performed at 84 hours after PGF treatment, resulted in a

better PR (84%) than the 33.39% reported by Saldarriaga *et al*. (2007) when FTAI was 48 hours. The higher PR may be attributed to improved follicle development, since Zuluaga *et al.* (2010) reported that allowing a greater interval between PGF administration and FTAI may have allowed for growth of larger follicles with greater steroidogenic activity at the time of insemination. Indeed, there is quite a lot of evidence that the timing of FTAI in GPG protocols affects PR. Zuluaga *et al*. (2010) and Esterman *et al*. (2016) reported 45% PR in *B. indicus* cows, in studies in which FTAI was performed 66 h after PGF treatment. Likewise, Geary *et al.* (1998) and Larson *et al*. (2006) reported higher PR in *B. taurus* (58% and 54%) respectively, when FTAI was performed 60 h after PGF.

Thus a conventional Cosynch programme, in which FTAI is performed 48 h after PGF appears to result in a low PR (33-39%) in *B.indicus* (Saldarriaga *et al*., 2007), although results are better in *B. taurus* (48%: Stevenson *et al*., 2003). In contrast, Larson *et al*. (2006) used a Cosynch protocol with FTAI 54 h after PGF and reported a compromised pregnancy rate (44%). Thus, there is not a consensus regarding the optimal time for FTAI in GPG protocols in *B. indicus,* but it is likely to be a contributory factor in determining pregnancy outcomes.

Semen quality and sire fertility may also affect PR. Macfarlone, (2003) observed that high fertility bulls used for FTAI gave better PR than did bulls of lower fertility. Fernandes *et al*., (2001) obtained different PR when sires of differing fertility were used in FTAI protocols (43% vs 17%), which is an indicator that bulls of high fertility should be selected.

5.4. Conclusion

The results of the present study are that, in the Muturu breed of *B. indicus* cattle, a higher pregnancy rate can be achieved from a single round of modified Ovsynch/FTAI than in six weeks of observation and insemination following detected oestrus. The results obtained indicate that the reproductive efficiency of Muturu cattle herds can be markedly improved, with beneficial effects upon the numbers of animals in the breed, the preservation of the trypanotolerate gene and the productivity of local beef herds. Moreover, it appears that the benefits from using the modified Ovsynch protocol are consequential regarding improvements in most of the endocrine cascade of physiological events that regulate fertility, all of which work in synergy to produce the desired reproductive outcome.

The initial hypothesis that hormonal treatment would improve reproductive performance of the Muturu breed, is supported by the results of improved follicle size, hormonal concentration, high ovulation and PR and greater response to oestrus. The animals in both Untreated and Treatment groups were of the same breed although kept in different locations, but the management of the animals was largely the same . The discrepancy in the reproductive outcome obtained for the two groups in the present study is an indication that hormonal treatment, rather than breed, location and animal management, was a major contributory factor to improvement of reproductive performance in the Muturu breed of cattle.

5.5. Summary

The Muturu cattle used in the present study were highly responsive to the hormonal treatment employed in the study when measured in terms of PR. The modified Ovsynch plus progesterone synchronization protocol is a relatively convenient and systematically functional synchronization protocol that is designed to achieve high FTAI PR. However, as this will help in conserving the trypanotolerant gene of the Muturu cattle through breeding and multiplication programme, further research is needed on the cost implications so that traditional farmers can employ it in their farms. Although there is no clear breeding season in Nigeria, pastures flourish in the rainy season, so getting the animals to calve at onset of the rainy season may be a better option to achieving enhanced reproductive performance.

5.6. Limitation

Availability and high cost of the ultrasonography scanner limited its use in the present study. A readily available scanner would avail the opportunity to investigate follicular wave, interval to ovulation from onset of oestrus and continuous monitoring of the ovarian activity over the treatment period.

5.7. Findings and recommendations

1. From the present study it is interesting to note that all the pregnant cows in the Treated group were not tick infested all through their gestation period, while the pregnant cows in the Untreated group were heavily infested with ticks. Tick infestation is common in the Muturu cows.
2. This is the first study to report such a long oestrus duration of 54 h in *B. indicus* cattle exposed to hormonal treatments.

3. This is the first study to report incidence of multiple ovulations in the Muturu beef cattle after eCG treatment.
4. The BCS of the cows were not taken into consideration, so BCS may or may not have contributed to the low fertility parameters seen in the Untreated animals. Further research may be needed to investigate the effect of BCS on the reproductive potential of the breed.
5. All the animals in the Untreated group were observed in oestrus, despite *B. indicus* very typically having silent oestrus and being anoestrus, so anoestrus may not be the key problem, rather the problem may be anovulatory follicles. Therefore, good nutrition and management need to be investigated to see if they would contribute to enhanced endocrine environment for a better reproductive outcome.

Future research is recommended to address the following points:

1. To investigate the rationale behind the hormonally Treated pregnant animal not being tick infested while other Untreated pregnant cows were heavily infested with ticks.
2. To investigate if replacing GnRH with oestradiol may produce similar reproductive outcome as seen in the present study while reducing the cost of the protocol.
3. It may be preferable to shorten the CIDR used in Muturu cattle from the size that is used in temperate breeds.
4. To ascertain the reason behind the unusual oestrus duration in the Treated group, i.e. whether eCG dosage or body weight of the Muturu has any contribution to this phenomenon
5. To investigate if reproductive technology such as superovulation and embryo transfer can be successfully carried out in the Muturu cattle, since there are cases of multiple ovulation in the Treated group

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