EVALUATION OF TWO DOSES OF GONADOTROPHIN-RELEASING HORMONE WITH PROSTAGLANDIN F_2ALPHA ON FOLLICULAR DYNAMICS OF BUNAJI AND FRIESIAN X BUNAJI COWS

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERISTY, ZARIA
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Declaration

I declare that the work in this Thesis entitled **"Evaluation of Two Doses of Gonadotrophin-Releasing Hormone With Prostaglandin F_2 Alpha on Follicular Dynamics of Bunaji and Friesian x Bunaji Cows"**has been carried out by me in the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. The Information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

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Certification

This Thesis entitled **"**EVALUATION OF TWO DOSES OF GONADOTROPHIN-RELEASING HORMONE WITH PROSTAGLANDIN F_2 ALPHA ON FOLLICULAR DYNAMICS OF BUNAJI AND FRIESIAN $_X$ BUNAJI COWS"by Aderemi Theophilus DARE meets the regulations governing the award of the Degree of Doctor of Philosophy of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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Dedication

This Thesis is dedicated first to God Almighty for the gift of life and to the entire DARE family.

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Abstract

Gonadotrophin–releasinghormone (GnRH) and its synthetic analogues (Buserelin, Goserelin, Leuprorelin, etc.) are widely used in veterinary practice to treat cystic ovarian disease and to synchronise oestrus and ovulation in cattle. The aim of this study was to compare the follicular dynamics, follicle stimulating hormone, luteinising hormone and progesterone profiles in response to the administration of two doses of GnRH in combination with Prostaglandin F_2 alpha ($PGF_2\alpha$; Ovsynch protocol) in apparently healthy Bunaji and Friesian x Bunaji cows. Two treatments were performed. Treatment 1: Bunaji cows (n = 15) and Treatment 2: Friesian x Bunaji cows (n = 15). In both treatments, it was hypothesised that follicular dynamics, FSH, LH and P_4 profiles did not differ between two ovsynch protocols administered to Bunaji and Friesian x Bunaji cows. Moreover, each treatment group was randomly divided into three groups. Group I: (control, n = 5) animals received 2 ml normal saline at time of hormonal treatments. Group II (n = 5) animals received 50 µg GnRH (Lecirelin®) on day 0 , day 7 a dose of 500 µg of $PGF_2\alpha$, and the second administration of 50 µg GnRH on day 9. Group III: (n = 5) animals received 100 µg GnRH (Lecirelin®) on day 0, day 7 a dose of 500 µg $PGF_2\alpha$, and administration of 100 µg GnRH on day 9. Ultrasound examination was done on all animals from day -1 to day 12. The animals were examined 12 hourly from day 9 until detection of ovulation. Blood samples were collected at 15 to 30 minutes intervals for 6 hours after the GnRH administration to determine serum concentrations of FSH and LH, and once daily for 12 days after the injection of the first GnRH to determine concentration of plasma progesterone. All animals had follicles > 8 mm at time of first GnRH administration. The ovarian responses of the various groups to first GnRH administration showed 0 % ovulation rate for both control groups and 40 % and 60 % ovulation rates for group ii and iii, respectively in Bunaji cows and 60 % each of cows in group ii and iii of Friesian x Bunaji cows. The organized event of follicular growth leading to ovulation following the second GnRH administration occurred in 20 % of controlled groups,

60 % each of treated groups in Bunaji and 60 % Group II; 80 % group III in Friesian x Bunaji cows. There was no difference in the day of new follicular wave emergence after the first GnRH injection in both treated groups and breeds. The changes in the small, medium, large and subordinate follicles did not differ significantly between the treated groups and breeds. The GnRH at the doses administered induced release of FSH and LH from the pituitary within 30 min of injection in treated groups than in control cows. Both hormones reached peak concentrations at about 120 min and then returned to pre-treatment concentrations by 300 to 360 mins after the first GnRH injection. There was a statistically significant difference ($P < 0.05$) in the peak values of serum FSH and LH obtained on day 0 (FSH Bunaji cows: 50 µg and 100µg dosages = 2.64 ± 0.21 mIU/ml and 2.70 ± 0.09 mIU/ml respectively; FSH Friesian x Bunaji cows: 50 µg and 100µg dosages = 3.38 ± 0.01 mIU/ml and 4.20 ± 0.25 mIU/ml respectively; LH Bunaji cows: 50 µg and 100µg dosages = 8.50 ± 0.50 mIU/ml and 10.20 ± 0.80 mIU/ml respectively; LH Friesian x Bunaji cows: 50 µg and 100µg dosages = 14.10 ± 0.46 mIU/ml and 16 ± 0.63 mIU/ml respectively) and those obtained on day 9 of GnRH administration (FSH Bunaji cows: 50 µg and 100µg dosages = 15.80 ± 0.66 mIU/ml and 26.00 ± 2.92 mIU/ml respectively; FSH Friesian x Bunaji cows: 50 µg and 100µg dosages = 31.00 ± 1.87 mIU/ml and 39.40 ± 1.96 mIU/ml respectively; LH Bunaji cows: 50 µg and 100µg dosages = 17.90 ± 0.90 mIU/ml and 20.20 ± 1.56 mIU/ml respectively; LH Friesian x Bunaji cows: 50 µg and 100µg dosages = 16.20 ± 0.66 mIU/ml and 26 ± 2.92 mIU/ml respectively). Plasma progesterone increased among treated groups following injection of GnRH reaching a higher peak on day 7 than in control, but did not differ among treated groups and breeds. Estimated cost benefit of using 50 µg as compared to 100µg dosages of GnRH in Bunaji and Friesian x Bunaji cows was N 6, 000:00 for each cow, N 30 000:00 for all cows and for each ovulation synchronised, N 10:000:00 and N 4, 458:00 in Bunaji and Friesian x Bunaji cows respectively. It was concluded that the GnRH analogue at

50 µg and 100 µg doses was effective in synchronisation of ovulation in Bunaji and Friesian x Bunaji cows but recommended the 50 µg for Bunaji and 100 µg for Friesian x Bunaji cows based on their synchronisation outcomes and cost-benefit analysis.

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Abbreviations

AI	Artificial Insemination
BS	Blood Sampling
CH	Corpora haemorrhegica
CIDR	Controlled internal device release
CL	Corpus luteum
DF	Dominant follicle
E	Oestrogen
E_2	Oestradiol
eCG	Equine choronic gonadotrophin
ELISA	Enzyme Linked Imminosorbent Assay
FSH	Follicle Stimulating Hormone
FSHr	Follicle stimulating hormone receptor
FTAI	Fixed Time Artificial Insemination
GnRH	Gonadotrophin Releasing Hormone
IFN-τ	Interferon-tau
IGF-1	Insulin-like growth factor 1
IGFBP-2	Insulin-like growth factor – bidning protein 2
LH	Luteinising Hormone
LHRH	Luteinising Hormone Releasing Hormone
MGA	Melengestrol acetate
Min	Minute
mRNA	Messenger Ribonucleic Acid
NNLRS	Nigerian National Livestock Resources Survey
OvSynch	Ovulation Synchronisation

P_4	Progesterone
PFSH	Pure human follicle stimulating hormone.
$PGF_2\alpha$	Prostaglandin $F_{2\alpha}$
PRID	Progesterore releasing introvaginal device
StAR	Steroidogenic acute reguilatory
TAI	Timed Artificial Inselah
US	Ultrasound

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information (Gonadotrophin-releasing hormone)

Gonadotrophin-releasing hormone (GnRH) also known as gonado-*liberin* and *luliberin* in its endogenous form and as *gonadorelin* in its pharmaceutical form, is a releasing hormone responsible for the release of follicle stimulating hormone (FSH) and luteinising hormone (LH) from the anterior pituitary. It is a decapeptide hormone synthesised and released from GnRH neurons in the basal hypothalamus and a key regulator of the mammalian reproductive system (Picard- Hagen *et al*., 2015; Zerani *et al*., 2021).

In veterinary medicine, the main clinical uses of GnRH treatments include: administration at the time of artificial insemination (AI) in normal and repeat breeder cows to improve fertility; treatment of cystic ovarian disease; management of anoestrus, oestrus and ovulation synchronisation programmes; post-insemination treatments to improve fertility; post-partum management to improve fertility later at the breeding period; and treatment of embryo transfer recipients to enhance embryo survival (Thatcher *et al.,* 1993; Peters, 2005; Jaiswal, 2007). Modifications of the decapeptide structure of GnRH to increase half- life have led to the GnRHanalogue medications that either stimulate (GnRH agonists) or suppress (GnRH antagonists) the gonadotrophins. These synthetic analogues have replaced the natural hormone in clinical use(Aliu, 2007).

The design of GnRH agonists has been directed toward stabilisation of the GnRH molecule and increasing the affinity of the agonist for the GnRH receptor (Padula, 2005). In particular, the replacement of the carboxy-terminal glycinamide terminus with alkyl amines has produced nona-peptides with a prolonged duration of action combined with increased

potency (Padula, 2005). Some of the synthetic analogues of GnRH are buserelin, goserelin, leuprorelin and triptorelin (Aliu, 2007).

At the pituitary, GnRH stimulates the synthesis and secretion of the gonadotrophins, FSH and LH. These processes are controlled by the size and frequency of GnRH pulses as well as by feedback from androgens and oestrogens. Low frequency GnRH pulses are required for FSH release whereas high frequency GnRH pulses stimulate LH pulses in a one to one manner (Jayes *et al.,* 1997). In male animals, GnRH is secreted in pulses at a constant frequency, however in females, the frequency of the pulses varies during the oestrous cycle and there is a large surge of GnRH just before ovulation (Ehlers and Halvorson, 2013). GnRH has a short half-life of approximately 2-4 minutes due to rapid cleavage by peptidases. As a result of this rapid degradation as well as massive dilution, the peripheral circulation does not contain biologically active concentrations of GnRH. Serum LH and FSH levels are used clinically as surrogate markers for the presence of pulsatile GnRH secretion. LH is a more accurate indicator of GnRH pulse characteristics (i.e. frequency and amplitude) than is FSH which has a longer half-life (Ehlers and Halverson, 2013).

For any of the clinical or management uses of GnRHanalogues, the key biological responses to evaluate efficacy are an adequate LH surge, the disappearance of the dominant follicle and the formation of an accessory corpus luteum (Picard- Hagen *et al*., 2015). However, the size and functional state of the dominant follicle at the time of GnRH administration and the phase of the oestrous cycle may be critical determinants of the response to GnRH treatments (Atkins *et al*., 2008; Colazo*et al.*,2008). The fixed-time artificial insemination (FTAI) programme, a pharmacological strategy that allows the insemination of a large number of animals without the need for oestrus detection, commonly called Ovsynch has been well described for use in dairy cattle (Pursely *et al.,* 1995;Schmitt *et al.,* 1996;Stevenson *et al.,*

1996;1999, de Lima *et al*., 2020).This programme consists of administration of GnRH on day 0 followed 7 days later with administration of $PGF_2\alpha$,and then, two days later, a second injection of GnRH is administered followed by insemination 8 to 18 h without oestrus detection. This programme uses 100 μg of GnRH for both injections because this was the labeled dose of GnRH in cattle for treatment of ovarian follicular cyst (Chenault*et al.,* 2014). Leslie*et al.* (2003) evaluated 100 μg and 200 μg of GnRH for first and second doses in a 48 h ovsynch programme and did not detect any overall difference in pregnancies to FTAI. Fricke *et al.* (1998) evaluated 50 μg or 100 μg of GnRH diacetate tetrahydrate for both administrations in an ovsynch programme in dairy cows and did not detect a difference in either the ovulation rate to the second dose of GnRH or pregnancies to FTAI. In general, a dose of 100 μg has been deemed acceptable for use in these FTAI programmes.

The study of ovarian follicular dynamics and other changes in the bovine reproductive tract has progressed significantly over the past two decades, primarily due to the use of non-invasive investigative approach such as ultrasound (Jose and Luize, 2015). Untrasonographic examinations have provided a better understanding of ovarian activity, uterine involution, oviducts and other segments of the reproductive tract in the bovine post-partum period. The use of ultrasound technology has also contributed immensely to bridge major knowledge gaps in ovarian follicle dynamics which has led to the development of improved protocols for the manipulation and control of the bovine oestrous cycle (Rajamahendran*et al.,*2001).

The ovaries of cattle contain two different pools of follicles, the non-growing pool and the growing pool. The non-growing pool contains the primordial follicles, whereas the growing pool contains the primary, secondary and tertiary follicles(Kanitz *et al.,* 2001). The primordial follicles continuously leave the arrested pool and undergo the primordial to primary follicles transition. The oocytes increase in size and the surrounding squamous pre-

granulosa cells become cuboidial and proliferate to form a layer of cuboidial cells around the growing oocyte called primary follicle (Fortune *et al.,* 2000). The mechanisms responsible for the initiation of follicular growth are poorly understood although some molecules (gonadotrophins, growth factors, C-Kit) have been mentioned (Webb *et al,* 1999).Fortune *et al.* (1999)reported that non-cortical portions of the ovary may regulate the flow of follicles from the resting reservoir. The number of follicles commencing growth in a given time is predictable because it is a function of the size of the primordial store which declines exponentially with time (Webb *et al.,* 1999; Fortune *et al.,* 2013).

According to Driancourt (2001), there is a window for follicular recruitment which lasts for two days in cattle, and only gonadotrophin-dependent follicles are recruited. In cattle 5 to 10 follicles (on average) are recruited by wave and all are potentially capable of being discharged during ovulation as oocytes (Gibbons *et al.,* 1997). The follicles are continuously recruited, with the variation in intensity dependent on the stage of follicular development and the oestrous cycle. The increase in the mitotic rate of the follicular epithelium cells, as well as the development of the antrum is more accelerated at the end of the oestrous cycle. The "chosen follicle" is selected to progress to ovulation according to maturity state and the onset of pre-ovulatory gonadotrophin (Adams, 1999). The number of follicular waves varies among animals of the same breed and even in an individual animal and can occur once, twice, three or four times (Bo *et al.,* 2000;Cummins *et al.,* 2012). Such variations can occur due to factors including nutrition, management, milk yield, lactation period and early post-partum period(Ginther *et al.,* 1996). In cattle, waves of growth of obligatory gonadotrophin-dependent follicles can be observed during the pre-pubertal period (Adams *et al.,* 1994; Melvin *et al.,* 1999); in pregnant cattle (Taylor and Rajamahendran, 1991); in post-partum periods (Murphy *et al.,* 1990) and during oestrous cycles (Roche *et al.,* 1999). During one inter-ovulatory interval, two (Ginther *et al.,* 1989; Burke *et al.,* 2000; Bellman, 2001), three

(Sirois and Fortune, 1988; Burke *et al*, 2000; Bellman, 2001) or four waves (Rhodes *et al.*, 1995) have been observed.

The first follicular wave emerges during ovulation on day 0 (d0). The emergence of the second wave occurs on days 9 and 10 (in cases of two wave cycles), or on days 8 or 9 (in three wave cycle). By the third wave, the follicular wave emerges on day 15 or 16. The dominant follicles under the influence of progesterone (P_4, dioestrus) undergo atresia. The dominant follicle (DF) present at luteolysis becomes the ovulatory follicle and the emergence of the next wave is delayed. By the second wave cycle, the CL begins to regress on day 16 and by the third wave cycle, the CL regresses by day 19, which results in 19-20 or 22 – 23 day oestrous cycles respectively (Adams *et al.*, 2008).Intervals from detection of dominance were shorter in animals with three waves (Ahmad *et al.*, 1997). Adams (1999) concluded from the available data that greater than 95% of oestrous cycles are composed of either two or three follicular waves. In addition, an increase in the proportion of three wave cycles has been associated with poor nutrition and heat stress (Bo *et al.*, 2003). The emergence of the follicular wave is characterised by two or three days of growth and the presence of 8 to 41 small follicles (3 – 4mm in diameter) that are detected by ultrasound (Figueiredo, 1995; Utt *et al.*, 2003). These waves proceed to find a small, medium and large follicle population in each ovary during all days of the oestrous cycle (Santos and Vasconcelos, 2007). From the cohort, one follicle is selected for continued growth and becomes dominant. If luteolysis occurs during the growth phase of dominant follicles, final maturation and ovulation occur. If luteolysis does not occur during the growing and maintenance phase of follicles, the fate is atresia (Kanitz, 2003). Changes in mRNA expression for the gonadotrophin receptors, key steoidogenic enzymes and growth factors (IGF-1 and – II) and their binding proteins (IGFBP) have been associated with different stages of follicular growth and atresia (Kanitz, 2003). In general, expression of mRNA for the gonadotrophin receptors, steroidogenic enzymes and

steroidogenic acute regulatory protein (StAR), increase with progressive follicular development and is highest when dominant follicles approach maximum size. Expression of mRNA declines rapidly and becomes low or undetectable in atretic follicles. The IGF-1 (granulosa cells) and IGF II (theca cells) are increased, whereas IGFBP – 2 (granulosa cells) is reduced in dominant follicles. Selection of dominant follicles is associated with expression of mRNA for LH receptors and 3B–H5D in granulosa cells. Thus, changes in gene expression are important in the recruitment, selection, dominance and atresia of ovarian follicles (Bao and Garverick, 1998; Beg *et al.,* 2001; 2002).

1.2 Research Problem

The Bunaji breed is the most populous indigenous breed of cattle in Nigeria (37.2 %; Resource Inventory and Management, 1992). There is a relatively poor understanding of the reproductive physiology of this breed and other zebu breeds (Degefa *et al*., 2016). The breed exhibits a short or low intensity period of oestrus; complete absence of behavioural oestrus or receptivity to bull in some cases; may refrain from repeated breeding attempts and often ovulate without displaying overt signs of oestrus; long post-partum anoestrus, and a high incidence of showing oestrus at night (Baruselli *et al*., 2004; Degefa *et al*., 2016). It has been established that *B. indicus* generally secrete insufficient luteinising hormone to cause adequate final growth and maturation of the pre-ovulatory follicle (Bo *et al*., 2003). These limitations appear to be the primary reasons why reproductive performances of the tropical breeds including Bunaji are impaired. Efforts to understand the biological functions and develop management schemes specific to *B. indicus*-influenced cattle raised in tropical and sub-tropical environments are critical to meet the increasing global demand for protein (Reinaldo *et al*., 2020). The cost of hormones needed to synchronise oestrus and ovulation has been one of the most important challenges to adoption of improved breeding techniques (Fricke, 1999). Reproductive intervention has to be cost effective.In routine clinical practice

of commercial dairy farms in developed countries, ultrasound scanning has been reported to be an accurate and reliable method of observing dynamic changes in ovarian structures and other related procedures such as, determining the patterns of post-partum resumption of ovarian activity and cystic ovarian conditions (Rajamahendran*et al*., 1994). The trans-rectal ultrasound machine is not widely used among bovine practitioners in developing countries. Research grade ultrasound machines are relatively expensive and they require a cart and external power source, making it cumbersome to operate under field conditions. The smaller battery operated machines lack the image quality of the larger console based units. There is a dearth of informationon the appropriate GnRH dosage to induce oestrus or ovulation synchronisation and the use of trans-rectal ultrasonography as an investigative tool for ovarian functions in Bunaji and Friesian x Bunaji cows.

1.3 Justification of the Study

Various studies have compared the effect of various doses and or types of GnRHanalogues in cattle (Martinez *et al.,* 2003; Driancourt*et al.,* 2008; Souza *et al.,* 2009; Chenault *et al.,* 2014).Fertirelin acetate has been reported to be 4-10 times more potent than gonadorelin as measured by LH and FSH releases during the luteal phase of the bovine oestrous cycle. Chenault *et al*. (1990) reported that buserelin was 50 times more potent than gonadorelin. Specific information on ovarian follicular dynamics (number, size, and growth patterns) in Bunaji and other indigenous breeds of cattle in Nigeria is scarce. The understanding of the fundamental development of antral follicles following administration of GnRH in Bunaji and Bunaji crosses would update current knowledge about folliculogenesis and fertility potentials of Bunaji cattle in Nigeria.There is little or no information on effects of graded doses of Lecirelin (GnRH agonist) in combination with $PGF_2\alpha$ on follicular dynamics, FSH, LH, and P_4 profiles in Bunaji and Friesian xBunaji cows.

1.4 Aim of the Study

The aim of the study was to evaluate the efficacy of two doses of GnRH in combination with $PGF_2\alpha$ on follicular dynamics, LH, FSH and P_4profiles in Bunaji and Friesian xBunaji cows.

1.5 Objectives of the Study

The specific objectives were to determine the following in Bunaji and Friesian x Bunaji cattle:

1. The temporal profile of the emergence, propagation and establishment of a dominant follicle and regression of ovarian follicular waves under the influence of two ovsynch protocols using a portable ultrasound device.
2. Detect ovulation and forming corpus luteum using a portable ultrasound device following administration of two ovsynch protocols.
3. Analyse the serum follicle stimulating hormone profile induced by two ovsynch protocols
4. Analyse the serum luteinising hormone profile induced by two ovsynch protocols
5. Analyse the plasma progesteroneprofile in response to corpus luteum formation following two ovsynch protocols
6. Compare endocrine and follicular dynamics responses between the two ovsynch protocols
7. Compare breed responses to each of the two ovsynch protocol
8. The cost benefit of using 50 μg or 100 μg GnRH in ovulation synchronisation protocols

1.6 Hypothesis

i. There is no difference in endocrine (FSH, LH, and P_4profiles) and follicular dynamics responses between two ovsynch protocols administered to Bunaji and Friesian x Bunaji cows

ii. There is no cost benefit advantage between Bunaji and Friesian x Bunaji cows treated with 50 μg or 100 μg GnRH in ovulation synchronisation protocols.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

The female reproductive system in mammals play a major role in the process of reproduction as important steps such as gametogenesis (oogenesis), fertilization, pregnancy, parturition and lactation occur in the female (Dugavathi, 2004). The ovary is the major functional unit of the female reproductive system. It is a very dynamic organ system involved with complex processes such as folliculogensis, oocyte maturation, cellular proliferation, differentiation and apoptosis taking place in a cyclic manner (oestrous /menstrual cycle and seasonality). Adding to the complexity, these activities are regulated at multiple levels, such as locally at the ovaryitself and also at the level of pituitary and hypothalamus.In the recent past, there have been remarkable advancements in the understanding of ovarian physiology in cattle due to pharmacological administration of biologically active agents and advances in real timeultrasongraphy (Rajamahendran*et al.*,2001; Flore *et al*., 2020). Diagnostic ultrasound scanning is an accurate and reliable tool if employed correctly, enabling non-invasive and potentially rapid evaluation of areas of interest (Rajamahendran*et al.*, 1994; Flore *et al*., 2020).

The technology of ovarian ultrasonography is one of the most significant advances in the area of reproduction biology and medicine (Kot and Ginther 1999;Pierson and Adams, 1999;Checura*et al.*, 2002; Flore *et al*., 2020).Gray-scale diagnostic untrasonography has been one of the most profound technologically advanced tools in the field of large animal research and clinical reproduction since the introduction of trans-rectal palpation and radio-immunoassay of circulating hormones (Gnither, 1986). The area that has arguably benefited more from the development of ultrasound technology than any other is reproduction in large animals (Fricke and Lamb, 2002). In many cases, rectal palpation has been replaced by trans-

rectal ultrasonography for pregnancy determination and diagnosis associated with uterine and ovarian infections. In addition, ultrasonography has added benefits such as foetal sexing, early embryonic detection and is less invasive than rectal palpation. Ultrasound has given researchers the ability to visually characterize the uterus, foetus, ovary, corpus luteum and follicles. More accurate measurements of the reproductive organs have opened doors to new areas of research and validated or refuted data from past reports (Fricke and Lamb, 2002).

2.2 Characteristics of Bovine Oestrous Cycle

The cow is polyoestrous, i.e. oestrous cyclescontinue throughout the year, unless the cow becomes pregnant. Thelength of the normal oestrous cycle is 21 ± 3 days in cows and 20 ± 3 days in heifers (Kim, 2018). For many breeds of cows, the duration of oestrus is generally 10 to 18 hours; however, considerable variation exists among individualanimals from 2 to 30 hours (O'connor andSenger, 1997).The oestrous cycle is commonly divided into four periods,pro-oestrus, oestrus, metoestrus and dioestrus (Goodman, 1994).

2.2.1 Pro-oestrus

This is the period preceding behavioural oestrus and is characterised by luteal regression, emergence and growth of the ovulatory follicle. The growing follicles on the ovary produce oestradiol-17β (E_2) in proportion to their sizes. The increased E_2 concentration causes a positive feedback on the hypothalamus that result in increased GnRH release (Kesner*et al*., 1981). TheGnRH also can self-prime the anterior pituitary causing additional GnRH release. Throughout the oestrous cycle, cohorts of follicle grow, regress, and are replaced continuously (Matton*et al*., 1981). For an unknown reason, one follicle will start to dominate E_2production, experience accelerated growth and produce inhibin (Hansel and Convoy,1983). During this time the dominant follicle has switched from FSH to LH- dependence for survival (Hansel and Convoy,1983). Inhibin serves as an inhibitor of FSH release from anterior pituitary, thus retarding the growth of FSH-dependant non-dominate follicles and soon these

follicles will undergo atresia (Ireland and Roche, 1983).The maturing dominant follicle produces gradually increasingE_2, which causes, with simultaneous absence of progesterone, behavioural and other signs of oestrus (Hansel and Convoy, 1983). Behavioural oestrus is observed during this time due to the effects of E_2.

2.2.2 Oestrus

Oestrus is regarded as the period of sexual receptivity and mating. In cattle, the primary sign of oestrus is standing to be mounted and secondary signs include frequent mounting, watery mucus from the vulva and restlessness. Pro-oestrus and oestrus are frequently referred to collectively as the follicular phase of the oestrous cycle (Dugavathi, 2004).

2.2.3 Metoestrus

Following the LH surge, $PGF_2\alpha$, E and P_4 are released from the ovary and aid in ovulation. In contrast to other livestock species, cattleovulate following the end of oestrus (approximately 28 to 32 h after the onset of oestrus or 12 to 20 h followingthe end of oestrus). Metoestrus is the period during which the remnants of the ovulated follicle transform into an endocrine gland, the corpus luteum (Keyes *et al,* 1983). Thenewly formed CL produces oxytocin, relaxin and most importantly P_4. The increased P_4 once again re-establishes the negative feedback on GnRH secretion and prevents a premature return to oestrus.

2.2.4 Dioestrus

The period of the oestrous cycle when there is a fully functional CL secreting large amounts of P_4 is referred as dioestrus. Metoestrus and dioestrus are frequently referred to collectively as the luteal phase of the oestrous cycle (Cline, 2002). The P_4 serves as a negative feedback retarding cycling GnRH release and preventing a return to oestrus. Around day 17, if a conceptus is not present, the non-pregnant bovine uterus produces $PGF_2\alpha$ causingluteolysis. The P_4 concentration decrease and GnRH secretion increases once the negative feedback

influence has been removed. TheGnRH stimulates the release of FSH and LH from the anterior pituitary. As follicular growth and maturity occur, FSH binds to its specific receptors on the growing follicle. The LH induces final follicle maturation and initiates the ovulation process. The LH pulse amplitude and frequency are influenced by steroid hormone concentrations. During periods of high E_2concentration, a high frequency, low amplitude LH occurs. In contrast, a low frequency, high amplitude LH pulse occurs under P_4 dominance (Rahe *et al*., 1980).

Although, characteristics of the oestrous cycle are similar among most beef breeds, important differences have been reported between*Bos taurus* and *Bos indicus* breeds (Galina *et al.,* 1987). In general, it is more difficult to detect oestrus in *B. indicus*female compared to *B. taurus* females. This is likely because*B. indicus* females are reported to have a shorter duration of behavioural oestrus compared to *B. taurus* females (Plasse*et al.,* 1970). In addition, *B. indicus* female has a decreased interval from onset of oestrus to ovulation (Randel, 1976); decreased magnitude of the pre-ovulatory LH surge (Randel, 1976), smaller CL(Irvin*et al.,* 1978) and lower luteal phase concentrations of P_4 (Adeyemo and Heath, 1980)than*B. taurus* females.

2.3 Hormonal Patterns duringthe Oestrous Cycle

The oestrous cycle is regulated by hormones secreted by the hypothalamus (GnRH), anterior pituitary gland (FSH and LH), ovary (oestradiol and progesterone) and uterus ($PGF_2\alpha$). These hormones serve as chemical messengers that travel in blood to specific target tissues which contain receptors that are hormone specific and regulate the phases of the oestrous cycle. The combination of hormone secretion and metabolism (liver, kidneys and lungs) maintain the correct hormonal balance during the follicular and luteal phases of the cycle (Dugavathi, 2004).

2.3.1 Gonadotrophin –Releasing Hormone

One of the most significant occurrences in the research into the neuro-endocrine control of reproduction was probably the isolation and chemical characterisation of Gonadotrophin-releasing hormone in 1971 by Schally and Guillemin who won NobelPrize for this accomplishment. The translation from discovering to clinical usefulness was swift in Human medicine (Conn and Crowley, 1991). Basic research and clinical application of GnRH inVeterinary Medicine developed soon thereafter. Synthesis of a deca-pepatide having GnRH activity (Geiger*et al.,* 1991) made the hormone readily available for research and its use in animal reproduction.

The GnRH is a straight chain deca-petide synthesised in cell bodies of neuro-secretory neurons located in the medio-basal hypothalamus and transported by their axons to circumscribed areas of the median eminence. Here, they are released into the capillary plexus of the portal vascular system and transported to the anterior pituitary gland (Beattie, 1982; Thatcher *et al.,* 1993; Picard-Hagen *et al.,* 2015; Zerani *et al*., 2021). Although the hypothalamus and pituitary gland are the main GnRH synthesis and action sites, several studies have reported an extra-hypothalamic presence of GnRH and its cognate receptor (GnRHr) in numerous peripheral tissues, including reproductive organs such as the gonads, prostate, uterine tube, placenta and mammary glands (Ramakrishnappa *et al*., 2005). Both the natural and synthetic decapeptides release LH and FSH and induce ovulation in all mammalian species so far examined (Cline, 2002; Zerani *et al*., 2021). Passive immunisation of a variety of mammalian species with an antiserum to GnRH reduces blood level of both FSH and LH and blocks ovulation collectively, indicating that the deca-peptide represents the part responsible for the release of LH and FSH (Beattie, 1982). The plasma half-life of GnRH is short, ranging from two to four minutes (Bennett and Mc Matin. 1978).

The GnRH is a pyroglutamic acid peptide. The amino-terminal tri-peptide and tetra-peptidefragment of GnRH do not stimulate gonadotrophin release. The carboxy-terminal octa-peptide and nona-peptide also have negligible gonadotrophin releasing activity. Individual amino acid substitutions drastically alter the gonadotrophin releasing activity of the native molecule. In general, the amino acid in positions 1 and 2 and from positions 4 to 10 appear to be involved only in the binding of GnRH to its target tissue receptors and in exerting conformational effects. However, histidine-2 and tryptophan-3 exert an important functional effect, since substitution of these L-amino acids with other D-amino acids produces analogues of GnRH with a significant loss in gonadotrophin releasing activity and even potentantagonistic properties. Deletion of positions 2 and 3 also leads to dramatic losses in gonadotrophin-releasing activity(Beattie, 1982).

Alterations in the chemical structure of the native GnRH molecule have led to the synthesis of potent GnRH agonist. Substitutions usually involve replacement of the glycine molecules at position 6 and 10 with a D – amino acid at position 6 and or an N- ethylamide group at position 10 (Thatcher *et al.,* 1993; Padula, 2005). The design of GnRH agonists has been directed toward stabilisation of GnRH molecule against enzymatic attack, increasing binding to plasmaproteins and membranes and increasing the affinity of the agonist for the GnRH receptor (Conn and Crowley, 1991; Padula, 2005). According to Jaiswal, 2007, the following GnRHanalogues and GnRH agonists are or have been available commercially: Gonadorelin (native-like GnRH; gonadorelin diacetate tetrahydrate or gonadorelin hydrochloride), buserelin (D-serin at position 6 and ethylamide at position 10), fertirelin acetate (ethylamide at postion 10) and deslorelin (D-tryptophane at position 6 and ethylamide at position 10).

Owing to alterations in chemical structure, marked differences exist between the various GnRHanalogues in their relative potencies to release LH and FSH in cattle (Chenault*et al.,* 1990; 2014); for example fertirelin acetate was approximately four to ten times more potent

than gonadorelin as measured by LH and FSH release during the luteal phase of the bovine oestrous cycle; while buserelinwas 50 times more potent than gonadorelin (Chenault*et al.*, 1990). A single intramuscular injection of native GnRH and GnRH agonists gave a predictable release of both LH and FSH into the peripheral circulation over a 3 h period (Thatcher *et al.*, 1993).

2.3.2 Release of Luteinising Hormone from the Anterior Pituitary

The release of luteinizing hormone(LH) from the anterior pituitary is under the control of complex interactions, all of which are not fully understood (Cline, 2002); including external and internal cues as well as both negative and positive feedback mechanisms govern LH release. External cues from the environment exert their effects by means of the central nervous system. Such factors include but are not limited to photoperiod, availability of food, temperature and sexual receptivity of the opposite sex (Fink, 1988). The body's internal environment also manipulates LH release. Warren (1983) reported metabolism, bodyweight and body fat percentage; while several nutrition associated diseases also exert feedback loops within the body that modulate gonadotrophin release. Diseases which result in malnutrition can restrict LH secretion. The precise mechanisms of the internal environment and disease modulation of LH are not known; however, it appears that the central gonadotrophin regulatory mechanism is affected which leads to altered pulse frequency (Warren, 1983). Oestrogens and progesterone can exhibit either a stimulatory or inhibitory effect on LH release. This relationship is thought of as the classical control mechanism. The LH response is largely dependent upon the immediate steroid surroundings. Increased production of $E_2$from the follicles especially the dominant follicle is responsible for initiating the cascade of events leading to the LH surge (Fink, 1979).

Classically, LH is thought to be synthesised and released from the anterior pituitary under control of itssecretogogue GnRH (Schally *et al.*, 1973). In the past, there was speculation that

LH and FSH secretion were directed by two independent secretogogues. However, White (1970) disproved this notion and concluded that both were under the control of a common secretogogue termed GnRH. Synthesis and release of GnRH is controlled by neurons in the fore-, mid-, and hind-brian and regulated by steroid hormones.

The release of GnRH occurs daily throughout the cycle but only at sub-threshold levels of what is required to induce LH surge (MacKinnon *et al.,* 1978). It has been demonstrated that high circulating concentration of E_2 is required for the natural LH surge and subsequent ovulation (Sherwood*et al.,* 1980; Ching, 1982). Inthe ovari-ectomised animal, release of LH is not continuous, but rather pulsatile with a frequency of 1 h in cows (Forrest *et al.,* 1980) and sheep (Butter *et al.,* 1972). Sarkar and Fink (1979) found ovari-ectomised rats did not experience a release of GnRH at the pituitary stalk. However, when oestradiol benzoate was administered, the GnRH release occurred as normal. Ovari-ectomised cattle treated with oestrogen (E) implants have higher blood mean LH concentrations than non-treated cows (Kinder*et al.,* 1991). These elevated LH levels were a result of increased LH pulse amplitude. The E_2levels are critical for LH pulse activity. During periods when$E_2$is low, LH pulse frequency is low, whenE_2 is high, frequency of LH pulses increases. Thus, backgroundE_2 enhances the pituitary's responsiveness to GnRH.

It has been demonstrated that high levels of P_4 hindered the positive feedback effects of E_2in ovari-ectomised ewes (Scaramuzzi *et al.,* 1971). In the early luteal phase, P_4 concentration was low and LH pulse frequency was elevated over mid-luteal levels (Peters *et al.,* 1994). When natural P_4levels are high such as during luteal phase, E_2cannot induce an LH surge in the ewe (Symons *et al.,* 1973), but whenP_4 levels are reduced, such as during the follicular phase, E_2 effectively elicits an LH surge. Several man-made agents are known to block the LH surge by hindering GnRH release. Sherwood *et al.* (1980) found the naturally occurring

LH surge was blocked in rats that were under the influence of the anestheticsalpha-chloralose, ketamine hydrochloride and urethane. Several other anesthetics includingalphaxalone and alphadolone acetate have been shown not to affect the LH surgein rats. There are several other known regulators of LH release other than GnRH, P_4 and E_2

2.4 Hormonal Control ofFolliculogenesis

Follicle stimulating hormone (FSH), a glycoprotein hormone produced and secreted by the anterior pituitary gland plays a primary role in the regulation of follicle growth. The FSH acts by binding to specific receptors, localized exclusively on the granulosa cells of follicles (Simoni *et al.*, 1997; Wang *et al*., 2021). The role of FSH in the regulation of small follicle growth has been debated. It has been argued that the FSH receptors (FSHr) are not coupled to the adenyl cyclase second messenger system and hence FSHr are non-functional until a follicle reaches the secondary stage of development in cattle (Wandji*et al.,* 1997) as well as in other mammalian species (Oktay*et al.,* 1997). These inferences were made because of the lack of noticeable effects of exogenous FSH on small follicles *in vitro* (Wandji*et al.,* 1997)which may be attributed to a minimal requirement of FSH during the early stage of follicular development (Govan and Black, 1975). The lower requirement of FSH may be further attributed to extremely slow rate of growth of small follicles (Scaramuzzii*et al.,* 1980; Lussier *et al.*, 1987) due to the longer time required for granulosa cells to double in number called the "doubling time" (Fortune, 1994). However, a large amount of evidence from *in vivo* and *in vitro* studies supports the notion that FSH is the primary regulator of follicular growth at all stages of folliculogenesis.

The granulosa cells in the ovary are the only target site for FSH action (Simoni *et al.*, 1997). Immediately after the activation of primordial follicles in cattle, FSH receptors are expressed on the granulosa cell membranes (Xu*et al.,* 1995; Bao and Garverick, 1998). Studies in

rodents have indicated that FSH is required for the maturation of flattened pre-granulosa cells into cuboidal granulosa cells (Arendsen, 1982), which marks the transition of an activated primordial follicle to the primary stage follicle. However, suppression of FSHlnhibits transition of an activated primordial follicle into the growing primary follicle (Gongeon*et al.,* 1992).

Studies have indicated a regulatory role of FSH in (i) the transition of flattened pre-granulosa cells to cuboidal granulosa cells (Arendsen, 1982; Gougeon*et al.,* 1992); (ii) growing of pre-antral follicles (Abir*et al.,* 1997; Cecconi*et al.,* 1999); and (iii) transition of a follicle from the pre-antral to the antral stage through its direct effect on antrum formation (Roy and Treacy, 1993). The actions of FSH and LH depend on the size of the follicle. Inexperiments where the circulatory concentrations of FSH in heifers were suppressed with GnRH agonist (Garverick*et al.,* 2002) or by an active immunisation against GnRH (Prendiville *et al.,* 1995) follicles did not grow beyond 4mm size. The late antral stage follicles were stimulated to grow only when the GnRH agonist treated heifers were challenged with exogenous FSH equivalent to the endogenous peak concentrations of FSH (Picton and Mc Neily, 1991).

The follicle attains dominance at a critical size of ≥ 8mm after which its development is dependent on the pulsatile secretion of LH. Hence, when the pulsatile secretion of LH was suppressed initially following the treatment of heifers with GnRH agonist, follicles did not grow beyond 7 to 9 mm (Gong *et al*., 1993). The action potential of FSH on follicle depends on the threshold concentrations of FSH. The minimum concentration of FSH required to recruit antral follicles varies among individuals as well as among similar-sized follicles within the same individuals. The dissected follicle of similar size from the same individual that were cultured with FSH expressed variable amounts of aromatase activity, which is

indicative of the differential response of similar sized follicles to the available FSH (Fry and Driancourt, 1996).

2.5 Follicular Development

Folliculogenesis is the development process in which an activated primordial follicle develops to a pre-ovulatory size following the growth and differentiation of the oocyte and its surrounding granulosa cells (Senger, 1997; Knight and Glister, 2001). During folliculogensis, a follicle may be classified as primordial, primary, secondary or tertiary. This classification is based on the size of oocyte and the morphology of granulosa cell layers surrounding the oocyte (Lussier *et al.,* 1987) Entry of primordial follicles into the growth phase occurs throughout the reproductive life (Van Wezel and Rodgers, 1996). When follicles leave the resting pool, the granulosa cells become cuboidal and begin to express markers of cell proliferation, such as proliferating cell nuclear antigen (Wandji*et al.,* 1997).

When primary follicles become secondary follicles, they have two or three layers of granulosa cells (Driancourt*et al.,* 1991). Early antral or tertiary follicles are the next stage of follicular development followed by the formation of a complete antrum (Lundy *et al.,* 1999). Antral follicles continue to grow under the influence of gonadotrophins and acquire steroidogenic capability to form mature follicles. The total time required by an activated primordial follicle to reach the pre-ovulatory size has been estimated to be 80-100 days (Britt, 1991). During this long developmental period, the exposure of a follicle to different hormonal environments may have an influence on its competence to reach the pre-ovulatory size and release a fertile oocyte. The dynamics of follicles $\geq$ 4mm and their response to different concentrations of circulating gonadotrophin and steroid hormones has been clearly demonstrated by Adams *et al.*,(1991). In contrast, little is known of the dynamics of ovarian

follicles during preceding stages of folliclogenesis which comprise small antral and pre-antral follicles.

2.6 Antral Follicular Waves in Cattle

There are two stages of ovarian antral follicle development in cattle as other domestic species (Mihm and Bleach, 2003). First a slow growth phase which is believed to be independent of gonadotrophins (Cahil 1981; Lussier *et al.*,1987); second, a fast growth phase that requires gonadotrophin support and it is usually described as a follicle wave. In evaluating ovarian antral follicular development, a few terms were introduced and become widely used to describe the patterns of antral follicle turnover (Goodman and Hodgen 1983; Evans 2003). In domestic ruminants the growing phase is defined as the time taken by the individual antral follicle to grow from emergence, as recorded by trans-rectal ultrasonography, to its maximum size. The regressing phase is the time taken by this follicle to regress to the minimal recordable size (2 or 2 mm in diameter using ovarian ultrasonography), and the time period between the end of the growing phase and the onset of regression is defined as the static phase (Schrick*et al.,* 1993; Ravindra *et al.,* 1994). Recruitment refers to the synchronised growth of a group of ovarian antral follicles that eventually gain the ability to fully respond to endocrine (gonadotrophic) stimuli. Selection is the process by which only a limited number of these cohort of follicles are rescued from atresia and continue to grow to an ovulatory size. Dominance is a characteristic of a large selected ovarian antral follicle (dominant follicle) of a wave or cohort of follicles, that permits its survival and further development in an endocrine environment suppressive to other co-existing follicles (subordinate follicles). Follicle emergence or follicular wave emergence is the beginning of the growth of a group of antral follicles from the minimum recordable size that subsequently ovulate or undergo ateresia (Ginther *et al.,* 1996).

Ovarian antral follicular dynamics have been most thoroughly studied in cattle (Adams and Pierson 1995). Antral follicles $\geq$ 4mm develop in an orderly succession, in a wave-like pattern; i.e synchronous emergence and growth of a group of follicles in response to a surge in the circulating concentrations of FSH (Ginther *et al.,* 1996). The initial proposition of two or three waves of follicular development (Rajakoski, 1960) during the oestrous cycle in cattle, with an inter-wave interval of 7-10days, was confirmed by more advanced studies using ultrasonography (Savio *et al.,* 1988; Sirois and Fortune, 1990). The development of follicles 1 to 3mm has been characterised as wave-like similar to the pattern described for follicles $\geq$ 4mm (Jaiswal *et al.,* 2004).

The emergence of a follicle wave in cattle is characterised by a significant increase in the number of small antral follicles 6 to 9 follicles in the 4 to 6 mm size range(Gong *et al.,* 1993; Ginther *et al.,* 1996). The emergence of each follicular wave is preceded by a transient peak in plasma FSH concentration. During the next few days one of the follicles becomes dominant and the others (subordinate) become atretic (Ginther *et al.,* 1989b). This stage of antral follicular development has been termed "deviation" (Ginther *et al.,* 1996). During the growth of the dominant follicle there is a gradual reduction in the number of small follicles (4 to 6mm in diameter). Initially, all emergent follicles of the wave have the ability to become the dominant follicle (Ginther *et al.,* 1996). Approximately 3 days after wave emergence the dominant follicle reaches a diameter of 8mm and it continues to grow at a higher rate compared to the subordinate antral follicles. The duration of the deviation process in cattle is very short, 8h (Ginther *et al.,* 1996). The ovulatory follicles (8 to 20 mm in diameter) in cows invariably originate from the last follicle wave of the inter-ovulatory interval (Ginther *et al.,* 1996).

Evaluating the difference in detection of follicles by ultrasound or rectal palpationconcluded that ultrasound was more effective at identifying follicles greater than 10mm in diameter than rectal palpation. Follicles of 10 to 15mm in diameter were detected in 90% of cases using ultrasonography versus 62% of the cases using rectal palpation. Follicles greater than 15mm were detected in 100% of the cases for both ultrasonography and rectal palpation. In a similar review (Hanzen*et al.,* 2000), manual diagnosis of follicles 10mm was inaccurate, but ultrasound offered the possibility to diagnose follicles <5mm and to measure the diameter of these follicles.

2.7 Follicular Dominance

Current theory on follicular dominance states that every follicle has the potential to achieve dominance and to suppress the development of subordinate follicles. Adams *et al*. (1993a) supplemented cattle with FSH injections, and found all follicles in the cohort present at injection were able to achieve dominant follicle (DF) diameter. Exogenous FSH is used to induce super-ovulation to provide a large number of embryos for use in embryo transfer procedures. Gibbons *et al*.(1996) using ultrasound-guided ablation to pre-select a dominant follicle at random by removing all other members of its cohort, found that any follicle has the potential and can be forced to become the dominant follicle. Ko *et al.* (1991) destroyed the dominant follicle of a cohort and showed that a subordinate follicle may inherit dominance if deviation had occurred within two days. When the dominant follicle was cauterized three days before expected ovulation (Adams *et al*., 1993b), the largest subordinate follicle reached a larger diameter than controls (11.7 mm vs. 8.0 mm) and reached maximum diameter later (9.2 d vs. 3.1 d).

2.8 Persistent Follicles

Cattle that were treated with progestins for oestrus synchronisation had elevated E_2blood levels greater than what is normally found during the luteal phase (Sirois and Fortune, 1990; Wehrman*et al*., 1993; Sanchez *et al*., 1995). Rathborne*et al*. (2001) speculated that increased E_2 levels may be due to persistent follicle. Elevated E_2 levels during this period are likely due to increased frequency of LH pulses and are likely similar to the follicular stage (Rathborne*et al.,* 2001).

2.8.1 Persistent Follicles Reduce Fertility

When typically used commercial doses of synthetic progestins, specifically, melengesrol acetate (MGA) or norgestomet, were used for oestrus synchronisation, pregnancy rates were reduced compared to administration of levels two to three times greater (Wehrman*et al*.,1993). Mihm*et al.* (1994), reported that as the time a persistent follicle existed increase (over 4 days), pregnancy rate declined. Wehrman*et al*. (1996) reported oocytes exposed to increased amounts of E_2 for longer periods than normal, and exposure to the uterus may be the mechanism of reduced fertility. Another theory of reduced fertility associated with persistent follicle involves LH. The first meiotic division of an oocyte is induced by the LH surge during oestrus (Rathbone *et al*., 2001). Revah and Butler (1996) proposed that the oocyte in persistent follicles may be exposed to an increased frequency of LH pulses which induces start of the first meiotic division prematurely. Wehrman*et al*. (1996) reported that conception rates are similar for normal and persistent follicles, however, in the latter, early embryonic death occurred at a greater frequency.

2.9 Prostaglandin $F_2\alpha$

Prostaglandin $F_2\alpha$ and its synthetic analogues have been successfully used for induction of oestrus in BunajiCattle (Rekwot*et al.,* 1999; Voh*et al.,* 2004).Prostagladins are naturally occurring compounds that are produced by most cells in the body and have a variety of

biological actions (Smith *et al.,* 2010). $PGF_2\alpha$ is anaturallyoccurring luteolytic hormone that has also been utilized to synchronise oestrus and induce abortion in cattle through induction of CL regression (Amare and Ayalew, 2021). Synchronisation of oestrus by it and its potent analogues is the drug of choice in the reproductive management of bovine species (Ahlawat *et al.,* 2015). In theabsence of embryo, uterine concentrations of $PGF_2\alpha$ increase during the luteal phase. The $PGF_2\alpha$ is secreted in pulses and transported to the CL via a counter-current mechanism. The mechanisms associated with $PGF_2\alpha$ induced luteolysis are not completely understood, However, $PGF_2\alpha$ probably has both a direct and indirect (decreased blood flow) action. Luteal cells are known to have $PGF_2\alpha$ receptors on their plasma membranes and direct inhibitory effects of $PGF_2\alpha$ on luteal progesterone secretion have been demonstrated (Niswender *et al.,* 2000). In addition, $PGF_2\alpha$ isknown to reduce luteal blood flow due to vasoconstrictor activity (Niswender and Nett, 1988).

Prostaglandin $F_2\alpha$ causes luteolysis and in doing so removes the negative feedback influence of P_4 on GnRH secretion. Inducedluteolysis is one of the oldest means of effectively manipulating the oestrous cycle (Teige andJakobsen, 1956). Inskeep (1973) reported that $PGF_2\alpha$ administered in the early stages (d 5and d 6) ofthe oestrous cycle in cattle was not as effective in inducing luteolysis as lateradministration. It was speculated that early administration was not effective because $PGF_2\alpha$ receptors were not yet present on the CL. This theory was discredited by Wiltbank *et al*. (1995) who removed ovaries from heifers on days 2, 4, 6, and 10 of the oestrous cycle and found high-affinity PGF2α receptors present at all the days sampled.

Wiltbank (1997) later speculated that a 4 to 6 day CL established a positive feedback loop for intra-luteal P_4 production after exogenous $PGF_2\alpha$ treatment. For purposes of oestrus synchronisation, injection of $PGF_2\alpha$ is only effective in cycling heifers and cows

(approximately d 6 to 16 following oestrus, [d 0 = oestrus]). Injection of $PGF_2\alpha$ into pre-pubertal heifers or anoestrous cows is not effective due to the absence of luteal tissue. Furthermore, $PGF_2\alpha$ treatment will not induce cycling activity in non-cycling cattle. Therefore, when using $PGF_2\alpha$ alone to synchronise oestrus, it is important to assess the proportion of cycling animals beforeinitiating the treatment.

The preferred treatment regimen for $PGF_2\alpha$ consists of two injections spaced 10 - 14 days apart (see figures 2.1 and 2.2). The theory behind thistreatment is at least one injection will be administered during the middle stage of the oestrous cycle, and in theory, all cattle should be responsive to$PGF_2\alpha$ at this time. When cattle wereinjected with $PGF_2\alpha$ during days 5-16 of the oestrous cycle a return to oestrus was observed within2 - 4 days (Rekwot*et al.,* 1999). Several factors mayinfluence the return to oestrus interval including age, breed, and physiological factors (Dare *et al*., 2010). The main factors affecting synchrony when using$PGF_2\alpha$ treatment was the stage of the oestrous cycle (King *et al*., 1982; Stevenson*et al.*, 1984). The sensitivity of the CL to $PGF_2\alpha$ administration was greatest on d 10 (King *etal*., 1982; Tanabe and Hann, 1984). The recommended protocol to synchroniseoestrus using $PGF_2\alpha$ is two injections spaced 10 - 12 days a part (Britt, 1979; Hansel and Beal, 1979). This protocol resulted inacceptableconception in the mid 1970's (35%) with fixed time AI at 80 h post secondinjectionCooper andRowson, 1975). However, different strategies are now available thatresult in higher conceptionrates (Pursley*et al.,* 1995; Gizaw *et al*., 2016).

In herds containing both cycling and non-cycling females, the most effective oestrus synchronisation protocols combine treatment with a progestin and an injection of$PGF_2\alpha$. In pregnant feed-lot heifers, $PGF_2\alpha$ is highly effective at inducing abortion before 100 days of gestation (Smith *et al.,* 2010).

Peters and Benboulaid (1998) investigated the occurrence of ovulation after $PGF_2\alpha$ / GnRH application in some animals by means of ultrasound. Ovulation occurred between 24 – 48h after GnRH injection and the size of the ovulatory follicles was between 15 and 20mm. Kot and Ginthers (1999) showed that the mean time from beginning to completion of evacuation of ovulatory follicles was 4.3 ± 3.3 min (min 6s; max 15.5min).

After the ovulation, the luteinization of the corpus haemorrhagicum ensues, which causes the dominant follicle (DF) to rupture resulting in the formation of a CL which produces P_4 (Skarzynski*et al.,* 2013). The morphology of the CL and plasma P_4 concentrations is a good indicator of CL formation. Intense angiogenesis, proliferation of granulosa and theca cells from the follicular wall after ovulation, and differentiation during the first 5to 6 days after ovulation results in a progressive increase in plasma P_4 concentrationsfrom <l ng/ml three days after ovulation, to approximately 3ng/ml six days following ovulation. The peak in plasma progesterone levels occurs between days 10 and 14 post ovulation (>4ng/ml).

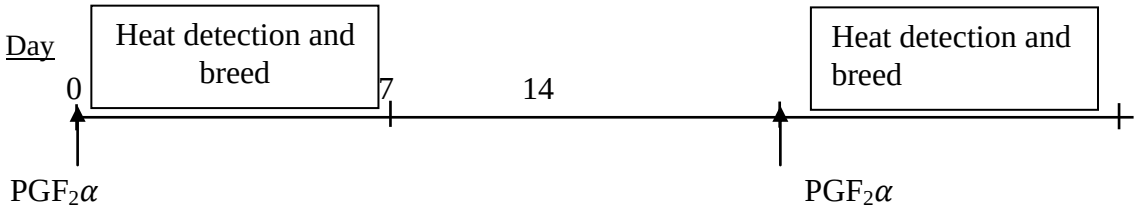


Figure2.1: Modified two injections of prostaglandin treatment.

Figure2.2: An illustration of two injections of prostanglandin treatment.

However, a decline occurs after the 16th day due to induced release of $PGF_2\alpha$ in the endometrum (Singh *et al.,* 2003), which promotes luteolysis. Some authors investigated results of artificial insemination in dependence on number of follicular waves or duration of dominance.Ahmad *et al.* (1997) found that pregnancy rate did not depend on number of follicular waves during oestrous cycle. However, Townson *et al.*(2002) found that fertility was higher in cattle with three waves per cycle in comparison to cattle with two waves. Mihm*et al.* (1994) reported that pregnancy rate was clearly dependent on duration or persistence of dominance of the ovulatory follicle in progestenone treated beef heifers.

2.10 Ultrasonography

Since the 1600's, studies of reproductive function have involved data gathered from gross and microscopic examination of excised tissues. Even the most detailed studies of static anatomic specimens excluded the dynamic process of life and the mechanisms controlling them (Adams and Singh, 2011). Although knowledge gained from the study of tissue samples has been considerable, understanding of structure-function relationships of the reproductive system was hindered until 1980's when real-time B-mode ultrasonography became available. The ability to visualize changes in structure in a serial fashion, without interruption or distortion of function has revitalized the study of reproduction in many species, but most notably in cattle (Adams and Singh, 2011). Studies on events associated with follicular dynamics have helped to improve the strategy involved in the efficient control of reproduction and other related procedures (Manjusha*et al.,* 2008;Safilho*et al.,* 2014).

2.11 Veterinary Ultrasound Equipment

Trans-rectal ultrasonography and ultrasonography have been used as diagnostic tools since the 1980s in large non-domestic species (Adams *et al.,* 1991), cattle (Pierson and Ginther, 1984; 1988; Rajamahendran*et al*., 1994), ewe (Schrick*et al*., 1993; Ravindra *et al*., 1994;

Bartlewski*et al.*, 2000) and swine (Soede*et al*., 1992). In general, linear-array, real-time B-mode ultrasound scanners are best suited for veterinary applications involving cattle reproduction. Most ultrasound machines consist of a console unit that contains the electronics, controls and a screen upon which the ultrasound image is visualized by the operator, and a transducer, which emits and receive high frequency ultrasound waves. Linear-array transducer results in a rectangular image on the field of scan as opposed to a pie shaped image produced by a sector transducer (Fricke and Lamb, 2002). Bovine reproductive organs are most commonly scanned per rectum using a linear array transducer specifically manufactured for trans-rectal use. However, specialized applications including ovum pick-up and follicle ablation involve a trans-vaginal approach using a sector transducer. Linear-array transducers of 5.0 and 7.5 MHZ frequency ranges are most commonly used in cattle and most veterinary ultrasound scanners are compatible with probes of different frequencies. Depth of tissue penetration of sound waves and image resolution are dependent upon and inversely related to the frequency of the transducer. Thus, a 5.0 MHZ transducer results in greater tissue penetration and lesser image detail, whereas a 7.5 MHZ transducer results in lesser tissue penetration and greater image detail. An ultrasound scanner equipped with a 5.0 MHZ transducer is most useful for bovine practitioners conducting routine reproductive examinations; however, small ovarian structures such as developing follicles are best imaged with a 7.5 MHZ transducer (Fricke and Lamb, 2002). Computer-assisted image analysis is a natural extension of the technological advances in ultrasonography (Singh *et al.*, 2003).

An ultrasonographic image is composed of thousand of picture elements or pixels (Pierson and Adams, 1995). Each pixel represents a discrete tissue reflector and can assume one of 256 shades of grey (ranging from black to white in an 8-bit grey-scale image (Pierson and Adams, 1995). Computer algorithms have been designed specifically for analysis of ultrasound images, in an effort to provide a qualitative approach to echotextural analysis.

These algorithms have been used extensively in studies characterizing the echotexture dynamics of antral follicles and CL at different stages of their growth (Pierson and Adams, 1995; Singh *et al.,* 1998).

2.12 Imaging the Ovaries

Nowhere have the effects of ultrasound imaging been more evident than in the understanding of ovarian physiology (Adams *et al*., 2008). More studies have been published on ovulation and follicular- and luteal-dynamics using the bovine model than in all other species combined (Singh *et al*., 2003). Controversy surrounded the wave theory of follicular development for nearly three decades, until the first ultrasonic study of the bovine ovaries was reported in 1984 (Adams *et al*., 2008). This new capability created a flurry of interest and led to a series of advancements in the understanding of folliculogenesis and the development of methods to control ovarian function in cattle and other species. One of the most important advantages arising from these studies is the ability to centralize follicular and hormonal data about a specific ultrasonically detectable point of reference (e.g ovulation, wave emergence, follicle selection). This capability led to the development of a conceptual model of follicular wave dynamics that provides an explanation of hormonal control of the oestrous cycle (Adams and Singh, 2011).The use of ultrasound technology to evaluate ovarian activity has been reviewed in great detail (Pierson and Ginther, 1988; Beal *et al.,* 1992). The Ovarian stroma, ovarian vessels, follicles, cysts, corpora hemorrhagica (CH) and corpora lutea (CL) are all structures that have been previously identified by real-time ultrasonography (Pierson and Ginther, 1988; Beal *et al* 1992; Singh *et al.,* 1997).

The most distinguishable ovarian structures are antral follicles. Because follicles are fluid-filled structures, they absorb ultrasound waves and are displayed as black on the screen (i.e anechoic or non-echogenic). In contrast, the ovarian stroma, CH and CL all contain varying

degrees of dense cells, which reflect the ultrasound waves and result in a gray image on the screen.Routine reproductive examinations should include visualization of the major structures (or the lack thereof) on both ovaries. However, rectal palpation is a poor method for resolving ovarian follicles (Pieterse *et al.,* 1990); by contrast ultrasonic imaging is a highly accurate and rapid method for assessing ovarian structures (Griffin and Gintherm, 1992). Too often, bovine practitioners proceed directly to scanning the uterus during reproductive examinations and neglect the ovaries all together. This is unfortunate because the ovaries contain a wealth of information that can be used to aid in diagnosing pregnant status of the cow and for selecting approximate therapies or reproductive interventions. For example, presence or absence of a corpus luteum aids in diagnosing pregnancy status, especially when conducting pregnancy examination early post-AI. The size and location (i.e. left / right ovary) of the CL indicate the location of the conceptus within the uterus, if the cow is pregnant (Fricke and Lamb, 2002). Because most twinning in cattle is dizygousthe presence of multiple corpora lutea is a diagnostic indicator of the presence of twin foetuses. Ovarian pathologies such as "static ovaries" and follicular and luteinised cysts can easily be distinguished. Use of ovarian structures as diagnostic aids during reproductive examinations, however, requires a thorough understanding of ovarian and reproductive anatomy and physiology. In addition, there are limitations to the conclusions that can be made from a single as opposed to serial ultrasound examinations (Fricke and Lamb, 2002).

2.13 Ovarian Follicles

Folliculogenesis is the process of forming mature follicles capable of ovulation from the pool of non-growing, primordial follicles in the ovary (Jaiswal, 2007). Ovarian follicles are fluid-filled structures surrounded by an inner layer of granulosa cells and an outer layer of theca cells. The oocyte is suspended within the antrum by a specialized pedicle of granulosa cells called the cumulus oophorus (Fricke and Lamb, 2002). Because fluid absorbs rather than

reflects ultrasound waves, fluid-filled structures such as follicles appear as black circular structures surrounded by echogenic ovarian tissue. Follicles with a diameter of 2 to 3 mm or greater can be resolved with most veterinary grade ultrasound scanners, and larger follicles can easily be tracked during serial scanning sessions (Pierson and Ginther, 1988). The ability to non-invasively track follicular growth during the oestrous cycle using ultrasound has revolutionised the understanding of reproductive physiology.

2.14 Follicular Waves

The first studies using ultrasound revealed that follicular growth occurs in waves, each wave culminating with the formation of a large follicle (Ginther *et al*., 1996). A follicular wave beginswith emergence of a group or cohort of small antral follicles just before the day of ovulation. During the next several days, one of the follicles in this cohort continues to grow and becomes dominant, thereby suppressing subordinate follicles within the wave from which it originated as well as emergence of follicles in an ensuing follicular wave. As the dominant follicle continues to grow, growth of the remaining follicles in the cohort ceases or slows, and these subordinate follicles eventually undergo atresia. A second wave of growth emerges on approximately day10 after ovulation and, for three-wave cycles, an additional wave emerges at day 16 after ovulation.

For both two and three-wave cycles, the ovulatory follicle arises from the final wave (Ginther *et al.*, 1996). Follicular wave phenomenon was demonstrated over five decades ago, using SwedishRed and White breeds of cattle (Rajakoski, 1960). Researchers concluded that the cowexperienced two such follicular waves over the course of the oestrous cycle by examiningovaries post-mortem (Rajakoski, 1960). Histological evidence later revealedthat three rather than two waves occurred during the cycle, and that each of these wavesproduced one dominant follicle (Ireland and Roche, 1983). When ultrasound technologybecame

available, many laboratories utilized this technique to resolve the questions of howmany follicular waves occur.

Pierson and Ginther (1984) judged ultrasound an effective tool for monitoring and evaluating ovarian follicles and CL in normal and superovulated heifers.Pierson and Ginther (1984) reported that locating the reproductive structures was not difficult after several practice sessions; however, a clear image was not always obtainable. Cattle wereevaluated trans-rectally and forbest results all faecal material needed to be removed from therectum to allow complete contactbetween the transducer and rectal wall. The instrument wasdeemed practical after ultrasound data was consistent with reports generated by marking structures with India ink and examination post-mortem. Utilizing ultrasound technology,Ginther *et al*. (1989b) found 81% of cattle scannedexhibited two follicular waves per cycle,the remainder experienced three waves. Other investigators found a predominance of cattlewith three follicular waves. Savio *et al*. (1988)found 81%of their herd had three dominantfollicles per cycle, 15% had two, and the remaining 4% had one. When all datawerecombined, it was concluded that over 95% of cows experienced either 2 or 3 follicular waves.Some cattle of *Bos indicus* origin may have a total of four wavesper cycle. Rhodes *etal*. (1995) studied 17 Brahman heifers using ultrasound. The dominant follicle and CL of these heifers were smaller than in *B.taurus* breeds, but the overall pattern of development wassimilar. Also, one heifer on this study experienced four follicular waves per cycle. Zeitoun *et al*. (1996) reported that cattle of *B. indicus* origin that experienced fourwaves per cycle also experienced a longer oestrous cycle. However, most of these four wavecycles were the result of extended inter-ovulatory intervals resulting from delayed luteolysis orovulation failure (Ko *et al*., 1991; Adams *et al*., 1992). Ko *et al*. (1991) induced a four-wavecycle in a heifer by performing a cautery on the dominant follicle on day 3.

2.15 Corpora Lutea

The CL is a transient endocrine gland that forms after ovulation from the tissues that previously composed the ovarian follicle. Thus, the CL can be viewed as the terminal stage of follicular development. Changes in the hormonal stimulation of a pre-ovulatory follicle may have a subsequent effect on luteal P_4 secretion (Smith *et al*., 2010).McNatty*et al*. (1979) suggested that development of a normal CL may depend upon a pre-ovulatory follicle meeting the following criteria: (i) adequate number of granulosa cells, (ii)adequate number of LH receptors on granulosa and theca cells and (iii) granulosa cells capable of synthesising adequate amounts of P_4following luteinization. Furthermore, the ability of luteinised human granulosa cells to screte P_4 increased when the cells were collected from follicles having increased follicular fluid concentration of E_2 compared to granulosa cells collected from follicles that had lower concentrations of E_2(McNatty*et al*., 1979).

Premature induction of ovulation in ewes was associated with luteal insufficiency. These data are relevant to fixed-time insemination protocols in which physiologically immature dominant follicles are induced to ovulate at AI and the subsequent circulating concentrations of $P_4$are lower than in cows in which a larger dominant follicle is induced to ovulate with GnRH (Perry *et al*., 2005). Inadequate luteal function following induced ovulation may be due to a reduced number of follicular cells and (or) inadequate preparation of follicular cells for luteinization and secretion of P_4(Smith *et al*., 2010). Ultrasonically,CL appears as distinctly echogenic areas within the ovarian stroma. Many CL appear as a solid tissue mass but may also contain fluid-filled cavities (Fricke and Lamb, 2002). Based on ultrasonographic examinations indairy heifers, 79% of otherwise normal CL contains cavities ranging from less than 2 mm to greater than 10 mm in diameter at some time during the

oestrous cycle and early pregnancy (Singh *et al*., 1997). The appearance of the CL may be used to estimate the stage of the bovine oestrouscycle. Ultrasonographic attributes of CL including cross-sectional diameter, luteal area and echogenicity have been correlated to luteal structure and function (Singh *et al*., 1997).

The use of luteal characteristics, to improve accuracy of pregnancy diagnosis, has been reported in dairy heifers (Kastelicand Ginther,1991). Luteal size and echogenic characteristics assessed at specific times post-breeding, may prove useful as a method to improve accuracy of early pregnancy diagnosis in dairy cattle. Although ultrasound is more accurate than rectal palpation for assessing ovarian follicles, it is difficult to distinguish between developing corpora lutea and older regressing corpora lutea using either technique (Pieterse *et al.*, 1990).

2.16 Imaging the Uterus

Of all the ultrasound applications utilized in the cattle industry, scanning of the uterus for infection and pregnancy are the most commonly practiced (Fricke and Lamb, 2002). In a non-pregnant cycling cow, the uterine tissue appears asa somewhat echogenic structure. Because the uterus is comprised of soft tissues, it absorbs a portion of the ultrasound waves and reflects others. In this way the uterus can be identified as a gray structure on the screen. A cross-sectional view of the uterus is displayed as a “rosette” and is easily distinguished from other peripheral tissues, whereas the longitudinal section is less recognizable (Fricke and Lamb, 2002). Characteristic changes of the tubular genitalia involve thickness of the uterine body, evidence of increased vascularity, oedema and accumulation of mucus (Pierson and Gnither, 1988). Adams and Singh (2011),reported that the period of pro-oestrus and oestrus (days -4 to -1; day 0= ovulation) is characterised by (i) increasing thickness of the uterine body, (ii) accumulation of luminal fluid first in the uterus followed in succession by fluid in

the cervix and vagina, and (iii) minimal curl of the uterine horns. Conversely, dioestrus (days 3-16) is characterised by minimal thickness, minimal luminal fluid and maximal curl to the uterine horns. Heterogenous endometrial echotexture (interspread areas of dark and bright gray) is reflective of uterine oedema and associated with impending oestrus and ovulation (Adams and Singh, 2011).

2.17 Ultrasonography and Embryonic Loss

The use of ultrasonography to diagnose pregnancy is relatively simple, but does require some expertise to avoid incorrect diagnoses. When the uterus is located cranial to the pelvic inlet, a false negative diagnosis is more likely to be given than when the uterus is within the pelvic cavity (Szenci *et al*., 1995). Since diagnosis of pregnancy can be made earlier using ultrasonography than by palpation, it is possible that more pregnancy losses (early embryonic death) may be detected. However, the technique *per se* does not appear to cause embryonic death (Ball and Logue, 1994; Baxter and Ward, 1997). Furthermore, the incidence of pregnancy loss (cows that aborted or returned to oestrus before term) after diagnosis was not different between cows that were 30, 40 or over 50 days pregnant when the diagnosis was performed (Baxter and Ward, 1997). Similar rates of pregnancy loss have been reported among studies involving trans-rectal ultrasonographic diagnosis of pregnancy in cattle: 5.5 % loss after ultrasound diagnosis on days 30 to 40 (Baxter and Ward, 1997); 8.6 % after diagnosis on days 26 to 58 (Szenci *et al*., 1998); and an estimated 10 % after diagnosis on days 25 to 45 (Mee *et al*., 1994). By comparison, in a study of 10 dairy herds involving 4,208 pregnancies (Forar *et al*., 1996), pregnancy loss (return to estrus, observed abortion, follow-up examination) following trans-rectal palpation at day 45 (median; range 38 to 80 days) of pregnancy was 10.8 % (range among herds, 7.6 to 13.0 %). The nature of embryonic loss has also been investigated in cattle, and ultrasonographic characteristics have been described (Kastelic, 1989; Baxter and Ward, 1997). Results of these studies have dispelled the mistaken

notion that uterine “resorption” is the common mode of eliminating the early conceptus. Results indicate that embryo / foetal fluids and tissues are retained until the ensuing oestrus when they are expelled through the cervix, usually unnoticed.

2.18 Diagnostic Limitations of Ultrasonic Imaging

Under most circumstances, practical application of ultrasound for routine reproductive management consists of a single ultrasound examination at a given point in time. It is important to understand that the physiological status of a follicle (e.g., dominant, subordinate, growing,regressing) or corpus luteum cannot be determined during a single ultrasound scan. In addition, ultrasonic imaging aids in distinguishing anatomical attributes of a structure but confers little information regarding physiological or endocrine status. For example, ovarian cysts can be categorized by anatomical attributes such as diameter and presence or absence of luteal tissue; however, no information regarding functionality such as plasma hormone concentrations can be conferred. One exception would be the visualization of foetal heart beat as a diagnostic indicator of a viable foetus (Fricke and Lamb 2002). The diagnostic limitation of ultrasonic imaging becomes important especially when the limitation is exceeded and an incorrect therapy or reproductive intervention is recommended. A thorough understanding of ovarian physiology and the mechanisms by which hormonal programmes succeed or fail is imperative for correct interpretation of ultrasonic imaging information(Fricke and Lamb 2002).

2.19 Gonadotrophin-Releasing HormoneSynchronisation Regimens

Both hormonal and physical methods have been used to eliminate the dominant follicle and initiate a new follicular wave (Rajamahendran*et al*., 2001). Hormonal methods include administration of P_4(Taylor and Rajamahendran,1994; Dare *et al*., 2010) and oestrogens (Bo *et al*.,1994); to induce dominant follicle regression, as well as GnRH and human chorionic

gonadotrophin to ovulate or luteinise the dominant follicle (Rajamahendran*et al.,* 1998). Physical methods include removal by electro-cautery and aspiration of the dominant follicle (Ko *et al*., 1991; Bergfelt, 1994). The most common methods of altering follicular turnover in conjuction with oestrus synchronisation have been the administration of GnRH or its analogues(Pursely*et al*., 1995; Twagiramungu*et al*., 1995) or oestrogen administration in conjunction with progestins (Bo *et al*., 1994). For simplicity, only the major treatments with GnRH will be described here.

2.19.1Select-synch

Select-sync was the first protocol established utilizing GnRHanalogues (Twagiramungu*et al.,* 1991). On day 0 all cows receive an injection of a GnRH analogue. Starting on day 6 after injection and for 6 successive days afterwards, the herd is monitored for oestrus activity, and bred 8 to 12hour after oestrus detection. On day 7; cows that have not been detected in oestrus are given an injection of $PGF_2\alpha$ to induce luteolysis. This is an effective protocol but the need for oestrus detection is still present and pregnancy rates can range from 20.8% (Lemaster *et al.,* 2001);40%(Kojima e*t al.,* 2000) and 53% (Stevenson *et al.,* 2000).

2.19.2 Cosynch

Cosynch was modeled after select-synch; however, the need for oestrus detection was eliminated with a second GnRH injection. On day 0 all cows receive a GnRH analogue; seven days later all cows receive $PGF_2\alpha$ to induce luteolysis. Two days after$PGF_2\alpha$ injection all cows receive a second GnRH injection followed by immediate insemination. Pregnancy rates after cosynch were 40 to 54% (Lamb *et al.,* 2001; Geary *et al.,* 2001 a, b). This system effectively reduces labour costs; however, higher levels of fertility are achievable. The second GnRH injection at breeding may cause premature ovulation and reduce pregnancy rates. Recent reports indicate that insemination at GnRH injection may not be ideal. Dalton *et*

al. (2000) reported AI of superovulated cattle 24h after oestrus increased fertilization rates compared with insemination at 0 or 12h after oestrus.Dalton *et al.* (2001) demonstrated that AI at 12 h post-oestrus optimized fertility of dairy cattle.

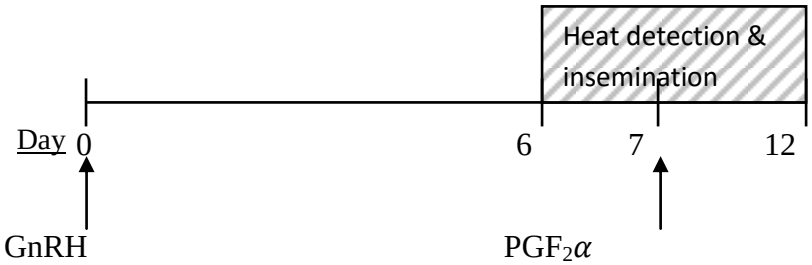


Figure2.3: Select-synch protocol. An Injection of GnRH followed 7 days later with $PGF_2\alpha$

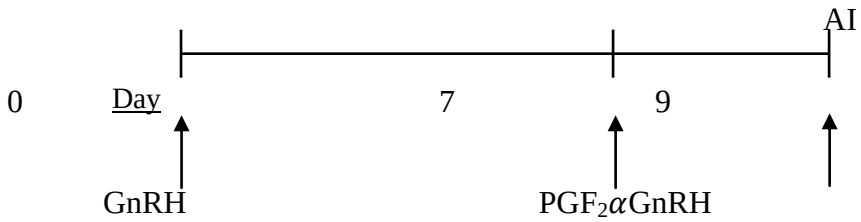


Figure 2.4: Cosynch Protocol. An injection of GnRH followed 7 days later with $PGF_2\alpha$ and insemination at an additional GnRHanalogue injection on day 9.

2.19.3 Ovsynch

The ovsynch protocol is a very popular synchronisation treatment for beef and dairy cattle utilizing GnRHanalogues in combination with $PGF_2\alpha$, since the need for oestrus detection is eliminated. Synchronisation of follicular waves and selection of new large follicle following GnRH at any stage of the oestrous cycle was used as a tool to further develop oestroussynchronisationprogrammes for fixed time AI (Twagiramungu*et al*., 1995).

The random administration of GnRH during the oestrous cycle results in LH and FSH release, causes ovulation or luteinization of large follicles present in the ovary, synchronises the recruitment of a new follicular wave (Thatcher *et al*., 1989; Martinez *et al*., 2000), and equalizes follicle development waves (Twagiramungu*etal*.,1995; Schmitt *et al*., 1996). Subsequent administration of $PGF_2\alpha$ induces the regression of an original or GnRH induced CL, and allows final maturation of the synchronised dominant follicle (Schmitt *et al*., 1996). Furthermore, there is no apparent detrimental effect of GnRH on the responsiveness of GnRH-induced CL or spontaneous CL to prostaglandin (Twagiramungu*et al*., 1995). Hamman *et al*.(2012) found no marked changes in blood parameters of cattle treated with ovsynch protocol.Conception rates were comparable to untreated cattle after this protocol. Pregnancy rates after timed AI with this protocol were similar for beef (Twagiramungu*et al.,* 1995) and dairy cattle (Pursley*et al.,* 1995; Schmitt*et al.,* 1996 and Witibank*et al.,* 1996). Over the 20 years since its first implementation, ovsynch has been modified many times to improve its reproductive outcomes and widen its use. Besides its original use for heat synchronisation, it is also used in many ovarian disorders as a therapeutic method (Nowicki *et al*., 2017).

The weaknesses of the protocol which makes it less effective are failure of the first ovulation or lack of luteolysis. The lower ovulation rate in numerous animals (only 54% of ovulation)

was described by Pursley*et al*. (1995; 1997), showing poorer reproductive results of Ovsynch in heifers. Studies performed by other authors also confirmed poor efficiency of the Ovsynchprotocol in heifers (Stevenson *et al*., 2008; Wiltbank and Pursely, 2014). Pursley*et al*. (1995) compared the percentage of pregnant cows and heifers after using the hormonal programme for ovulation synchronisation. The pregnancy rates in the groups of heifers differed starkly, with 35.1 % in the Ovsynch group and 74.4% when using $PGF_2\alpha$ in the control group. In contrast, the rates in cows were quite similar, 37.8 % – in the Ovsynch group vs 38.9 % in the control group (Pursely *et al*., 1997). These differences were caused by a lower ovulation rate after the first GnRH injection in heifers. This phenomenon was in line with the lower concentration of progesterone in the blood on the day of $PGF_2\alpha$ injection in heifers than in cows. The concentration of this hormone in 86.2% of cows was typical for the luteal phase (>1ng/mL), whereas in the group of heifers only 59.5% of animals reached this level (Stevenson *et al*., 2008). This fact suggests that in heifers reproductive performance could be better using another protocol, such as double Ovsynch (Stevenson *et al*., 2008). The second phenomenon that can reduce the efficiency of Ovsynch is non-occurrence of luteolysis after $PGF_2\alpha$ injection (Carvalho *et al*., 2015). As a consequence, the development of the second follicular wave will be suppressed, which makes the synchronisation of ovulation and timed AI (TAI) impossible. Many studies stress the detrimental effect of this phenomenon on fertility. A second $PGF_2\alpha$ injection 24 h later has been proposed to overcome this problem and to improve the fertility in Ovsynch-synchronised cows (Brusveen *et al*., 2009).

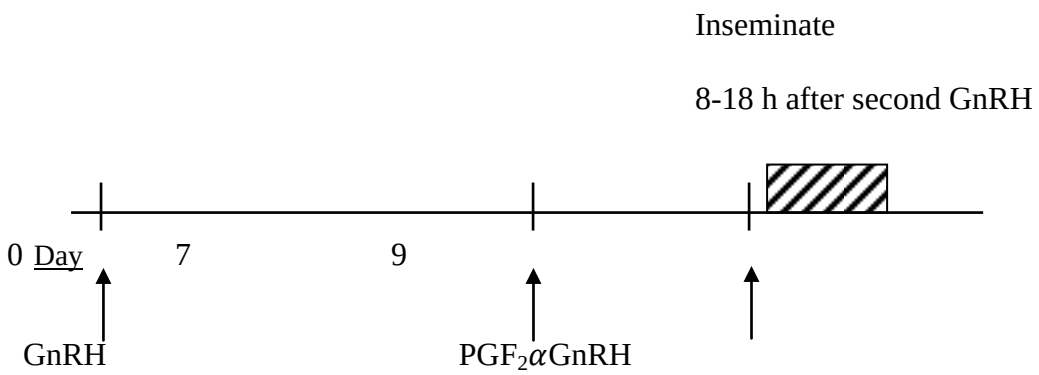


Figure2.5: Ovsynch Protocol: cattle are injected with a GnRHanalogue on day 0 followed 7 days later with $PGF_2\alpha$ treatment. 2 days post $PGF_2\alpha$ treatment cattle receive a secondGnRHanalogue injection followed by insemination 8 to 18hours later without oestrus detection.

2.19.4 Other indications for ovsynch

2.19.4.1*Silent heat*

Silent heat or lack of behavioural oestrus symptoms when genital organs undergo cyclical changes are an increasing problem on many dairy farms (Nowicki *et al*., 2017; Geofery, 2019). Unlike several decades ago when the prevalence of this phenomenon was 5 %, over 10 % to 40 % of dairy herds are currently being affected (Nowicki *et al.,* 2017). The reason for silent heat may be due to insufficient production of progesterone, heat or wet stress and iodine deficiency (Geofery, 2019). Progesterone acts along with oestrogen to manifest itself in visual signs of heat in a cow or heifer. In heifers, the oestrus associated with first ovulation is usually silent due to insufficient production of progesterone. However, during second ovulation, progesterone from regressing corpus luteum acts synergistically with oestrogen to produce oestrus symptoms (Geofery, 2019). Thus, silent heat can be prevented by ensuring proper feeding and mineral supplementation of dairy cows including adequate quantity and good quality roughage, *adlibitum* mineral supplementation, timely and regular heat detection. For treatments, the use of hormonal protocols that allow precisely timed insemination of cows at the same optimum moment as timed artificial insemination without heat detection is a reasonable solution (Nowicki *et al.*, 2017; Geofery, 2019).

2.19.4.2 *Heat stress*

Environmental heat stress is an important nutritional and reproductive concern for cattle in the tropical areas of the world (Dash *et al*., 2016). Increased body temperature is related to the impairment of ovarian function, heat expression and embryo development (Samal, 2013). Milk production is also negatively influenced by high temperatures with reported reductions of 40 % to 60 % (Usman *et al*., 2013). The effects of heat stress on livestock can be minimized via adapting suitable scientific strategies comprising physical modifications of the environment, nutritional management and genetic development of breeds that are less

sensitive to heat stress. In addition, infertility due to heat stress can be countered through advanced reproductive technologies involving hormonal treatments, timed artificial insemination and embryo transfer which may enhance the chances for establishing pregnancy in farm animals (Krishnan *et al*., 2017).

2.19.4.3 *Cyst treatment*

Ovarian cyst in dairy cows is defined as enlarged anovulatory follicle-like structures of at least 17 mm that persist for more than 6 days in the absence of corpus luteum and clearly interfering with normal ovarian cyclicity (Jeengar *et al*., 2014). Injection of GnRH causes higher LH secretion from the pituitary gland which leads to luteinization of cystic follicles or promotes other follicles to ovulate. Occasionally, a rupture of the cyst may occur (Garverick, 1997). The next step is the injection of $PGF_2\alpha$ which causes luteolysis of luteal tissues. A second GnRH injection should then stimulate the follicles of the last wave to ovulate thereby conditioning the animal for AI (Nowicki *et al.,* 2017). Administering the same hormones as in the classic ovarian cyst treatment, the Ovsynch protocol can be used for treatment of cysts based on similar hormonal changes (Nowicki *et al.,* 2017).

2.19.5 Ovsynch modifications

To improve insemination and pregnancy rates after ovsynch, some modifications of its basic protocol are currently being tested (Nowicki *et al*., 2017). These modified hormonal protocols are intended to overcome the problems with the development of follicles or corpora lutea that are not responding to GnRH or $PGF_2\alpha$.

2.19.5.1*Pre-synchronisation of ovaries with $PGF_2\alpha$*

The presynch protocol starts with a $PGF_2\alpha$ injection given 12 days before ovsynch (Cordoba and Fricke, 2001). This causes the synchronisation of the cyclic activity of the ovaries. There

is therefore a much higher probability that during the first GnRH injection according to the ovsynch protocol, the follicles of the second wave present on the ovary will be able to develop (Cordoba and Fricke, 2001). Some researchers (El-Zarkouny *et al*., 2004; Navanukraw *et al*., 2004) have suggested that presynchronisation with $PGF_2\alpha$ should include two injections 14 days apart and the ovsynch protocol should begin 11 or 12 days after the second injection. Other research has suggested the option of starting the ovsynch seven days after the second $PGF_2\alpha$ (Dirandeh *et al*., 2015).

2.19.5.2 *Double ovsynch as a pre-synchronisation schedule*

Another modification of the basic ovsynch is double ovsynch which proceeds through two ovsynch protocols seven days apart followed with timed AI after the second protocol (Souza *et al*., 2008; Dirandeh *et al*., 2015). The obtained results showed an even higher pregnancy rate compared to presynch outcomes (Dirandeh *et al*., 2015). The reason for this may be that cows with inactive ovaries after parturition often do not respond to $PGF_2\alpha$ injection during presynch. In contrast the two additional doses of GnRH in double ovsynch stimulate the ovaries to return to activity (Nowicki *et al*., 2017). One interesting observation was that the double ovsynch protocol was much more efficient in heifers than in cows (Souza *et al*., 2008). Presynch with $PGF_2\alpha$ should be preferred in cows because of its better efficiency (Cartmill *et al*., 2001; Roth *et al*., 2001).

2.19.5.3 *Second injection of $PGF_2\alpha$ during ovsynch protocol*

One important reason for the decreased pregnancy rate after ovsynch protocol is lack of luteolysis after the $PGF_2\alpha$ injection (Nowicki *et al*., 2017). The most probable explanation for this phenomenon is the formation of of a young corpus luteum after the first GnRH which is not responsive to $PGF_2\alpha$ (Carvalho *et al*., 2015; Dewey *et al*., 2010). The recommended time for the second $PGF_2\alpha$ injection is 24 h after the first injection without any changes to the time

of second injection or timed AI (Brusveen *et al*., 2009; Carvalho *et al*., 2015). It has been demonstrated that a double $PGF_2\alpha$ injection during ovsynch protocol is able to boost luteolysis (Carvalho *et al*., 2015).

2.19.5.4 *Intravaginal devices for maintenance of corpus luteum*

Intravaginal inserts (PRID- Progesterone releasing intravaginal device or CIDR- Controlled internal drug release) containing progesterone may also be included into the ovsynch protocol. Bisnotto *et al*. (2015) reported a negative influence of PRID on conception rate in ovsynch-synchronised cows, whereby a higher pregnancy rate was noticed in the control group than in the experimental group. On the other hand, El-Zarkouny *et al.* (2004) reported a positive influence of PRID on conception ratein ovsynch synchronised cows. Based on the above references, there is difficulty in clearly defining the role of progesterone supplementation during such an ovsynch protocol. The effect on conception rate is as a result of progesterone concentration which is subject to several variables such as, level of dry matter intake, milk yield and metabolic status (Sangsritavong *et al*., 2002; Vasconcelos *et al*., 2003). Thus, the application n of an intravaginal device and the concentration of progesterone caused by this treatment can be insufficient in many cows because of a very high turnover rate of this hormone (Nowicki *et al*., 2017).

2.19.5.5 *Resynchronisation*

Besides pre-synchronisation, there is also possibility to re-synchronise ovulation using the ovsynch protocol (Bartolome *et al*., 2005). This schedule assumes a consecutive AI if the cows were detected as not pregnant on days 30 - 32 after the first AI. The next ovsynch can be started at that moment, but it is also possible to shorten the time needed for the next AI by injecting the first GnRH dose seven days before the pregnancy check (Dewey *et al*., 2010). This allows the injection of $PGF_2\alpha$ at the time of pregnancy diagnosis and as a consequence

three days later, a cow can be re-inseminated according to the basic ovsynch protocol (Nowicki *et al*., 2017).

2.19.6 Superstimulation and superovulation

Super stimulation is based on the principle that subordinate follicles that would otherwise undergo atresia can be 'rescued' with continued development and ovulation (Mapletoft *et al*., 2002; Fernanda, 2008). The main objective is to prevent atresia of many follicles and ultimately have several ovulate, to maximise the number of fertilized ova and transferable embryos with a high probability of producing pregnancies (Valeria *et al*., 2019). Gonadotrophin treatments provide FSH activity resulting in super stimulation (Mapletoft *et al*., 2002). Historically, equine chorionic gonadotrophin (eCG) was commonly used in super stimulation protocols, but more recently purified porcine pituitary extract (pFSH) is more widely used (Fernanda, 2008). Equine chorionic gonadotrophin has primarily FSH activity in cattle with varying degrees of LH activity. A single injection at the recommended dose of (2500 IU) can remain in circulation for up to 10 days and cause excessive super stimulation resulting in few embryos of low quality (Mapletoft *et al*., 2002). However, the use of pFSH increased rates of ovulation and transferable embryos when compared with eCG treatments (Fernanda, 2008). The extreme variability in the response to stimulatory treatments is one of the major limitations of superstimulation. This challenge affects both the efficiency and profitability of embryo transfer programmes (Valeria *et al*., 2019). The variability in response has been attributed to environmental factors related to reproductive, sanitary or nutritional conditions and the type, source, batch and biological activity of gonadotrophin used (Bo and Mapletoft, 2014).

2.20 Indigenous Cattle Breeds of Nigeria

2.20.1 General

Individual breeds and races of cattle in Nigeria have been characterised, but overviews of thecountry as a whole are rare (Blench, 1999). Standard references such as Epstein (1971) and Mason (1988) constitute useful reviews of the general literature on West Africa but do not cover the specific situation in Nigeria. A specific study of the breeds of cattle recognised by indigenous producers was undertaken as part of the Nigerian National Livestock Resource Survey (NNLRS)in 1990. Using the locally named categories, village survey data using the grid square method was plotted to map the presence or absence of individual breeds. All types of cattle interbreeds were regarded as a single species. However, lower levels of sub-classification remain confused, conflating biological, cultural and linguistic differentiation. Standard texts such as Mason (1988) or FAO (1987) tend to confound different levels of variation; thus Rahaji (=Red Bororo), West African Dwarf shorthorns and Kuri were all regarded as "breeds". The primary distinction was taken to be that between the broad categories of Zebu and Taurine. Within West Africa, there are three categories of Taurines: West African Dwarf Shorthorn (henceforth Muturu), N'dama andKuri. The N'dama, a humplesslonghorn, is not indigenous to Nigeria but was imported from Sene-Gambia during the twentieth century and is now established in some rural areas, albeitcrossbred (Blench, 1999). Although there is a long history of introductions of European breeds,very few have passed into village production and these have not persisted. Moreover, occasionalsuccessful farms using European breeds, though usually based on economically unrealistic management practices, seemed to short-circuit the otherwise slow process of breeding more productive stock. In reality, however, in West-Central Africa such ranches or farms were never sustained on a long-term basis. The European stock that was imported either died as a result

of diseases or was cross-bred with local animals to such an extent that whatever desirable characteristics it originally possessed disappeared. High-input farms in Kenya and SouthernAfrica, supplying urban markets with relatively developed infrastructure may be said to constitute a partial exception. However, in terms of the continent, imported breeds have largely been a failure. This is particularly the case in West Africa, where the traditional sector continues to supply almost all of the meat requirements in the region (RIM, 1992).

2.20.2 Cattle and their distribution in Nigeria

Nigeria had a mean (i.e. averaged between wet and dry seasons) cattle population of some 13.9million in 1990, of which 11.5 million were kept in pastoral systems and 2.4 million in villages (Blench, 1999). These were predominantly Zebu, but included 115,000 Muturu, some Keteku, N'dama and Kuri. Country-wide, the mean density of cattle is approximately 15/km^2, or 6.6 hectare/head. Cattle numbers increase steadily with declining rainfall, so that much of the south has low cattle densities while most of the population is in the north. There are some seasonal changes in the relative proportions of cattle in the various ecozones. Approximately 45% of the national herd stays within the sub-humid zone throughout the year, with almost all of the remainder in the semi-arid or arid zones. In both seasons, there are several hundred thousand cattle in and around Lake Chad (Blench, 1999). The great majority of the cattle in Nigeria are owned by pastoralists, and even those owned by settled farmers are often managed by pastoralists for part of the year. These explain the considerable intra- and inter-annual fluctuations in numbers and distribution. Herders respond to the various constraints on production such as disease, pasture, political developments and of recent, cattle rustling and banditry extremely rapidly; moving their stock and largely ignoring international borders, control posts and veterinary regulations. Although there is a variety of pastoral groups, especially in the Northeast, throughout most of Nigeria the pastoralists belong to a single ethnic group, the (FulBe), or Fulani. The FulBes are the best known and most

numerous of all the pastoral groups in West Africa (Blench, 1999). They are also the major suppliers of cattle to settled farmers. As a consequence, and to a large extent, their breeding strategies and choice of livestock breeds define the situation in the country as a whole.

2.20.3 Overview of Zebu types

As shown in Table 2.1, Zebu are divided into six distinct resident breeds as well as animals that areof doubtful breed status or are only seen as trade stock. These breeds are of uneven numerical importance, with three breeds constituting 90% of the Zebu. Zebu, in turn represent the great majority of cattle with perhaps 115,000 Muturu and statistically insignificant numbers of otherbreeds. By combining densities noted from aerial survey with distributional data, it was possible to calculate approximate percentages of individual breeds in relation to the National herd. These are shown in Table 2.2

2.20.4 Bunaji

Bunaji or White Fulani cattle is a white, black-eared and medium-horned breed, and is the mostnumerous and widespread of all Nigerian cattle breeds. The NNLRS estimated that they represent some 37% of the national herd. They are found from Lagos to Sokoto, Katsina and Kano States and spread across North Central Nigeria. The only areas from which they are significantly absent are Borno, where *Rahaji* and *Wadara* predominate, and in the south-east, where there are no resident Zebu (Blench, 1999). The movement into the derived savannah and to the edge of the humid zone has largely been of *Bunaji* and pastoralists generally agree that they are superior to all other breeds of Zebu in resisting disease (Blench, 1999).

Table 2.1: Cattle subspecies and breeds in Nigeria

Status	Nigerian Reference Name	Other Names	Fulfulde
Zebu Types			
Resident:	Bunaji	White Fulani	Daneeji, Yakanaaji, Akuji
	Rahaji	Red Bororo, Abore	Bodeeji, WoDaaBe
	Sokoto Gudali		Bokolooji
	Adamawa Gudali		
	Azawak	Tagama Azawa	
	Wadara	Shuwa, Choa	
NotResident:			
	Jali (=Diali)		Jaleeji
	Ambala	Arab, Bahr elGhazal	
Taurines	West African Dwarf	Muturu	
Shorthorn			
	Keteku (Shorthorn x Zebu)	Borgu	Kataku, Ketari, Kaiama
	Keteku (N'dama x Muturu)		
	N'dama (humpless longhorn)		
	Kuri (humpless longhorn)		
Doubtfulbreed status:			
	Daleeji? = Azawa		
	Buzaye? = Azawak x Sokoto Gudali cross		
	Noori? = Colour name and not race.		

Source: Blench, 1999.

Table 2.2: Estimated percentages of different zebu breeds in theNigerian National Cattle Herd

Breed	Proportion of Cattle Population (%)	Calculated Number of Each Breed
Bunaji	37.2	5,118,547
Sokoto Gudali	31.6	4,351,523
Rahaji	22.0	3,029,541
Wadara	6.6	904,731
Adamawa Gudali	1.9	263,019
Azawak	0.7	103,280
Total	100.0	13,770,641

Source: RIM, 1992, II: 436

2.20.5 Sokoto Gudali

There are two quite distinct types of *Gudali* in Nigeria – the *Sokoto Gudali* (or *Bokolo'oji*) and the *Adamawa Gudali.* The *Sokoto Gudali* is a uniform cream, light grey or dun, the dewlap and skin folds are highly developed and the horns almost absent. Although the *Sokoto Gudali* stereo-typically occurs mainly in the North-West of Nigeria, in reality it is now distributed widely throughout the country. The Nigerian National Livestock Resources Survey estimated that they represent some 32 % of the National herd (Resource inventory and management, 1992).

2.20.6 Rahaji

The *Rahaji* is one of the largest zebu breeds and is distinguished by its deep burgundy-colouredcoat, pendulous ears and long, thick horns. It is the third most numerous breeds of cattle in Nigeria, some 22 % of the National herd. The *Rahaji* is adapted to arid and semi-arid regions and rarely goes further south than Kaduna in the wet season, except for the isolated population on theMambila Plateau in the South-east. *FulBe* pastoralists consider the *Rahaji* an extremely prestigious breed and many herds of 'white'cattle includes a few *Rahaji* for crossbreeding. Nonetheless, it tolerates neither humidity-related diseases nor poor nutrition. A lot of herdsmen have been obliged to exchange their stock for *Bunaji* as they moved south into the North Central Nigeriabecause of high mortality among the 'red' animals.

2.20.7 Wadara

The *Wadara* cattle are medium-sized, lightly built cattle, and are usually dark red, black, pied or brown.They are shorthorned and have a small erect hump, representing some 6.6% of the National herd. *Wadara* cattle are the 'indigenous' cattle of Borno and are referred to by the Koyam and related pastoralists as 'our' cattle. They are frequently called '*Shuwa*' in the literature; after the *Shuwa* Arabs who also herd them (Blench, 1999). A related breed with a

white coat, the *Ambala*, is often traded into Nigeria from Chad. *Wadara* breed has a high propensity for milk than any of the other zebu breeds in Nigeria (Nuru *et al*., 1981).

2.20.8 Adamawa Gudali

The Adamawa *Gudali* resembles the *Bunaji* in conformation. It is medium to large sized, withmedium-length horns, and usually pied, or with a white, black, red or brown coat. It has thick, crescent-shaped horns, a pendulous hump, and a short head and muzzle. The pendulous hump is the feature that most reliably distinguishes it from the *Bunaji* (Blench,1999). The NNLRSestimated that Adamawa *Gudali* represent some 2 % of the national herd. At least two local types were originally recognized in Nigeria: The*Banyo*, with *Rahaji* blood and rather large horns, often with a white face and red eye patches, and the *Yola*, which had an admixture of *Muturu*(Gates, 1952). The *Muturu*element has been progressively diluted since the 1950s and the *Yola* breed is no longer recognised as a distinct variety by local herders. The Adamawa *Gudali*, as its name implies, is restricted toAdamawa. Both Kanuri and *FulBe* pastoralists own Adamawa*Gudali* cattle. It is rare for them to have complete herds of Adamawa*Gudali*, and often they are mixed with *Wadara*, *Bunaji* or *Rahaji*. Adamawa*Gudali* is regarded by many farmers as the indigenousrace of the region and they are common in villages. Adamawa*Gudali* are favoured for ploughing, but when they become too large to pull a plough effectively, they are further fattened in the compound and sent to market (Blench,1999).

2.20.9 Azawak

The *Azawak* is said to be native to the *Azawak* valley north-east of Nigeria and is distributed along its north-western border. It is lightly built with medium-length horns. Although *Azawak* in Niger Republic are commonly described as red, the *Azawak* that enter Nigeria are usually a light fawncolour, though they can also be white, brown, pied and black (Blench,1999). The

NNLRS estimated that they represent just 0.7% of the national herd. A small population of *Azawak* cattle exists in Nigeria throughout the year, but the majorities are seasonal trans-humans. *Azawak* are generally only found on the border north and west of Sokoto but are also seen in the north-west of Borgu, and dotted along the frontier from Sokoto to Katsina (Blench,1999).

2.21 Taurines

This covers animals usually described as 'trypanotolerant' (ILCA, 1979) that include; the WestAfrican dwarf shorthorn or *Muturu*and the various types of *Keteku*, Zebu x *Muturu*and Zebu x*N'dama* crosses. No judgement is offered on their powers to resist disease (Blench,1999).

2.21.1 West African dwarf shorthorn

The West African dwarf shorthorn or *Muturu* is small bodied, and blocky in conformation withshort, fine-boned limbs. It has a compact body, no hump, a straight back, and a broad head. Theface is slightly dished, and the horns a.re very short. In South-Central Nigeria, the *Muturu* isgenerally black, or black and white. These animals on the Jos Plateau itself are usually black and white but are distinctly larger than lowland animals. There are more variations in the northern populations; brown, red or tawny animals are seen. Within Nigeria, Muturu cattle have avery disjunct distribution suggesting the gradual retreat of a once more widespread population (Blench,1999). The history, distribution, management and productivity of *Muturu* have recently been reviewed in Blench *et al.* (1998a). *Muturu* are widely dispersed and often stall-fed, and so are less visible than Zebu. As a result, published population figures are little more than informed guesses. Moreover, since Northern *Muturu*is barely known and their trypanotolerance is unmeasured, they have usually been excluded from estimates of 'trypanotolerant' cattle. ILCA's (1979) estimate of 120,000 *Muturu* should be contrasted with that of Ngere (1983) who gave a figure of 60,000 or 0.7% of the National

herd. Akinwumi and Ikpi (1985) surveying five States in the South, give an estimate of 85,000 for *Muturu.* The NNLRS, the first survey to take all the population islands into account, gave an estimate of some 115,000 for 1990 (RIM, 1992).

2.21.2 Keteku (Taurine x Zebu crosses)

Apart from the *Muturu* there are essentially three cattle types that fall under the broad rubric oftrypano-tolerant: (i) crosses of the West African Dwarf Shorthorn (=Muturu with ZebuwithN'dama) ii) Pure-bred N'dama.

The first two are known as *Keteku* within Nigeria, although they are very different in conformation. The *N'dama*, unlike the Zebu and *Muturu*, are not indigenous to Nigeria but has been imported from the Sene-Gambia during the twentieth century. The distribution and productivity of *Keteku* have been reported in more detail by Blench *et al*. (1998b).

The definition of *Keteku* has become more problematic in recent years with an increasing proportion of Zebu blood in '*Keteku'* herds. As *FulBe* pastoral herds push even further south and increasingly inhabit regions previously restricted to trypanotolerant stock, more Zebus are bought in for village herds. For example, the '*Biu*', a Zebu x Savannah M*uturu* cross found near Biu in Southern Borno and described in the literature (Gates, 1952), has effectively become submerged in the local Zebu gene pool. The application of the name *Keteku* to an individual animal mayreflect as much the owner's cultural background as its actual genetic composition. *Ketekus* are significantly less common than previously thought and their distribution quite different. It is unlikely that there are as many as 100,000 of all types (Blench, 1999).

2.21.3 BorguKeteku

The Borgu*Keteku* also known as *Kataku, Ketari, Borgu, Borgawa and Kaiama*, is a trypanotolerant, stabilized *Muturu* x Zebu cross (Gates, 1952). It combines *Muturu*and *Bunaji* features with white, grey and black types predominating, and more occasionally red and brown. The horns are long compared with a *Muturu*, but the hump smaller, and the legs shorter than a*Bunaji*. In Nigeria, *Keteku* in herds are restricted to a narrow band along the Benin Republicborder in the region usually known as 'Borgu'. Further east, *Keteku* are occasionally kept adjacent to villages in Northern Yorubaland. West African dwarf shorthorns were once common through this region and the *Keteku* fills the same niche. *Keteku* are sometimes bought as investment stock in the Ondo area by farmers who value their combination of size and trypanotolerance. *Keteku* were formerly distributed from breeding farms as part of livestock extension programmes and the Government Livestock Centre in Ado-Ekiti still keeps a stock of *Keteku*. In contrast to other West African countries, there has been very little 'new' crossing of Zebu and *Muturu* in Southern Nigeria (Blench, 1999). In some ways, it is surprising that the cross-breeding ofZebu and *Muturu* did not take place all along the line where the two types came into contact. Further east, among the Igbo, farmers tend to assume that the two breeds are incompatible; attempts at crossbreeding would conflict with religious strictures. The continuing genetic separation on the Jos Plateau probably reflects ethnic competition between the owners' as much as animal production considerations (Blench, 1999).

2.21.4 N'dama

The N'dama cattle are native to Sene-Gambia and adjacent parts in the west of West Africa (Starkey, 1984; Blench *et al*. 1998b). They were first brought in to Nigeria from Guinea in 1939 on anexperimental basis, because they were trypano-tolerant and yet were larger than *Muturu*. The *N'dama* has a medium-sized compact body with lyre-shaped black-tipped horns

and no hump. There is a small dewlap in the male, but a fairly large head. Although those imported into Nigeria are generally light brown, there are black and pied animals in Guinea. *N'dama* cattlehave been sold to farmers and pastoralists with a view to improving the resistance of local herds to trypanosomiasis. In most cases, herders cross them with Zebu and there are now few pure *N'dama* outside institutions, although some were recorded in Northern Yorubaland (Blench, 1999).

2.21.5 Kuri

The *kuri* is a large-bodied humpless longhorn whose exact historical origin is unknown (Blench,1993; Meghen*et al.*, 1999). The *kuri* has distinctive, inflated, spongy horns unknown in any other breed and with a mean height of 1.5 m, and weight of some 550 kg; is one of the largest breeds of African cattle. *Kuri* are noted for their extremely variable colours and their ability to thrive in semi-aquatic conditions. The nucleus of the *Kuri* cattle population is within the region of the Lake Chad, and along its Eastern shores. In Nigeria, *Kuri* are found not only on the Lake but on its shores and along the Yobe valley, as far west as *Gashakar*. There is also a restricted export of *kuri* as traction animals to the region North-East of Kano. The breeds along the Komadugu Yobe are crossed with Zebu and are generally referred to as *Jetkoram* in the literature (Blench, 1999).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Study Location

The study was conducted at two locations within Kaduna State due to insufficiency of the required number of animals in a single location. Location "A" was Livestock Section, Samaru College of Agriculture, Division of Agricultural Colleges, Ahmadu Bello University, Zaria, while Location "B" was Rahou Farms, Maraban-Jos, Kaduna. The two locations are situated in the Nothern Guinea Savannah zone between latitudes 11^0 and 12^0N and longitudes 7^0 and 8^0E at an elevation of 650 m above sea level. The average annual maximum and minimum temperatures were 31.0 $\pm$ 3.2and 18.0 $\pm$ 3.7^0C respectively. The average annual rainfall is 1100mm lasting from May to October with a mean relative humidity of 72%. The dry season lasts from November to April with mean daily temperatures ranging from 15^0C – 36^0C and mean relative humidity of 20% - 37% (Osuhor,2007).

3.2 Experimental Animals

Thirty female adults (n = 30), apparently healthy, Bunaji (n = 15) and Friesian x Bunaji (n = 15) breeds of cattle (4 to 6 yrs of age, multiple parities and average body weight: 200 – 250kg) were used in this study. Both locations had facilities consistent with semi-intensive method of production. All cows receiveddaily supplementary feeding (3 kg/cow) of a mixture of 48 % cottonseed cake,13.7% wheat bran,35.3%maize,2% bone meal and 1 %common salt. Water and mineral licks were provided *ad libitun*. All cows were identified with large plastic tags and were dipped once a week in coumaphos (Asuntol® Bayer) an organophosphate insecticide / acaricide at a dilution rate of 1 litre of 16 % coumaphos to 800 litres of water for effective control of ectoparasites. Before the commencement of the study, the cows were screened for blood and helminth parasites and appropriate treatments and vaccinations

against contagious bovine pleuropneumonia, blackleg and haemorrhagic septicemia were conducted.

3.3 Pre-treatment Assessment

All cows were examined ultrasonographically before the start of treatment for identifying the state of ovulation and presence of corpus luteum on the ovary. Only cows with a body condition score of 3.0 and above were included in the study using a scale of 0.0 – 5.0 i.e. from the most emaciated to the fattest (Pullan, 1978). Only cows with corpus luteum and not being treated with any reproductive control products (GnRH, $PGF_2\alpha$, P_4, and E_2) within 20 days before enrolment were included in the experiments.

3.4 Treatment

3.4.1 Experiment 1 (Bunaji cows)

Fifteen Bunaji cows (n = 15) were randomly allocated to three groups of five animals each (n = 5) and treated as follows:

Group 1 (n = 5) All animals received 2ml normal saline at time of hormonal treatments.

Group 2 (n=5) was treated with ovysnch protocol according to Pursely*et al.,* (1995). All animals received 50 μgi.m injection of Lecirelin (Bioveta, a.s., Komenskeho 212/12, Czech Republic) on day0 followed by 500 μg i.m injection of synthetic $PGF_2\alpha$, Cloprostenol (Estrumate, Schring-plough Animal health, equal to 2ml) on day 7 then a second i.m injection of 50μg Lecirelin on day 9.

Group 3 (n=5) All animals received 100μg i.m injection of Lecirelin on day 0 and day 9. On day 7, 500μg i.m injection of synthetic $PGF_2\alpha$ Cloprostenol (Estrumate, Schring-Plough, Animal health, equal to 2 ml).

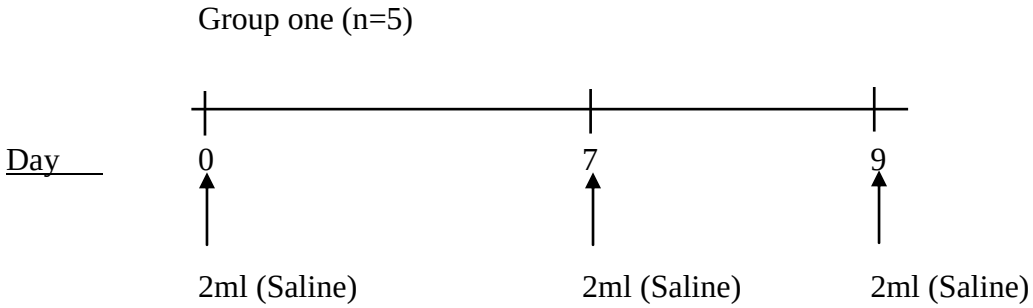


Figure 3.1: Schematic representation of treatment protocol for control cows (2 ml normal saline i.m injection / cow)

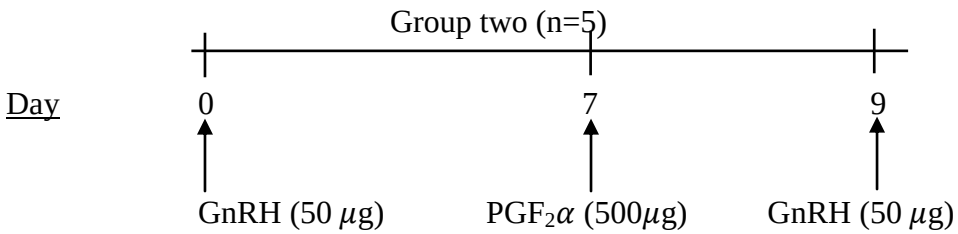


Figure 3.2: Schematic representation of ovsynch treatment protocol of animals in group two

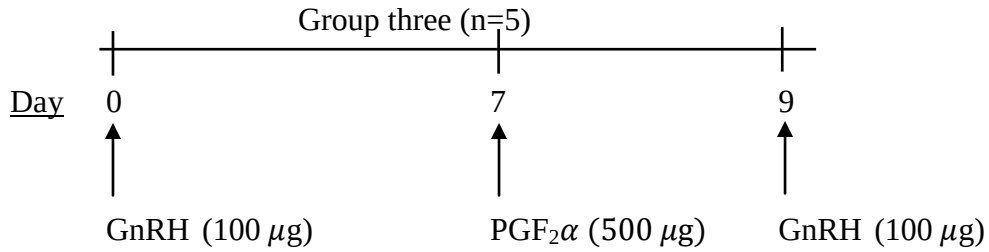


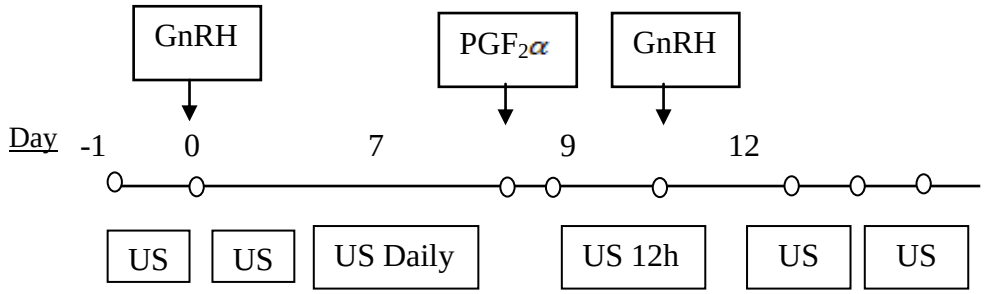
Figure 3.3: Schematic representation of ovsynch treatment protocol of animals in group three.

3.4.2 Experiment 2 (Friesian x Bunaji cows).

Fifteen Friesian x Bunaji cows (n = 15) were randomly allocated to three groups of five animals each (n = 5) and treated as described earlier for Bunaji cows.

3.5 Ultrasonography

By using trans-rectal ultrasonography connected to a 5.0 MHZ transducer (DP-2200 Vet, Mindray Co Ltd, China); ovarian morphology was monitored daily starting from the day before (day – 1), the first (day 0) to the second GnRH injection (day 9). Thereafter, ovarian morphology was monitored twice daily at 12 h interval till occurrence of ovulation. The changes in follicle dynamics after first and secondGnRH treatments were analysed. The observed follicles were classified based on diameter as follows (i) small (<0.5cm), (ii) medium (0.5-1.0 cm) and (iii) large (>1.0cm). The location of the largest ovarian structure and the diameter of the largest follicle were recorded during each examination. A large follicle of 1.0cm or greater in diameter on the day before GnRH administration was considered morphologically to be a dominant follicle. Ovulation was determined by the disappearance of the dominant follicle. The day of the emergence of a new follicular wave was defined by the small follicles group above 0.4 to 0.5cm (Gnither 1993). Desired images were frozen on the screen and measurements were taken.

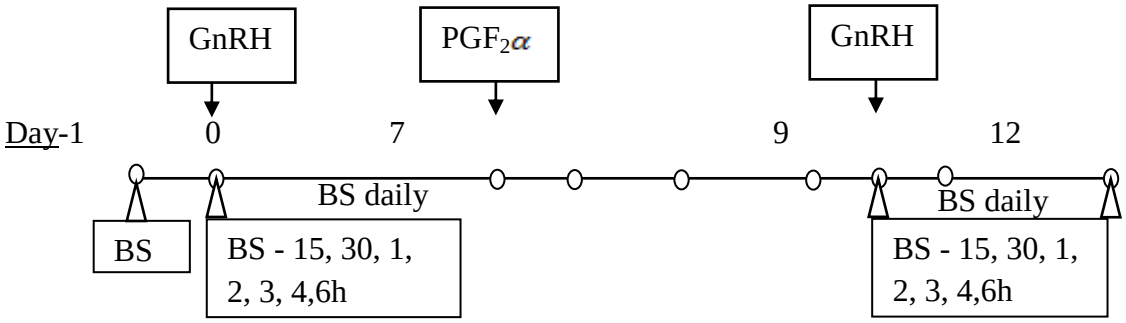


Key: US - Ultrasound examination

Figure 3.4:Schematic representation of ovsynch regimen and ultrasonographic monitoring of ovaries in Bunaji and Friesian x Bunaji cows.

3.6 Blood Sampling

A 5 ml blood sample was collected from the jugular vein of each cow and placed into heparinized vacutainers. Blood samples for progesterone determination were obtained from d 1 after the first GnRH administration up to d 3 after secondGnRH administration. The blood samples in heparinized vacutainers were placed on ice immediately after collection and thereafter centrifuged at 3000g for 10 minutes within 2 hours of collection. The plasma samples were then stored at -20^0C until assayed. Blood samples for FSH and LH determination were obtained just before the GnRH administrations and at 15 and30 min, thereafter at 1 h, 2 h, 3 h, 4 h, and 6 h after the first and second GnRH administrations. The blood samples for the determination of FSH and LH were collected into vacutainers devoid of anticoagulant and kept on ice until centrifugation at 3000g for 10 mins. The serum samples harvested were placed in separate vacutainers and stored at -20^0C until assayed.



Key: B.S - Blood Sampling

Figure 3.5: Diagramaticschemeof blood sample collection.

3.7 Hormonal Assays and Test Procedures

3.7.1 Follicle Stimulating HormoneAssay

Serum FSH was determined by an immunoenzymometric FSH ELISA kit (Accubind® ELISA, Monobind Inc. 100 North Pointe Drive Lake Forest, CA 92630, USA) intended for the quantitative determination of FSH in serum using IEMA/ELISA reader (Spectramax ID5 Hybrid Multi-Mode). The sensitivity of the assay was 0.006 mlU / well. The mean intra-assay coefficients of verification for level 1, level 2 and level 3 pooled control sera were 5.4 %, 3.8 % and 4.0 % respectively. The inter-assay precision coefficients of variation for level 1, level 2 and level 3 pooled control sera were 9.0 %, 8.6 % and 8.4 %, respectively.

3.7.1.1*Follicle stimulating hormoneenzyme linked imminosorbent assaytest procedure*

Before proceeding with the assay, all reagents, serum references calibrators were brought to room temperature (20 – 27 ^{0}C). The assay procedure was then carried out as follows:

i. Fifty microlitres (50 µl) of each serum reference for FSH (0, 5, 10, 25, 50, 100mlU/ml) was pipetted and coated in duplicates into wells A1 through A12.

ii. Fifty microlitres (50 µl) of respective serum samples was pipetted into wells B1 to H12.

iii. One hundred microlitres (100 µl) of FSH enzyme reagent solution was added to all wells and swirled gently for 20 – 30 s to mix.

iv. The Plate was covered and incubated for 60 min at room temperature

v. The contents of the microplate were discarded by decantation and the plate blot-dried with absorbent tissue paper

vi. Three hundred and fifty microlitres (350 µl) of wash buffer solution was added to the plate, decanted and blot-dried. The Process was repeated two (2) additional times for a total of three (3) washes.

vii. One hundred microlitres (100 µl) of working substrate solution was added to all wells

viii. The Plate was covered and incubated at room temperature for 15 min

ix. Fifty microlitres (50 µl) of stop solution was added to each well and gently mixed for 15 – 20 s

x. The absorbance in each well was read at 450 nm wavelength using Spectramax ID5 Hybrid Multi-Mode Microplate reader within 20 mins of adding stop solution

3.7.2 Luteinising HormoneAssay

Serum LH was determined by an immunoenzymometric LH ELISA kit (Accubind® ELISA, Monobind Inc. 100 North Pointe Drive Lake Forest, CA 92630, USA) intended for the quantitative determination of LH in serum using IEMA/ELISA reader (Spectramax ID5 Hybrid Multi-Mode). The sensitivity of the assay was 0.003 mlU/well. The mean intra-assay coefficients of verification for level 1, level 2 and level 3 pooled control sera were 6.8 %, 3.9 % and 3.6 %, respectively. The inter-assay precision coefficients of variation for level 1, level 2 and level 3 pooled control sera were 7.8 %, 10.8 % and 9.6 %, respectively.

3.7.2.1*Luteinising hormoneenzyme linked imminosorbent assay test procedure*

Before proceeding with the assay, all reagents, serum references calibrators were brought to room temperature (20 – 27 ^{0}C). The assay procedure was then carried out as follows.

i. Fifty microlitres (50 µl) of each serum reference for LH (0, 5, 10, 25, 50, 100 mlU/ml) was pipetted and coated in duplicates into wells A1 through A12.

ii. Fifty microlitres (50 µl) of respective serum samples was pipetted into wells B1 to H12.

iii. One hundred microlitres (100 µl) of LH enzyme reagent solution was added to all wells and swirled gently for 20 – 30 s to mix.

iv. The Plate was covered and incubated for 60 min at room temperature

v. The contents of the microplate were discarded by decantation and the plate blot-dried with absorbent tissue paper

vi. Three hundred and fifty microlitres (350 µl) of wash buffer solution was added to the plate, decanted and blot-dried. The Process was repeated two (2) additional times for a total of three (3) washes.

vii. One hundred microlitres (100 µl) of working substrate solution was added to all wells

viii. The Plate was covered and incubated at room temperature for 15 min

ix. Fifty microlitres (50 µl) of stop solution was added to each well and gently mixed for 15 – 20 s

x. The absorbance in each well was read at 450 nm wavelength using Spectramax ID5 Hybrid Multi-Mode Microplate reader within 30 min of adding stop solution

3.7.3 Progesterone assay

The plasma progesterone concentration was determined by a competitive P_4 ELISA kit (Accubind® ELISA, Monobind Inc. 100 North Pointe Drive Lake Forest, CA 92630, USA) intended for a quantitative determination of P_4 concentration in serum or plasma using ELISA microplate reader (Spectramax ID5 Hybrid Multi-Mode). The sensitivity of the assay was 0.105 ng/ml. The mean intra-assay coefficients of verification for the low, normal and high pooled control sera were 15.3 %, 3.6 % and 6.1 % respectively. The inter-assay precision coefficients of variation for the low, normal and high pooled control sera were 8.9 %, 7.5 % and 6.4 % respectively.

3.7.3.1 *Progesterone enzyme linked imminosorbent assaytest procedure*

Before proceeding with the assay, all reagents, serum references calibrators were brought to room temperature (20 – 27 ^{0}C). The assay procedure was then carried out as follows.

i. Twenty five microlitres (25 µl) of each serum reference for progesterone (0.0, 0.3, 2.0, 5.0, 15.0, 30.0 and 60.0 ng/ml) was pipetted and coated in duplicates into wells A1 through B2.

ii. Twenty five microlitres (25 µl) of respective plasma samples was pipetted into wells B3 through H12.

iii. Fifty microlitres (50 µl) of Progesterone Enzyme Reagent was then added to all the wells. Contents were mixed by gently swirling the microplates for 20 s.

iv. Fifty microlitres(50 µl) of Progesterone Biotin Reagent was also pipetted into all the wells. Contents were again mixed by gentle swirling for another 20 s. The wells were covered with a foil and incubated at room temperature for 60 min.

iv. The contents of the microplate were discarded by decantation and the plate blot-dried with absorbent tissue paper

v. Three hundred and fifty microlitres (350 µl) of wash buffer solution was added to the plate, decanted and blot-dried. The process was repeated two (2) additional times for a total of three (3) washes.

vi. One hundred microlitres (100 µl) of Substrate solution was added to all wells. Care was taken to add reagents in the same order to minimize reaction time differences between wells

viii. The Plate was incubated at room temperature for 20 min

ix. Fifty microlitres (50 µl) of stop solution was added to each well and gently mixed for 15 – 20 s

x. Absorbance in each well was read at 450 nm wavelength using Spectramax ID5 Hybrid Multi-Mode Microplate reader within 20 mins of adding stop solution.

3.7.4 Calculation of Results

A dose response curve was used to determine the concentrations of FSH, LH and P_4 in the unknown specimens as follows:

i. The absorbance obtained fron the printout of the Microplate reader was recorded.

ii. The absorbance for each duplicate serum reference was plotted against the corresponding FSH and LHconcentrations in mlU/ml, and P_4concentration in ng/ml. The duplicates of the serum were not averaged before plotting.

iii. The points were connected with a best-fit curve.

iv. The concentrations of the unknown samples of FSH, LH and P_4 were determined by locating the mean absorbance values of the duplicates for each specimen on the vertical axis of the graph and the intersecting point on the curve. The corresponding concentration was read from the horizontal axis of the graph (Figures 3.6; 3.7 and 3.8).

3.8 Analysis

Descriptive statistics was used and the data was presented as the mean ± standard error of mean (SEM). The effect of time after ovulation on various parameters was assessed by one-way ANOVA.Mean values were compared using two way repeated measures ANOVA and Fisher's least significant difference (LSD).All tests were performed using Graphpad Prism® Version 8.0 for windows, Graphpad software, San Diego California, USA, www.graphpad.com. Values of $P < 0.05$ were considered statiscally significant.

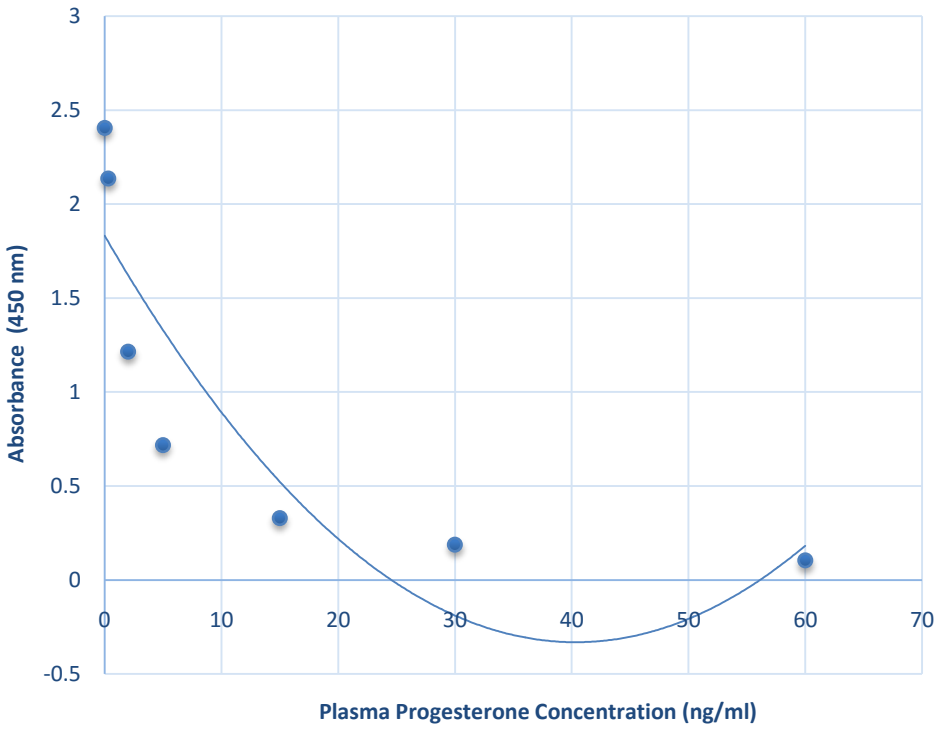


Figure 3.6: Construction of best-fit curve by plotting Absorbance (450nm) vs Concentration for plasma progesterone concentrations.

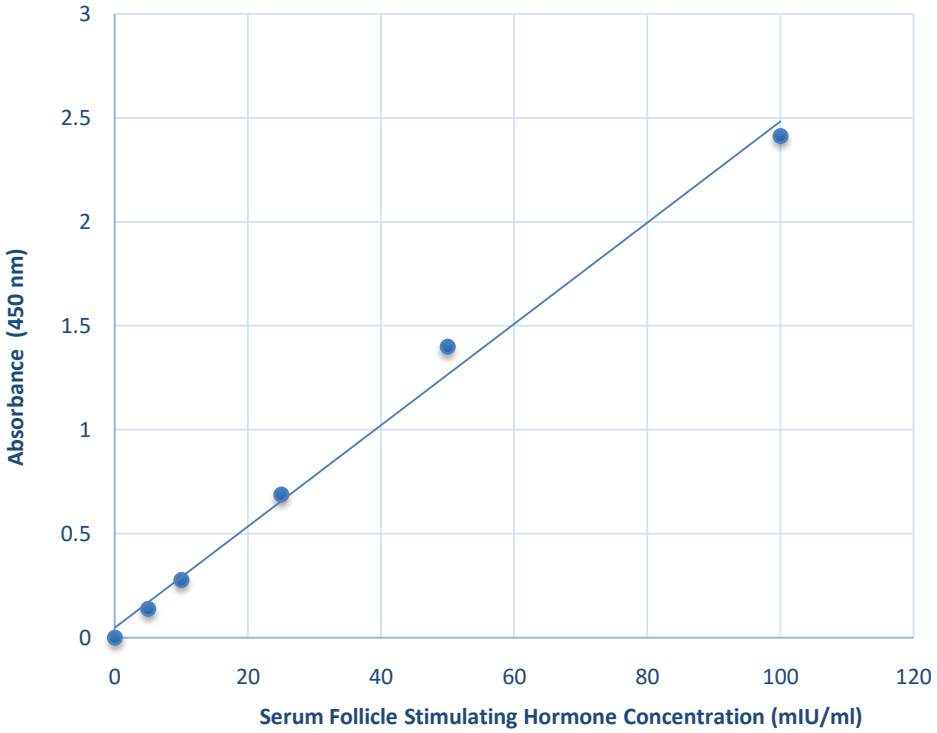


Figure 3.7: Construction of best-fit curve by plotting Absorbance (450nm) vs Concentration for serum Follicle Stimulating Hormone concentrations.

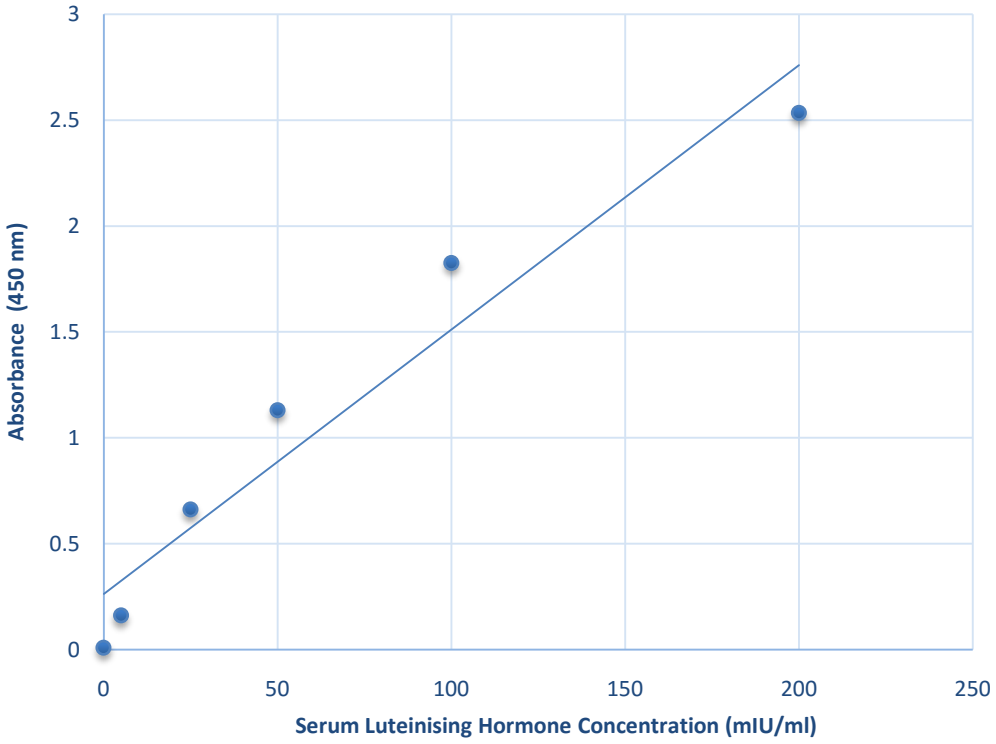


Figure 3.8: Construction of best-fit curve by plotting Absorbance (450nm) vs Concentration for serumLuteinising Hormoneconcentrations.

CHAPTER FOUR

4.0 RESULTS

4.1 Ovarian dynamics

Table 4.1 depicts results of cows that ovulated following first and second GnRH analogue injections and other parameters associated with ovarian follicular dynamics. The cows in the control groups had 0 % and 20 % ovulation rate at the time of first and second GnRH administrations, respectively. The Friesian x Bunaji cow that ovulated from the control group displayed more visible signs of oestrus when compared to the Bunaji cow in the control group and other treated cows. Following the first GnRH administration, ovulation occurred in 40 % and 60 % of Bunaji cows in groups 2 and 3, respectively as compared to 60 % each in groups 2 and 3 of Friesian x Bunaji cows. This was followed by the emergence of a new follicular wave 1 – 4 days after the first GnRH treatment. The percentage of cows that synchronised the emergence of a new ovarian follicular wave was similar between treatments and breeds. The percentage of cows ovulating after the second GnRH treatment was high in Bunaji (60 %) and was not altered by treatments. However, the percentage of Friesian x Bunaji cows ovulating following second GnRH administration was higher in group 3 compared with group 2 (80 % vs 60 %). The sizes of dominant follicles at time of first GnRH analogue injection, sizes of pre-ovulatory follicle at second GnRH analogue injection, peak sizes of pre-ovulatory follicles after second GnRH injection and diameters of largest subordinate follicles at ovulation were not affected by ovsynch dosage or genotype ($P > 0.05$).The mean (± SEM) maximum diameter of the largest ovarian follicle was 14.80 ± 1.39 mm and 14.80 ± 0.37 mm for Bunaji and Friesian x Bunaji cows, respectively. This diameter was not affected by genotype or treatment. However, the sizes of pre-ovulatory follicle at second GnRH analogue injection and rate of pre-ovulatory follicular growth from emergence

to second GnRH injection differed significantly (P > 0.05) among treatment groups (Table 4.2). Representative ultrasonographic images of the ovaries of Bunaji and Friesian x Bunaji cows in response to ovsynch synchronisation protocols are shown in plates 1 – 5.

4.2 Follicle Stimulating Hormone

Concentrations of FSH in serum during a 360 minute period after an injection of saline (control) or GnRH at 50 µg or 100 µg are illustrated in figure 4.2a-f. No significant increase in serum FSH was observed in the control cows during a 6 h sampling period. The concentrations of serum FSH in treated cows irrespective of breeds increased above those in control groups within 15 – 30 minutes and remain elevated up to 300 minutes post GnRH administration on day 0 and day 9. Peak concentrations of FSH in treated groups on day 9 tend to be higher than those of day 0 (Figure 4.2e) Serum FSH concentration reached baseline concentrations at approximately 360 minutes post treatment on day 0 but FSH levels remain elevated in serum 360 minutes after the GnRH administration on day 9 (figures 4.2c and 4.2d). There was no significant difference in the pattern of FSH response to GnRH treatments between breeds.

Table 4.1: Summary of ovarian follicular dynamics as determined by ultrasonography in Bunaji and Friesian x Bunaji cows subjected to ovsynch synchronisation protocol. (n = 5)

	Bunaji			Friesian X Bunaji		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Occurrence of regression / ovulation after 1st GnRH analogueinjection (%)	0	40	60	0	60	60
New wave emergence after 1st GnRHanalogue injection (%)	0	40	60	0	60	60
Day of new follicular wave emergence post GnRH injection (days)	0.00 ± 0.00^{a}	1.40 ± 0.60^{b}	1.60 ± 0.68^{b}	0.00 ± 0.00^{a}	1.60 ± 0.68^{b}	1.40 ± 0.60^{b}
Occurrence of ovulation after 2nd GnRHanalogue injection (%)	20	60	60	20	60	80
Day by which ovulation had occurred post 2nd GnRHanalogue injection (days)	0.40 ± 0.40^{a}	1.60 ± 0.68^{b}	1.40 ± 0.60^{ab}	0.20 ± 0.20^{c}	1.40 ± 0.60^{ab}	2.00 ± 0.55^{b}

Values with same superscript alphabets along the same row are not statistically different at $P < 0.05$.

Group 1. All animals received 2ml normal saline at the time of hormonal treatments.

Group 2. GnRH: 50 μg + $PGF_2\alpha$: 500μg + GnRH: 50 μg

Group 3. GnRH: 100 μg + $PGF_2\alpha$: 500μg + GnRH: 100 μg

Table 4.2: Diameter of the dominant and subordinate follicles in Bunaji and Friesian x Bunaji cows subjected to ovsynch synchronisation protocol. (n = 5)

	Bunaji			Friesian X Bunaji		
	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
Size of dominant follicle at time of 1st GnRHanalogue injection (mm)	8.60 ± 1.07^{a}	10.00 ± 1.30^{a}	11.40 ± 1.63^{a}	8.80 ± 1.02^{a}	9.60 ± 2.64^{a}	9.00 ± 3.69^{a}
Size of pre-ovulatory follicle at 2nd GnRHanalogue injection (mm)	14.20 ± 0.66^{a}	11.40 ± 1.03^{b}	10.80 ± 1.39^{b}	11.80 ± 1.20^{b}	12.00 ± 1.52^{ab}	11.80 ± 1.50^{ab}
Rate of pre-ovulatory follicle growth from emergence to 2nd GnRHanalogue injection (mm / day)	0.86 ± 0.16^{a}	0.71 ± 0.09^{a}	0.83 ± 0.13^{a}	1.43 ± 0.04^{b}	1.13 ± 0.12^{b}	0.99 ± 0.15^{ab}
Peak size of pre -ovulatory follicle after 2nd GnRHanalogue injection (mm)	14.40 ± 0.81^{a}	14.80 ± 1.39^{a}	13.60 ± 1.50^{a}	13.80 ± 0.37^{a}	14.40 ± 0.68^{a}	14.80 ± 0.37^{a}
Diameter of largest subordinate follicle at ovulation (mm)	11.40 ± 0.60^{a}	12.80 ± 1.39^{a}	11.40 ± 1.47^{a}	11.60 ± 0.51^{a}	11.60 ± 0.75^{a}	12.00 ± 0.45^{a}

Values with same superscript alphabets along the same row are not statistically different at $P < 0.05$.

Group 1. All animals received 2ml normal saline at the time of hormonal treatments.

Group 2. GnRH: 50 μg + $PGF_2\alpha$: 500μg + GnRH: 50 μg

Group 3. GnRH: 100 μg + $PGF_2\alpha$: 500μg + GnRH: 100 μg

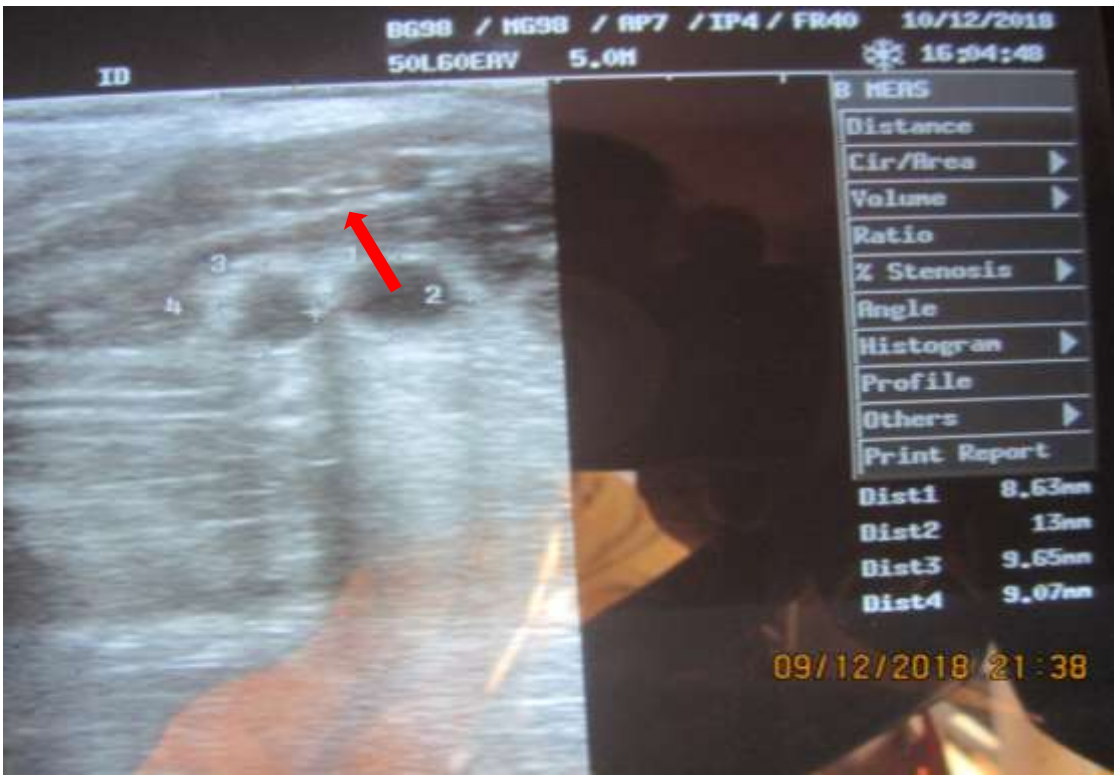


Plate I: Ultrasonographic picture of non echogenic follicles with arrow pointing at small growing ovarian follicles in Bunaji cows at 4 days after first 50 *µ*g GnRH treatment

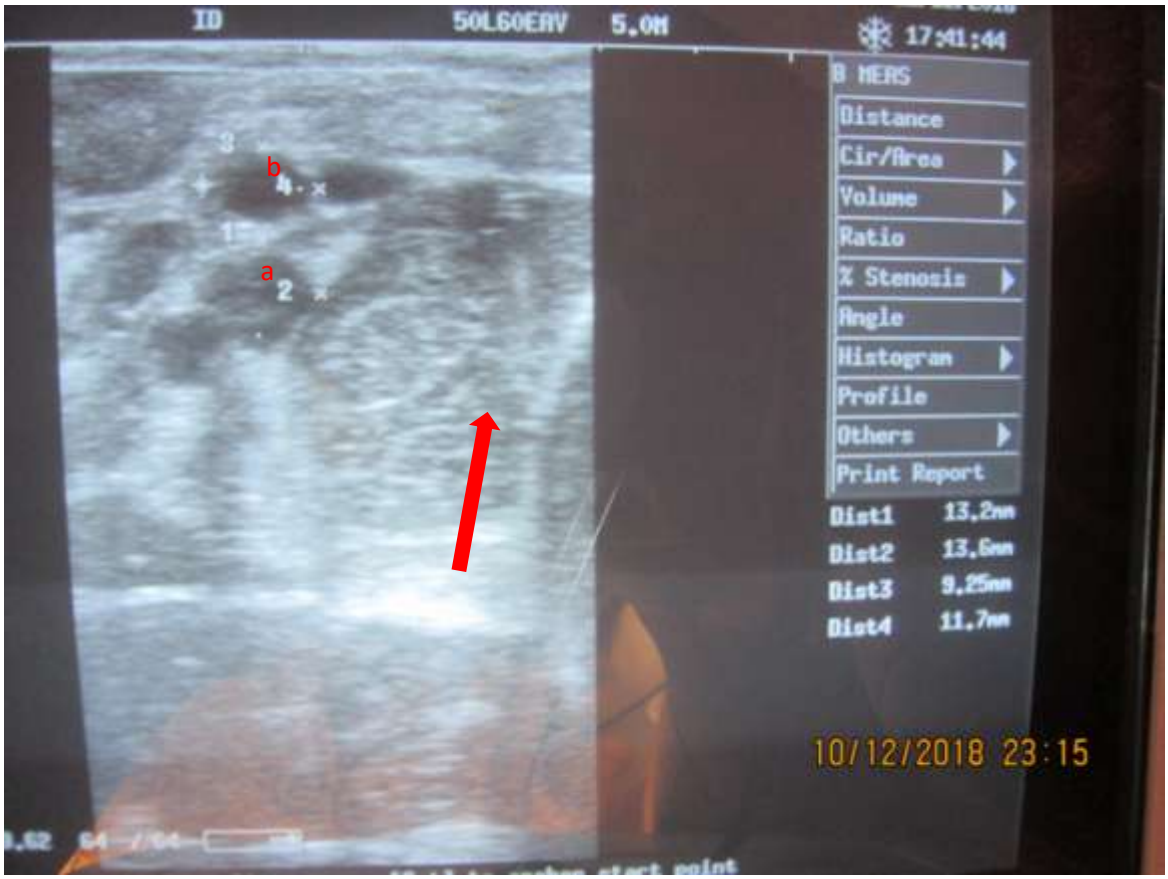


Plate II: Ultrasonographic image of gray, granular, hypo-echogenic matured corpus luteum (arrow) contrasted by anechoeic follicles a and b, large (13.2 x 13.6 mm) and medium (9.25 x 11.7), respectively in Friesian x Bunaji cow 7 days after treatment with first 100 μg GnRH.

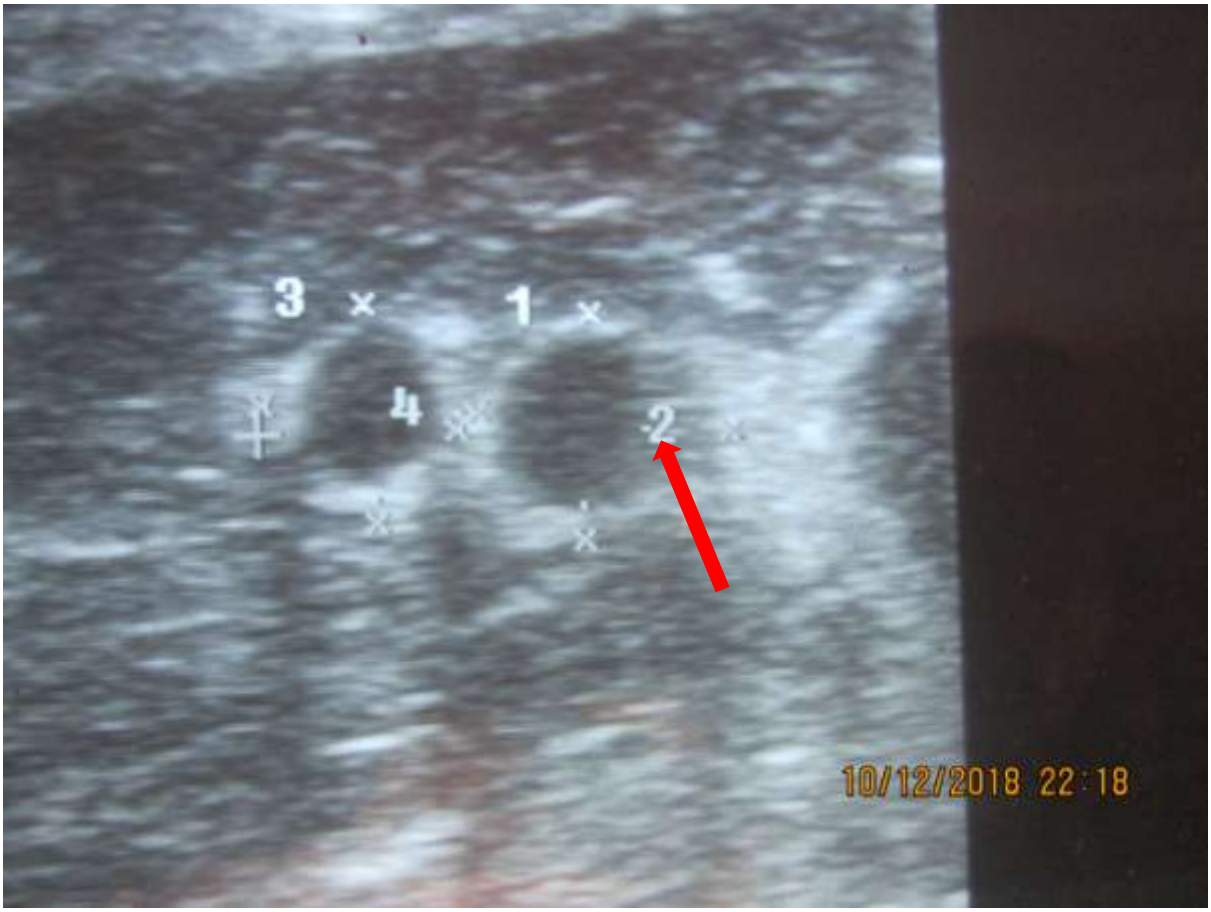


Plate III: Ultrasound image of non-echogenic large pre-ovulatory follicle and subordinate follicle (1x2 and 3x4 distance measurements, respectively) in a Bunaji cow at time of treatment with second 100μg GnRH. Magnification (x5) Infinix Hot 8 mobile phone camera

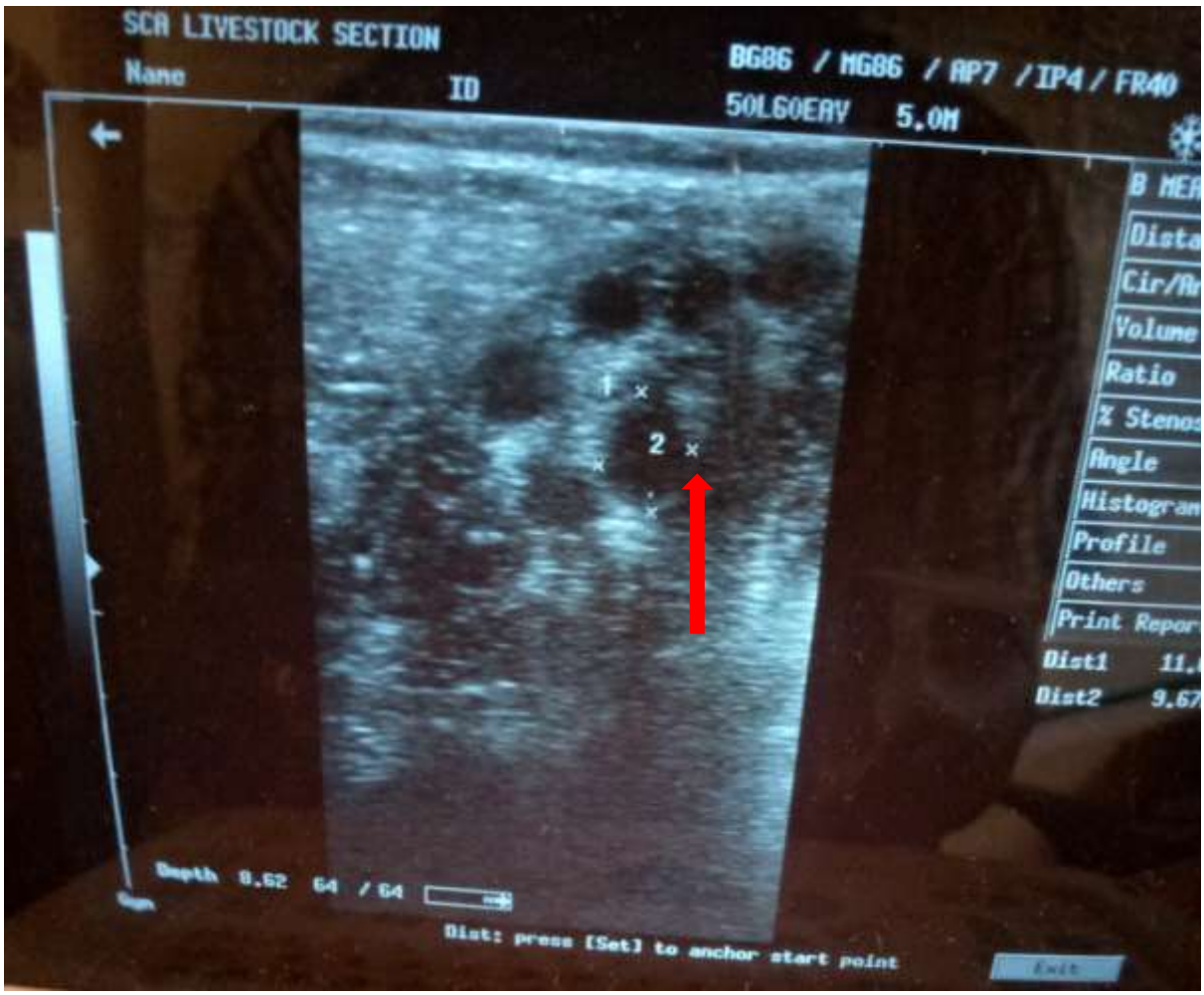


Plate IV: Ultrasonographic picture of a non-echogenic pre-ovulatory follicle with several medium sized follicles in Bunaji cow 48 h after second 100 μg GnRH treatment

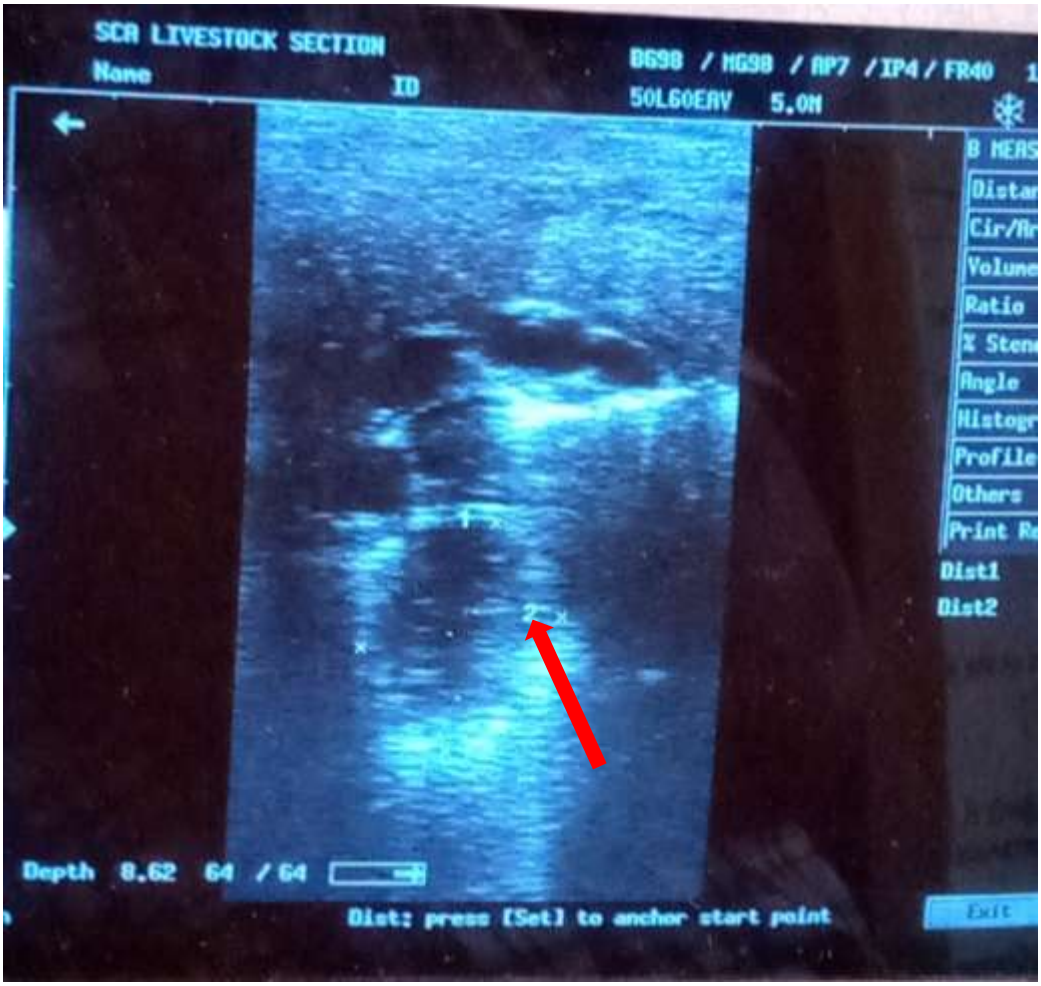


Plate V: Ultrasonic image of a large anechoic ovulatory follicle with slight echogenicity at the borders in a Bunaji cow 48 h after treatment with second 50μgGnRH. Magnification (x5) Infinix Hot 8 mobile phone camera

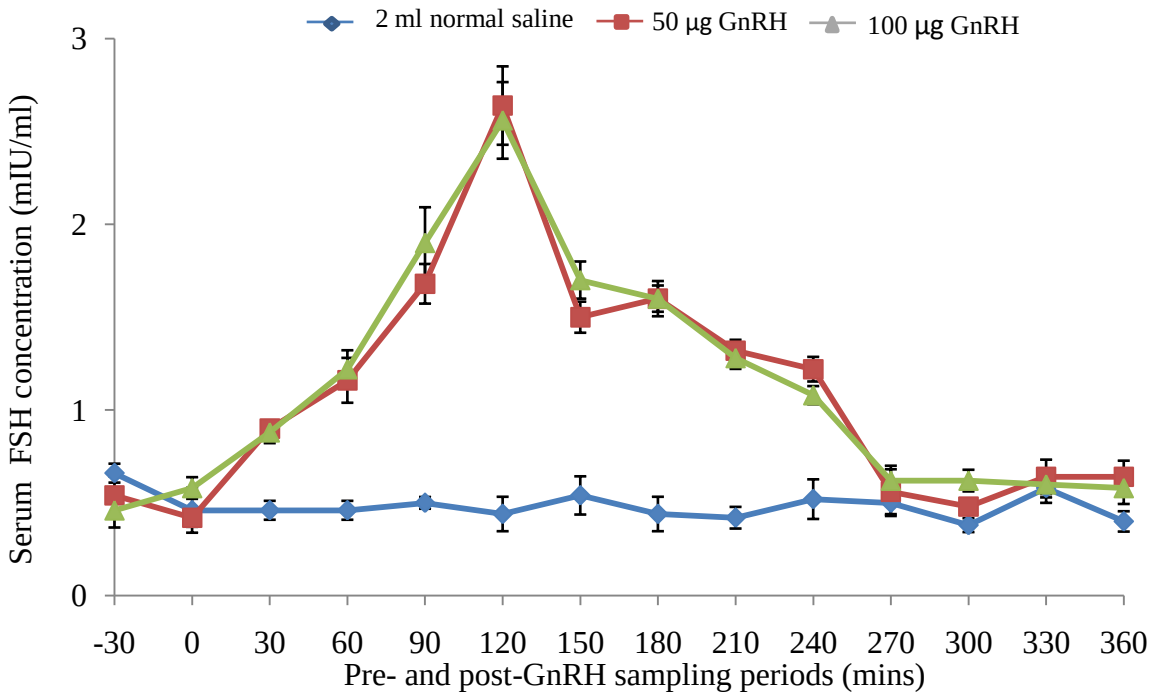


Figure 4.2a Mean (±SEM) serum concentrations of follicle stimulating hormone in Bunaji cows treated with saline, 50 µg and 100 µg GnRH at day 0 of experiment.

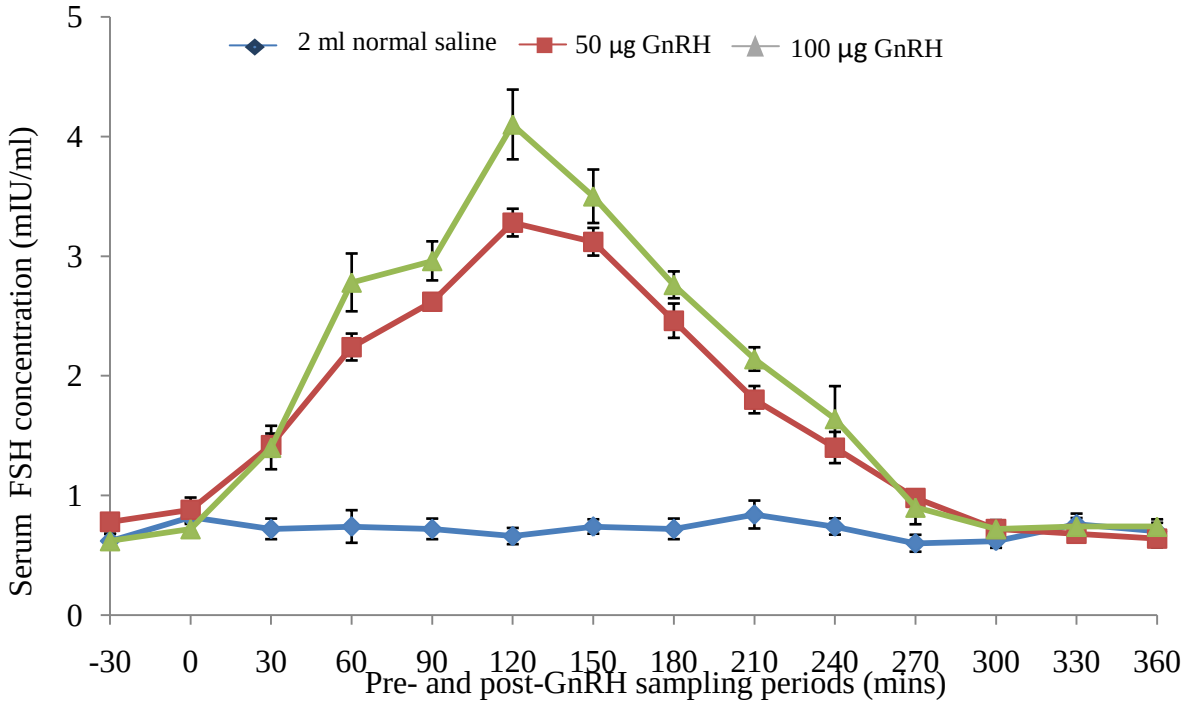


Figure 4.2b: Mean (±SEM) serum concentrations of follicle stimulating hormone in Friesian x Bunaji cows treated with saline, 50 µg and 100 µg GnRH at day 0 of experiment.

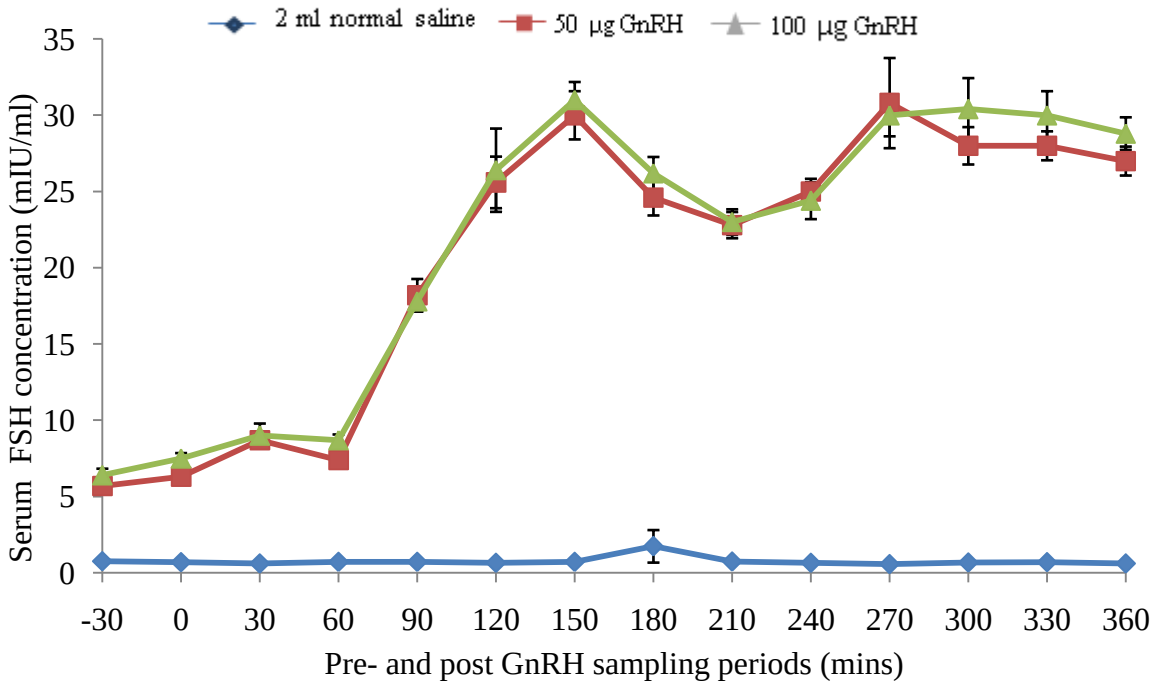


Figure 4.2c Mean (±SEM) serum concentrations of follicle stimulating hormone in Bunaji cows treated with saline, 50 µg and 100 µg GnRH at day 9 of experiment

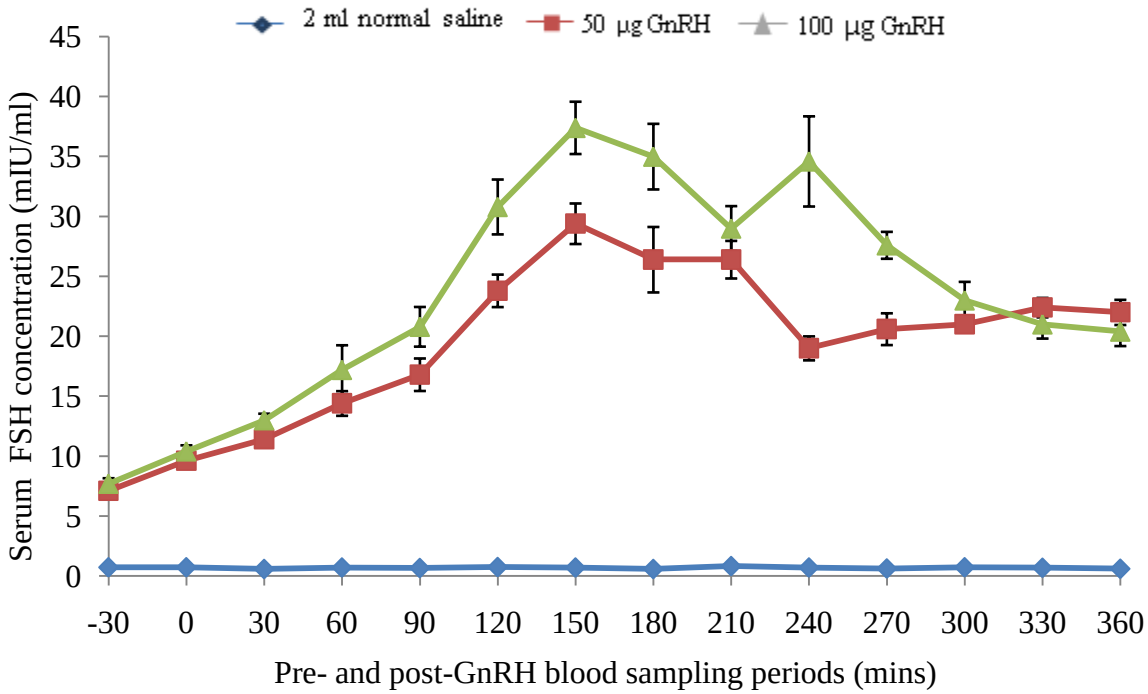


Figure 4.2d: Mean (±SEM) serum concentrations of follicle stimulating hormone in Friesian x Bunaji cows treated with saline, 50 µg and 100 µg GnRH at day 9 of experiment.

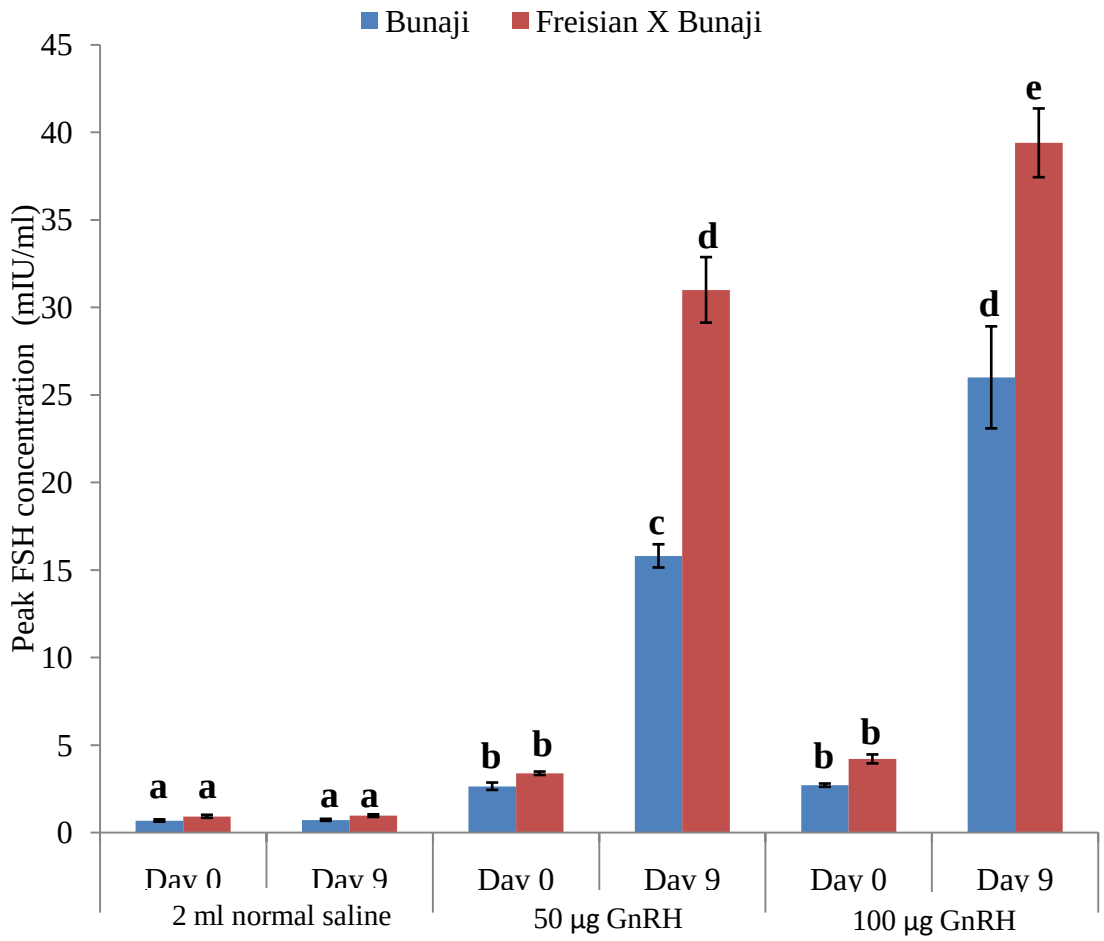


Figure 4.2e: Comparative profiles ofPeak follicle stimulating hormone concentrations of Bunaji and Friesian x Bunaji cows at days 0 and 9 of experiment. Mean values with the different alphabets are statistically different at P < 0.05

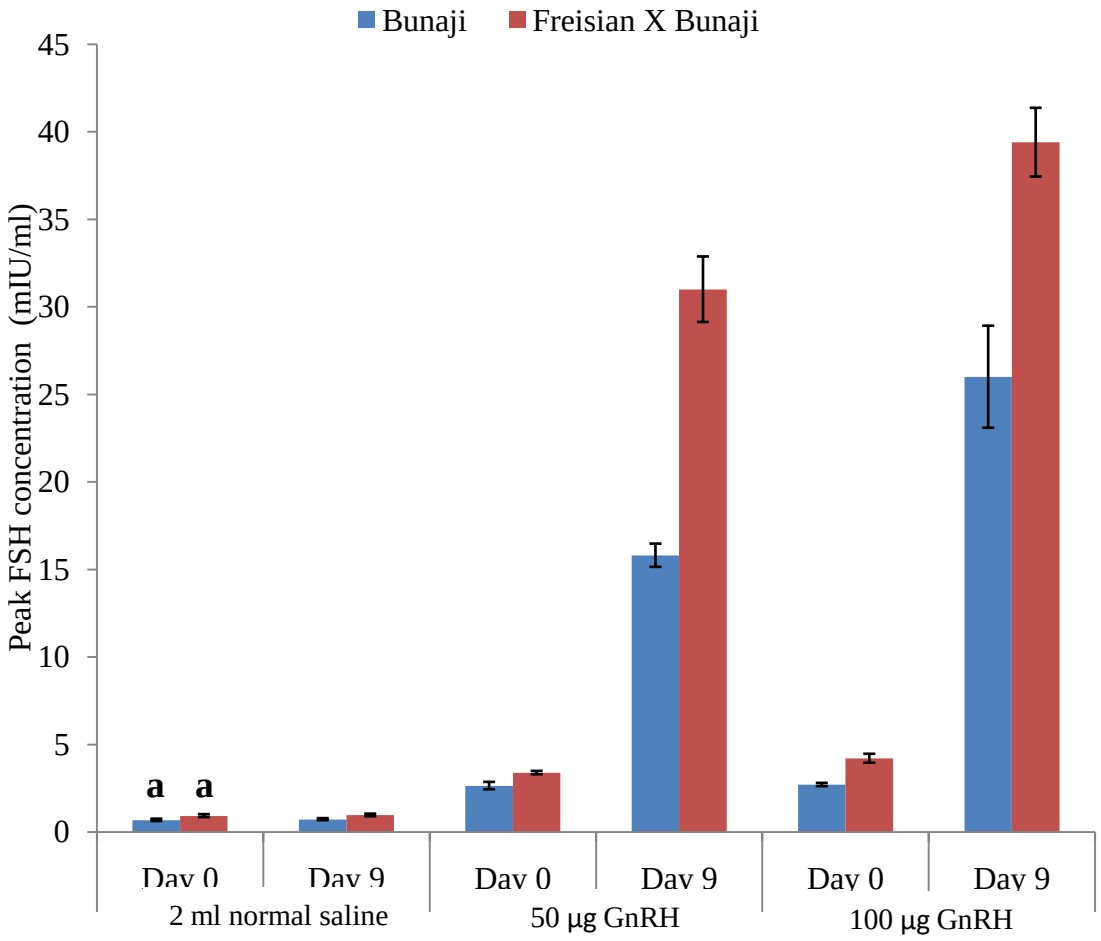


Figure 4.2f:Comparative profiles of time interval to peak follicle stimulating hormone concentrations of Bunaji and Friesian x Bunaji cows at days 0 and 9 of experiment. Mean Values with the different superscript alphabets are statistically different at P < 0.05

4.3 Luteinising hormone

Average serum concentrations of LH during a 360 minute period after injection of saline (control) or GnRH agonist at 50 µg or 100 µg on day 0 and day 9 are illustrated in Figures 4.3a-f. No significant changes in serum LH were observed in Bunaji or Friesian Bunaji cows administered with saline during the 360 minutes sampling period. Serum LH in both Bunaji and Friesian x Bunaji increased above that of the control within 30 minutes and significantly by 60 or 120 minutes after GnRH treatment and decreased to basal levels by 240 minutes. Peak concentrations tend to be higher in cows treated with GnRH 48 hours after $PGF_2\alpha$ than those treated on day 0. The LH concentrations peaked 120 minutesafter GnRH treatments (figures 4.3a-d).

4.4 Plasma Progesterone

A highly significant variation in P_4 levels among different days of the protocol (days 0, 7, and 9) was observed in both breeds with a higher value recorded on day 7. Concentrations of P_4in plasma increased progressively after injection of GnRH and remained elevated until day 7 post injections (Fig 4.4a-bs). The plasma P_4 concentrations declined from day 8, the day after $PGF_2\alpha$ treatment and reached their lowest around day 11. Plasma P_4 continue to rise in the control group after d 8 reaching a peak on d 12. The plasma P_4 also tended to peak at higher concentrations in treated groups than in controls. In all the groups, plasma P_4 on the day of initiating treatment was low. There was no significant difference in the pattern of P_4 response to GnRH treatments among breeds.

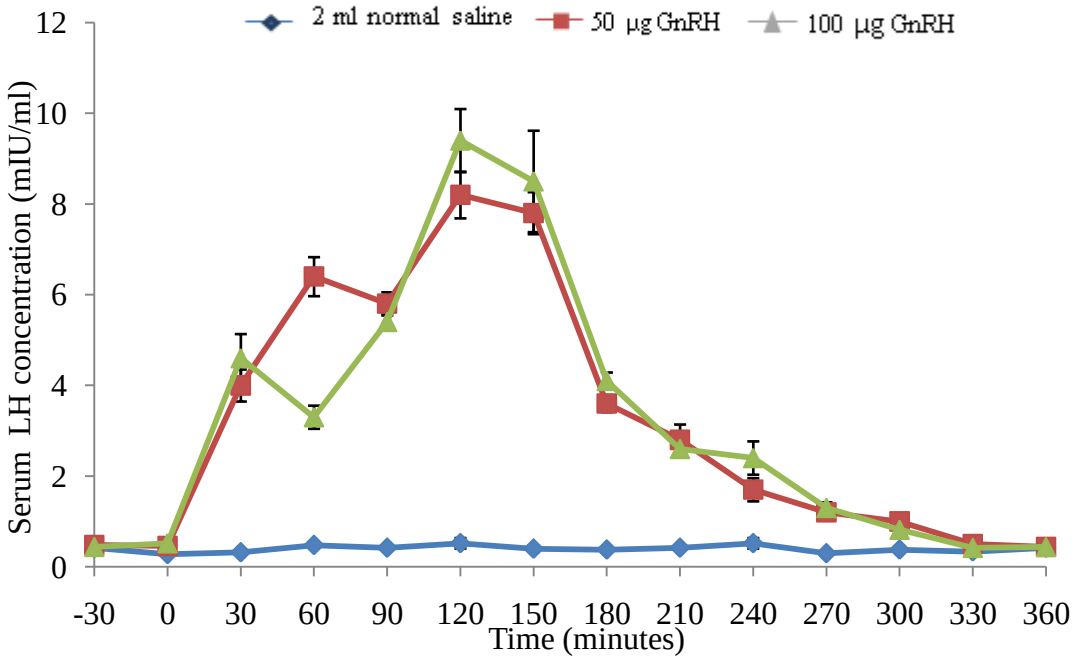


Figure 4.3a: Mean (±SEM) serum concentrations of Luteinising hormone in control and treated Bunaji cows (n = 5 each) administered with saline, 50 µg and 100 µg GnRH at day 0 of experiment.

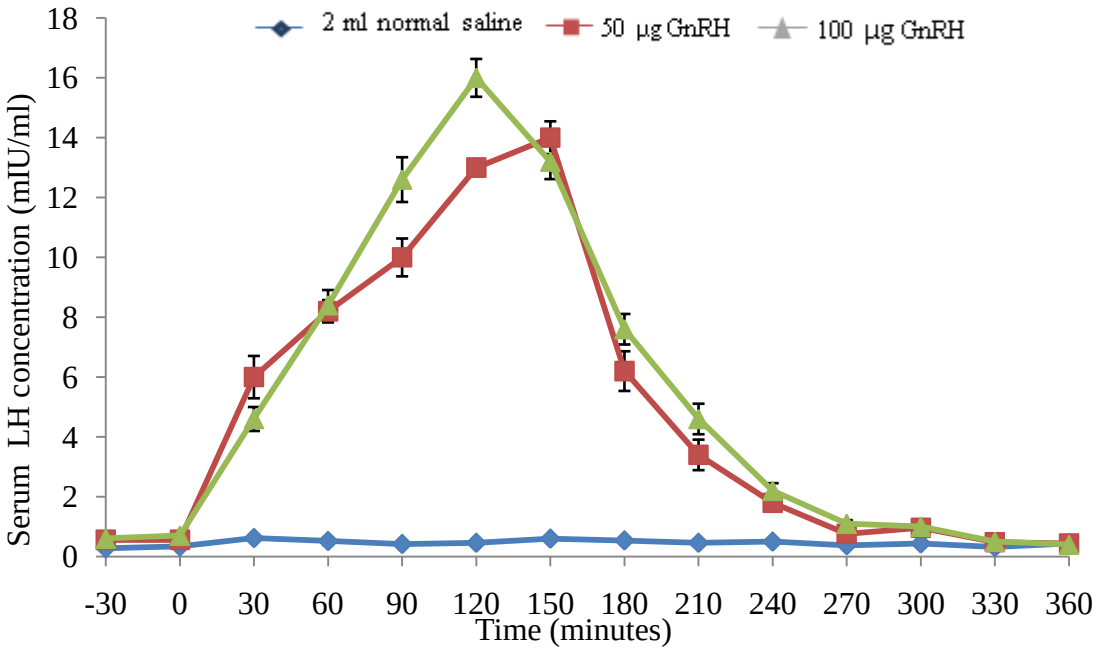


Figure 4.3b: Mean (±SEM) serum concentrations of luteinising hormone in Friesian x Bunaji cows (n = 5 each) administered with saline, 50 µg and 100 µg GnRH agonist at day 0 of experiment.

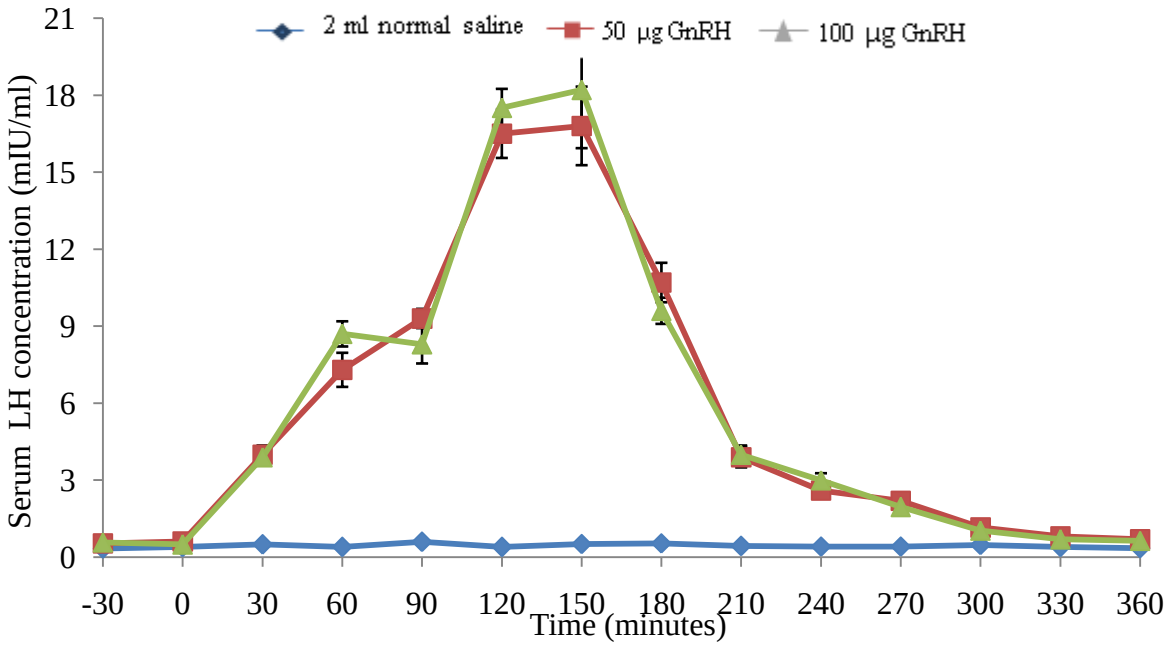


Figure 4.3c: Mean (±SEM) serum concentrations of luteinising hormone in Bunaji cows (n = 5 each) administered with saline, 50 µg and 100 µg GnRH agonist at day 9 of experiment.

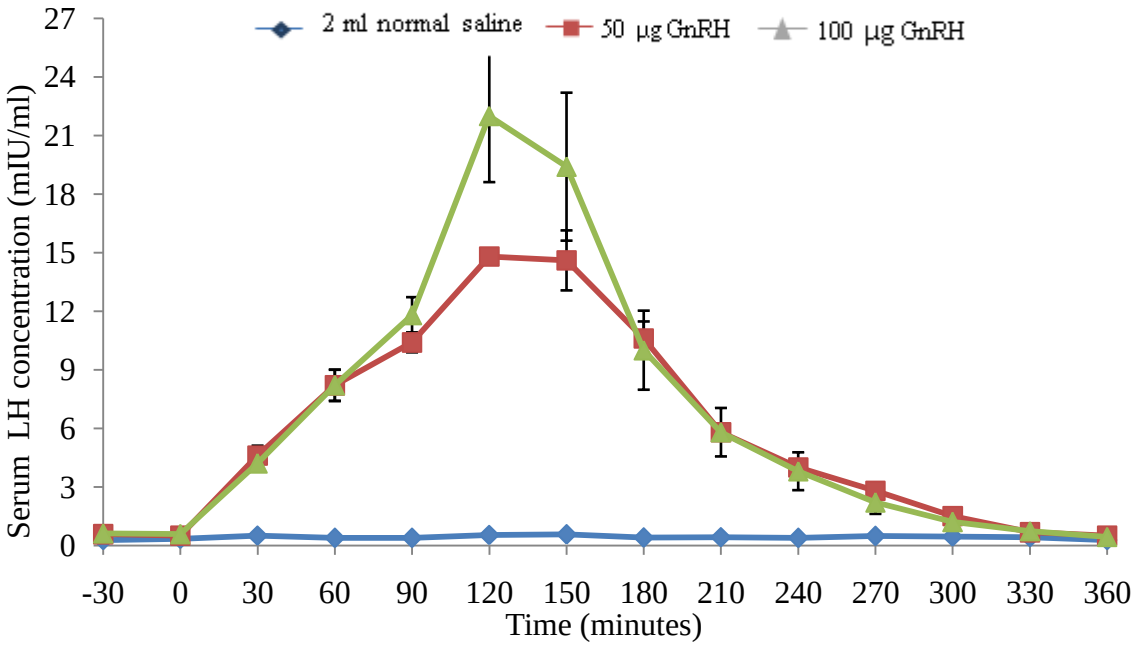


Figure 4.3d: Mean (±SEM) serum concentrations of luteinising hormone in Friesian x Bunaji cows (n = 5 each) administerd with saline, 50 µg and 100 µg GnRH agonist at day 9 of experiment.

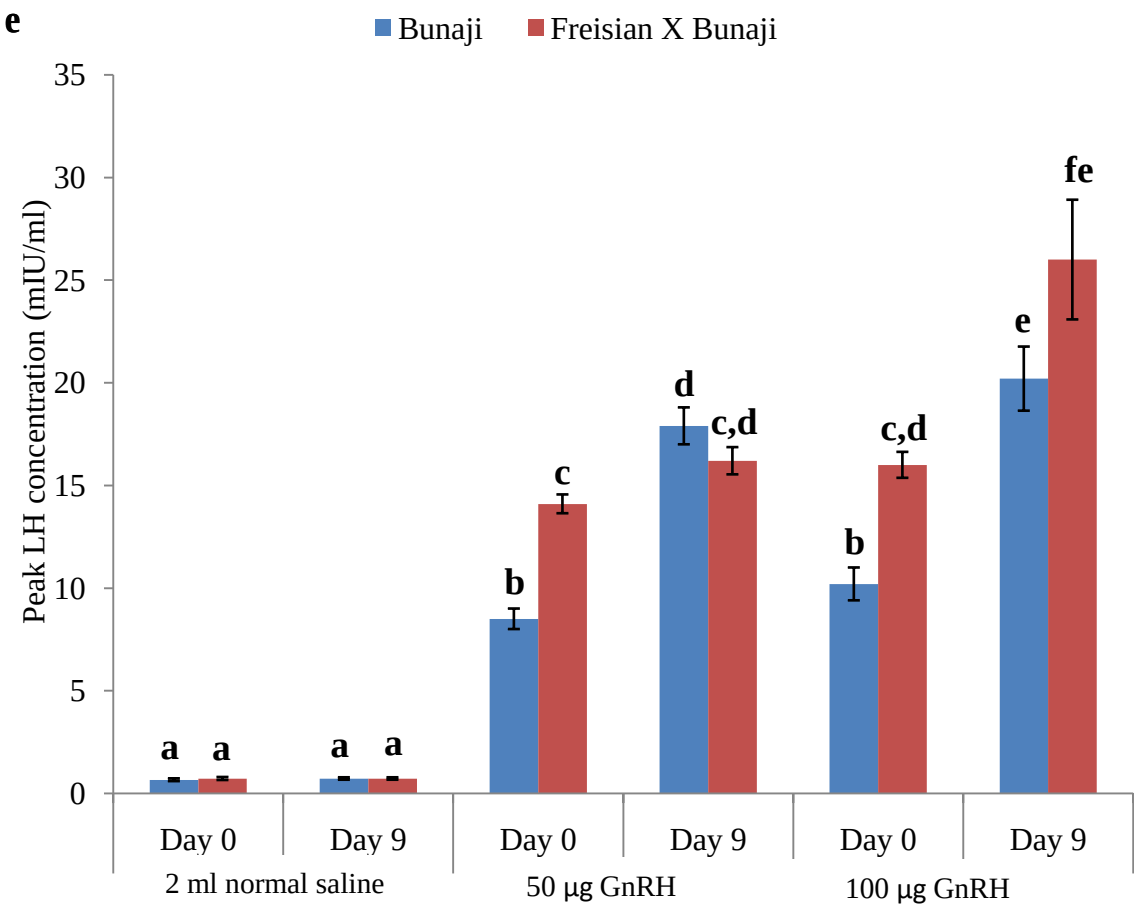


Figure 4.3e: Comparative profiles ofpeak luteinising hormone concentrations of Bunaji and Friesian x Bunaji cows at days 0 and 9 of experiment. Mean values with the different superscript alphabets are statistically different at P < 0.05

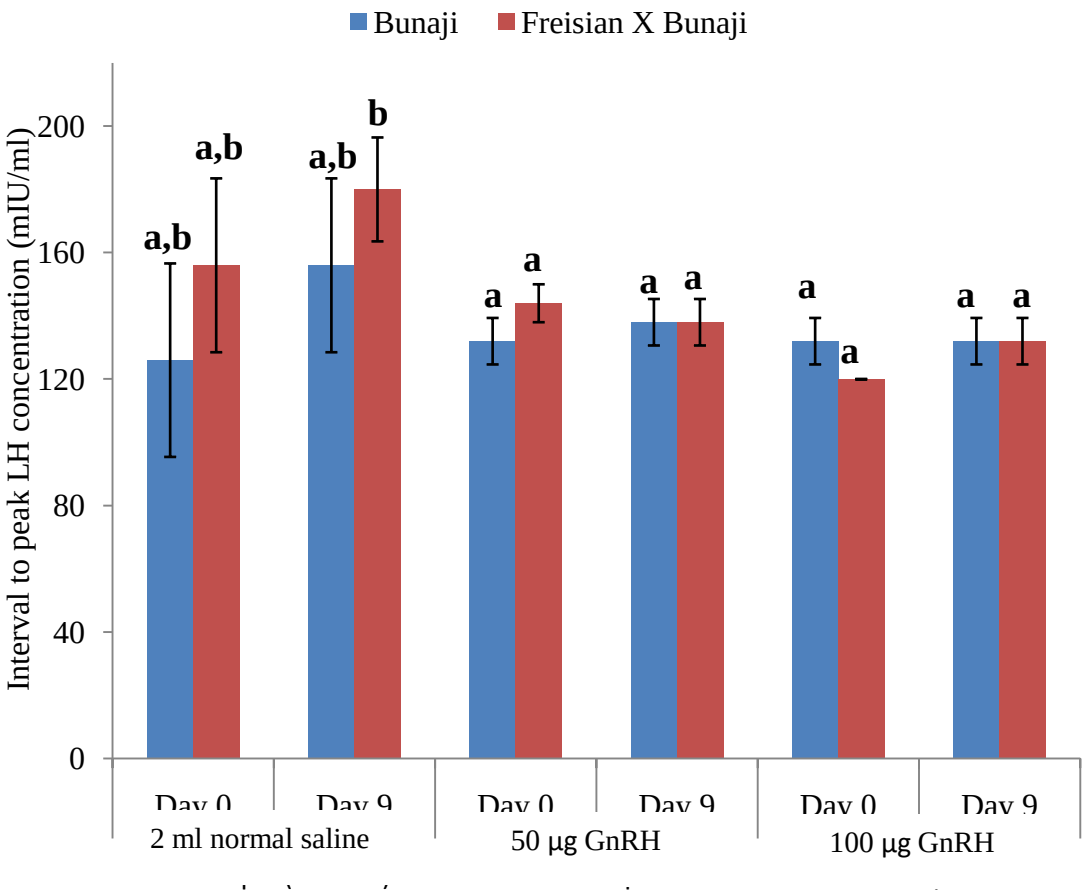


Figure 4.3f: Comparative profiles of time interval to peak luteinising hormone concentrations of Bunaji and Friesian x Bunaji cows at days 0 and 9 of experiment. Mean Values with the different superscript alphabets are statistically different at P < 0.05

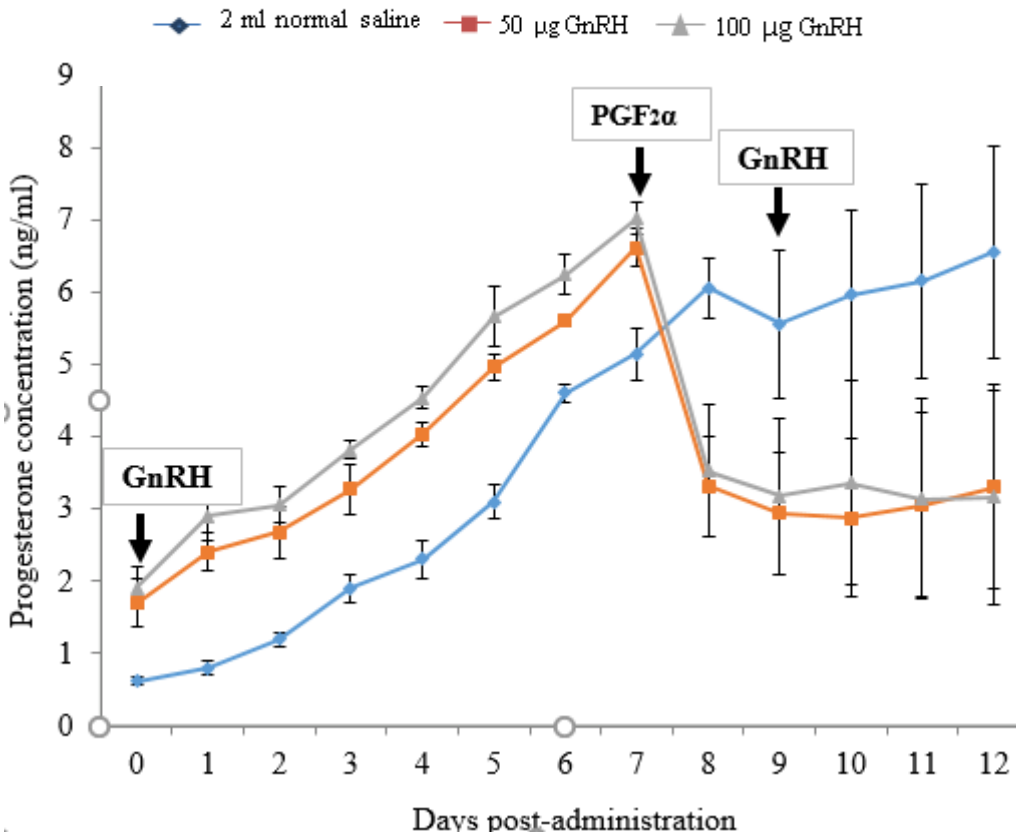


Figure 4.4a Mean (±SEM) plasma concentrations of Progesterone in Bunaji cows (n = 5 each) treated with 2 ml normal saline, 50 µg and 100 µg GnRH agonist.

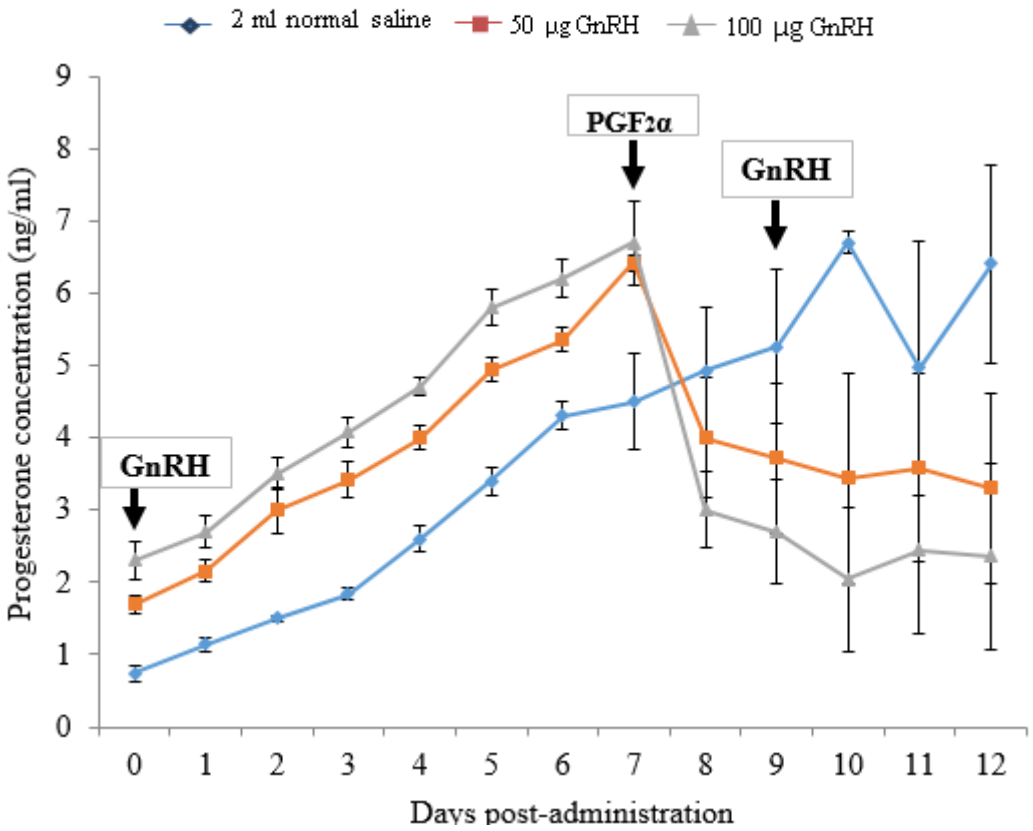


Figure 4.4b: Mean (±SEM) plasma concentrations of Progesterone in Friesian x Bunaji cows (n = 5 each) administered with saline, 50 µg and 100 µg GnRH agonist.

4.5 Cost – Benefit

The cost benefit was based on the number of cows that ovulated out of the treated groups in Bunaji and Friesian x Bunaji cows. Costs included in the analysis were the cost of hormones using the half dose or the full dose ovsynch protocol. For the purposes of this study, no labour cost was included because it is similar for both treatment groups. At the time of this study a dollar was assumed to exchange for 500 naira and the market price of 100 µg GnRH (Lecirelin® Bioveta, a.s., Komenskeho 212/12, Czech Republic) and 500 µg $PGF_2\alpha$ Cloprostenol (Estrumate®, Schring-Plough, Animal health, equal 2 ml) were N 12,000 ($24) and N 1300 ($2.6) respectively. Thus the cost of GnRH treatment in groups ii and iii were N 6, 000 ($12) and N 12, 000 ($24) respectively for both Bunaji and Friesian x Bunaji cows and the cost of GnRH treatments for all cows in groups 2 and 3 were N 30,000 ($60) and N60, 000 ($120) respectively for both Bunaji and Friesian x Bunaji cows (Table 4.3). Therefore, the cost of GnRH treatment per cow in group 2 was N 6, 000 ($ 12) less than for group 3 while the cost of GnRH for all cows in group 2 was N30, 000 ($60) less than for group 3 for both Bunaji and Friesian x Bunaji cows.

Furthermore, the cost of $PGF_2\alpha$ for each cow in groups ii and iii were the same for both Bunaji and Friesian x Bunaji cows (N 1, 300; $2.6), likewise, the cost of $PGF_2\alpha$ for all cows in groups 2 and 3 for both Bunaji and Friesian x Bunaji cows (N 6, 500; $13). Whereas, the total cost of hormones used to synchronise ovulation for each cow in groups ii and iii were N 7, 300 ($14.6) and N 13,000 ($26) respectively for both Bunaji and Friesian x Bunaji cows (Table 4.3), the total cost of hormones used for treatment of all cows in groups 2 and 3 were N 36, 500 ($73) and N 66, 500 ($127) respectively for Bunaji and Friesian x Bunaji cows. The cost per ovulation synchronisation in groups 2 and 3 were N 12, 167 ($24.33) and N 22, 167 ($44.33) respectively in Bunaji cows, whereas in Friesian x Bunaji cows, the cost for each ovulation synchronisation in groups ii and iii were N 12, 167 ($24.33) and N 16, 625

($33.25) respectively. Thus, the estimated cost benefit of using half dosage when compared with full dosage of ovsynch regimen was N 6, 000 ($12) for each cow, N 30, 000 ($60) for all cows and N 10, 000 ($20) for each ovulation in Bunaji cows. In Friesian x Bunaji cows, the estimated cost benefit of using half dosage when compared with full dosage of ovsynch regimen was N 6, 000 ($12) for each cow, N 30, 000 ($60) for all cows and N 4, 458 ($8.92) for each ovulation

Table 4.3: Cost-Benefit of using either 50 µg GnRH or 100 µg GnRH ovsynch Protocol in Bunaji and Friesian x Bunaji cows

Parameters	Bunaji			Friesian X Bunaji		
	Group 2 (50 µg)	Group 3 (100 µg)	Cost benefit	Group 2 (50 µg)	Group 3 (100 µg)	Cost benefit
Number of cows treated	5	5	-	5	5	-
Number of ovulation [a]	3	3		3	4	
Cost of GnRH[b]						
Cost of GnRH for each cow (₦)	6,000 ($12)	12,000 ($24)	6,000 ($12)	6,000 ($12)	12,000 ($24)	6,000 ($12)
Cost of GnRH for all cow (₦)	30,000 ($60)	60,000 ($120)	30,000 ($60)	30,000 ($60)	60,000 ($120)	30,000 ($60)
Cost of $PGF_2\alpha$ [b]						
Cost of $PGF_2\alpha$ for each cow (₦)	1,300 ($2.6)	1,300 ($2.6)	00	1,300 ($2.6)	1,300 ($2.6)	00
Cost of $PGF_2\alpha$ for all cows (₦)	6,500 ($1.3)	6,500 ($1.3)	00	6,500 ($1.3)	6,500 ($1.3)	00
Total Cost of Hormone						
Total Cost for each cow (₦)	7,300 ($14.6)	13,300 ($26.6)	6,000 ($12)	7,300 ($14.6)	13,300 ($26.6)	6,000 ($12)
Total Cost for all cows (₦)	36,500 ($ 73)	66,500 ($ 133)	30,000 ($60)	36,500($ 73)	66,500($ 133)	30,000 ($60)
Total Cost of hormone for each ovulation (₦) [c]	12,167 ($ 24.33)	22,167($ 44.33)	10,000($ 20)	12,167($ 24.33)	16,625($ 33.25)	4,458($ 8.92)

Present currency exchange rate was assumed at $1 = ₦500

(a) For each treatment group, number of cows that ovulated after second GnRH treatment.
(b) Hormone cost was ₦12,000 per 100 µg of GnRH and ₦1,300 per 500 µg of $PGF_2\alpha$
(c) Total cost of hormone per ovulation in each treatment group was calculated as the total cost of hormones for all cows treated from each group divided by the number of cows that ovulated after the second GnRH administrated in each group.

CHAPTER FIVE

5.0 DISCUSSION

One of the major benefits of Ultrasound in animals is that it facilitates the study of reproductive functions without interrupting or distorting that function(s) as can occur when studying the same events via laparatomy or laparascopy (Zacarias *et al*., 2015; Degefa *et al*, 2016). The use of ultrasonography was reported herein to obtain current data on ovarian follicular dynamics of the Bunaji and Friesian x Bunaji *B. indicus* breed of cattle, subjected to ovulation synchronisation protocol. To the best of our knowledge, this is the first report comparing the ovarian and hormonal responses of Bunaji and Friesian x Bunaji cows to induction of ovulation with ovsynch protocol using two different dosages. The present study indicates that the ovulation rates obtained in the untreated groups of Bunaji and Friesian x Bunaji cows were 0 and 20 % as at the time of first and second hormonal administration respectively. This may be as a result of hormonal insufficiency. Bo *et al.* (2003) reported that *B. indicus* breeds of cattle lack adequate LH to cause final growth and maturation of the pre-ovulatory follicles. Sartori and Barros (2011) also showed that *B. indicus* cattle are characterised by small CL and as such may be incapable of synthesising sufficient progesterone required for follicular dynamics and pregnancy establishment. The low hormonal measurements and ovulation rates recorded in the untreated groups as compared with the treated groups confirm some of the reasons for reproductive inefficienceies associated with *B. indicus*cattle. Hormonal treatments that can improve reproductive efficiencies must be included in the breeding programmes of the most populous indigenous breed of cattle in Nigeria and its hybrid counterpart, the Friesian x Bunaji. The response to first GnRH administration was similar among treatment groups and breed. Ovulation rates of 40 % - 60 % were recorded to first GnRH administration in all treated cows. This is in contrast to earlier reports on ovsynch protocols in cattle (Pursely *et al*., 1995; Ullah *et al.*,

1996; Perry *et al*., 2005) where 66 % - 90 % ovulation inductions by first GnRH administration have been reported. The relatively low ovarian response in the treated groups could be as a result of fewer numbers of FSH dependent follicles, low level LH secretion from the pituitary in response to GnRH injection, lower levels of LH receptors in the pituitary gland or breed of animals. It has been reported that ovsynch protocol may be a less efficient means of oestrus synchronisation in *B. indicus* than in *B. taurus* cattle (Larson *et al.,* 2006; Saldarriaga *et al*., 2007; Vasconcelos *et al.,* 2009). Several authors (Pursely *et al*., 1995; Bello *et al*., 2006; Walters *et al.,* 2008) have reported that high ovulation rates after GnRH administration depends on the maturation stage of the follicles (> 9.00 mm) at the time of treatment. In the present study, all cows had follicles between (8.60 ± 1.07 mm – 11.40 ± 1.63 mm). This may probably explain the ovulation rates obtained. On the other hand; some authors (Martinez *et al.,* 1999; Perry and Perry 2009; Tortorella *et al*., 2013) have reported a low ovulation rate despite large follicle diameters at the time of GnRH administration. It therefore implies that other factors apart from follicle sizes may influence ovulation rate. Uchechukwu (2017) reported that the hormonal environment, rather than the follicle size may be responsible for oocyte competence and subsequent fertilization. Progesterone has been reported to be of significant importance in this regard. Biehl *et al*. (2013) reported ovulation rates of 85.7 % and 25.8 % in Nellore heifers with low and high P_4 concentration respectively. Likewise, Melo *et al* (2016) reported a negative correlation between circulating P_4 concentration and ovulatory response to GnRH in *B. taurus* cattle.

Results from the present study indicate that 80% of Friesian x Bunaji cows ovulated in response to a second dosage of 100µg GnRH compared to 60 % of Bunaji cows of the same dosage. This is not surprising because the Friesian x Bunaji breed responded better to the first GnRH injection even though the difference was not statistically significant. Several authors (Geary *et al.,* 2000; Bridges *et al.,* 2014) have reported that the better the response in the first

GnRH injection, the better the synchrony of the final ovulation and the higher the probability of a successful reproductive outcome. An ovulation rate of 86% - 100% has been reported in cyclic cows receiving second GnRH (Pursley *et al.,* 1995; Fricke *et al.,* 1998; Uchechukwu, 2017). The importance of the second GnRH injection was to control the synchrony of the final ovulation although some studies consider it as of marginal value (Uchechukwu, 2017). However, the secondGnRH has been reported to consistently induce an LH surge which improves oestradiol production before ovulation (Perry *et al.,* 2007); improves synchrony of ovulation in *B. taurus* (Pursely *et al.,* 1995) and *B. indicus* (Barros *et al.,* 2000), thus, allowing effective use of fixed time artificial insemination.

Similarly, ovulation occurred in both Bunaji and Friesian x Bunaji cows 30-36 h after treatment. This agrees with earlier findings (Pursely *et al.,* 1995; Wiltbank *et al.,* 2011) that GnRH causes ovulation between 24 and 32h after treatment when a dominant follicle greater than 8.5mm is present at time of treatment. Furthermore, the results show that follicular wave emerged within two days of GnRH treatment in those animals that ovulated. This also agrees with the findings of Marinez *et al*., 1999 and Bo*et al.,* 2002, that reported that only if the first GnRH treatment results in ovulation do a synchronised follicle wave emergence occur. It is obvious that GnRH did not induce ovulation in some cows that received $PGF_2\alpha$. This can be attributable to lack of luteolysis after $PGF_2\alpha$ treatment thereby suppressing the development of the second follicular wave; or possibly that inadequate dose of GnRH or possibly that the follicle has lost its dominance (Carvalho *et al*., 2015). Jaiswal (2007) reported that ovulation could occur as late as 4 – 5 days after GnRH treatment suggesting the possibility of inappropriate dosage in the affected cows. The intensity of oestrus in the treated cows that ovulated was not as high as those of the control group at the time of second GnRH administration. This observation agreed with some previous studies (Pattabiraman *et al*.,

1986; Barkawi *et al*., 1995) who reported poor signs of oestrus in GnRH treated cows and buffaloes.

The hypothesis that there is no difference in follicular dynamics among Bunaji and Friesian x Bunaji breeds of cattle subjected to different dosages of ovsynch protocol was supported. The narrow range of diameters of follicles that was present on day of second GnRH injection in treated groups argues the fact that there was an effective response to the first GnRH in terms of disappearance of the existing DF and recruiting a new follicular wave. The diameter of the DF of Bunaji and Friesian x Bunaji cows in our study compared favourably to that obtained in *B.taurus* (14 – 18mm: Sartori *et al.,* 2016) and *B. indicus* (Degefa *et al.,* 2016; Uchechukwu, 2017).

The present result supports our hypothesis thatendocrine responses do not differ significantly among breed and ovsynch dosage. The treatment of both Bunaji and Friesian x Bunaji cows with 50µg and 100 µg of lecirelin a GnRH agonist on days 0 and 9 were able to elicit sufficient FSH and LH responses to guarantee the disappearance of the dominant follicle in most cows and initiate the emergence of a new follicular wave. This is in complete agreement with some research groups that investigated the potency of GnRH agonists (Kaltenbach *et al.,* 1974; Chenault *et al.,* 1990; Rettmerer *et al.,* 1992; Martinez *et al.,* 2003; Picard Hagen *et al.,* 2015). The similar patternsin the serum FSH and LH response found in both treated Bunaji and Friesian x Bunaji cows are in partial agreement with reports showing no difference in serum LH concentrations between 50µg and 100µg doses of GnRH in cows (Colazo *et al.,* 2009). Lasdun and Orlowski (1990) ascribed the increased potency of the GnRH agonists to their reduced susceptibility to degradation by pituitary endopeptidases.

The findings from this study also suggest that higher serum FSH and LH release occurred on d 9 of GnRH administration than d0. Several factors have been attributed to the magnitude of GnRH induced release of FSH and LH. These include the stage of the follicular wave

(Kastellic and Mapletoft, 1998) and circulating steroid hormone concentrations (Karsh, 1987l; Nette *et al.,* 2002). Oestradiol is one of the factors involved in the regulations of FSH and LH during the oestrous cycle (Bleach *et al.,* 2001). The decrease in FSH and LH levels in the treated groups on d0 and the increased concentrations on d9 might have been regulated by the high and low E_2 levels of the days respectively and differential P_4concentrations. Low levels of E_2 suppress FSH and LH release (Adams *et al.,* 2008; Aerts and Bols, 2010). However, as E_2 increases during follicular phase of the oestrous cycle, following a decrease in P_4 due to luteolysis, the pituitary becomes sensitized and FSH and LH responses increase (Aerts and Bols, 2010, Breda and Kozicki, 2015). High levels of P_4produced by a functional CL in dioestrus or pregnancy decreases the pulsatile LH response to GnRH (Collan, 1998, Breda and Kozicki, 2015).

The result of the present study showed that average plasma P_4 concentrations, up till d 7 after GnRH treatment, were significantly higher in treated cows than those in the control cows. This finding agrees with those of Pursely and Martins (2011) and Picard-Hagen *et al*. (2015) who also reported elevated plasma P_4 concentrations following GnRH treatment. The increase in plasma P_4 could be viewed as further evidence for the luteo-protective and anti-luteolytic effects of the GnRH agonist on the secretion of E_2 (Rettmer *et al*., 1992). It could also be attributed to the formation of an accessory CL as a result of the acute increase in LH after administration of GnRH or to the hypertrophy of the luteal cells in the spontaneous CL (Twagiramungu *et al*., 1995; Davis *et al*., 2003).

The $PGF_2\alpha$ given on day 7 to lyse all CL and result in a new oestrus and ovulation, caused a rapid decline in P_4 concentrations within 24 h of $PGF_2\alpha$ treatment in 60 % to 80 % of treated cows indicating complete luteolysis in the affected animals. This agrees with previous findings (Waldmann *et al*., 2006; Stevenson, 2016; Berg *et al*., 2020) and clearly suggests that a fully functional status was achieved for induced CL 7 days after GnRH treatment in all

groups. Incomplete regression of CL could be responsible for cows that did not respond to $PGF_2\alpha$ treatment. Pinheiro et al., (1998) and Rekwot *et al.* (1999) have reported incomplete regression of CL and increased concentration of P_4during the follicular phase thereby limiting oestrus. A clear understanding of the effects of P_4 and $PGF_2\alpha$ on the oestrous cycle is crucial for oestrus synchronisation.

One of the most common objections to using ovsynch is the overall cost of the hormones needed to synchronise ovulation. In the present study, the cost of hormones for each cow was reduced from N 13,300 ($26.6) for the 100 µg GnRH treated groups to N7, 300 ($14.6) for the 50 µg GnRH treated groups in both Bunaji and Friesian x Bunaji cows. Thus, a cost benefit of N 6, 000 ($12) in cost of purchase was achieved in hormones for ovulation synchronisation using 50 µg GnRH. This agrees with earlier reports on cost benefits of using half dosage of ovsynch synchronisation (Fricke, 1999; Bugau, 2014).

Furthermore, the total cost of hormone for each synchronised ovulation in this study was reduced from N 22, 167 ($44.33) to N 12, 167 ($24.33) for the full and half-dosage treatment respectively in Bunaji cows and from N 16, 625 ($33.25) to N12, 167 ($24.33) for the full and half - dosage treatment respectively in Friesian x Bunaji cows. The cost benefit was higher in Bunaji cows than in Friesian x Bunaji cows because there was no difference in ovulation synchronisation rate between Bunaji cows in the full-dose and half-dose ovsynch groups. Fricke *et al*. (1998) reported no difference in conception rates at 28 or 56 days post AI between cows in the full - dose and half - dose ovsynch groups. The cost reduction in hormone treatment offered a cost benefit advantage of N 10, 000 ($20) and N 4, 458 ($8.92) in Bunaji and Friesian x Bunaji cows respectively for each ovulation synchronised with half – dose ovsynch regimen. This agrees with previous studies that reported cost reduction in reproductive outcomes such as conception rate and pregnancy rate following the use of half - dosage ovsynch regimen (Fricke *et al*., 1999; Mckee, 2004; Bugau, 2014).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the findings of this study, it is concluded that:

i. Possibly for the first time ultrasonographic data on ovarian follicular dynamics in response to ovulation synchronisation has been established in Bunaji and Friesian x Bunaji cows in Nigeria.

ii. The GnRH at 50 µg and 100 µg when used in conjunction with produced ovulation synchronisation in Bunaji and Friesian x Bunaji $PGF_2\alpha$ cows, with Bunaji responding better to 50 µg and Friesian x Bunaji cows to 100 µg .

iii. Cows subjected to ovulation synchronisation had better ovarian follicular dynamics and CL activity compared to untreated cows.

iv. The GnRH administered at 50 µg and 100 µg was enough to stimulate LH and FSH responses for ovulation synchronisation in Bunaji and Friesian x Bunaji cows irrespective of oestrous cycle stage when compared to the untreated animals.

v. To maximize the FSH and LH responses, GnRH treatment should be given 48 hours after $PGF_2\alpha$ treatment

vi. Using the half – dose ovsynch protocol to manage ovulation synchronisation is a cost effective method of improving reproductive efficiency in Bunaji cows without compromising the effectiveness of the protocol

6.2 Recommendations

Based on the conclusions of this study, it is recommended that:

i. To optimise herd health reproductive efficiency, ovulation synchronisation using GnRH - $PGF_2\alpha$ – GnRH protocol should be used to stimulate appropriate endocrine responses and follicular activity in Bunaji and Friesian x Bunaji crosses.
ii. To maximise the FSH and LH responses GnRH treatment should be given 48 h after $PGF_2\alpha$ treatment.
iii. For cost effectiveness and optimum response, half the standard cattle dose of ovsynch (50 µg GnRH) should be used in Bunaji cows
iv. However, for optimum response, the standard dose of (100 µg GnRH) should be used in Friesian x Bunaji crosses.
v. Ultrasonography should be used as an effective diagnostic tool in the clinical evaluation of ovarian activity in Bunaji, Friesian x Bunaji, and other indigenous breeds of cows.
vi. The role of oestradiol in the endocrine cascade of physiological events that regulate ovulation synchronisation in Bunaji and Friesian x Bunaji cows should be investigated
vii. Further research should be carried out to determine the point of deviation of follicles in the synchronised new wave where serum concentration of FSH is expected to decline and the role of inhibin on serum FSH concentrations in Bunaji and Friesian x Bunaji cows.

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APPENDICES

Appendix 1: Mean (±SEM) serum LH concentrations in Bunaji cows following treatment on d 0 and d 9 with 50 µg lecirelin (group 1) or 100 µg lecirelin (group 2) or 2 ml saline (control) over a 6 h period.

Time (mins)	Control		Group 1		Group 2	
	Day 0	Day 9	Day 0	Day 9	Day 0	Day 9
-30	0.42 ± 0.12	0.34 ± 0.05	0.48 ± 0.04	0.54 ± 0.05	0.44 ± 0.05	0.58 ± 0.04
0	0.28 ± 0.04	0.40 ± 0.07	0.46 ± 0.05	0.62 ± 0.06	0.52 ± 0.04	0.50 ± 0.07
30	0.32 ± 0.06	0.50 ± 0.07	4.00 ± 0.35	4.00 ± 0.35	4.60 ± 0.53	3.90 ± 0.29
60	0.48 ± 0.07	0.40 ± 0.07	6.40 ± 0.43	7.30 ± 0.66	3.30 ± 0.25	8.70 ± 0.49
90	0.42 ± 0.04	**0.60 ± 0.07**	5.80 ± 0.25	9.30 ± 0.37	5.40 ± 0.19	8.30 ± 0.75
120	**0.52 ± 0.12**	0.40 ± 0.07	**8.20 ± 0.51**	16.50 ± 0.95	**9.40 ± 0.70**	17.50 ± 0.74
150	0.40 ± 0.07	0.52 ± 0.10	7.80 ± 0.46	**16.80 ± 1.53**	8.50 ± 1.12	**18.20 ± 2.27**
180	0.38 ± 0.04	0.54 ± 0.10	3.60 ± 0.19	10.70 ± 0.77	4.10 ± 0.19	9.60 ± 0.51
210	0.42 ± 0.04	0.44 ± 0.09	2.80 ± 0.34	3.90 ± 0.40	2.60 ± 0.19	4.00 ± 0.35
240	0.52 ± 0.12	0.42 ± 0.06	1.70 ± 0.25	2.60 ± 0.19	2.40 ± 0.37	3.00 ± 0.27
270	0.30 ± 0.04	0.42 ± 0.09	1.20 ± 0.12	2.20 ± 0.12	1.30 ± 0.12	1.96 ± 0.04
300	0.38 ± 0.06	0.48 ± 0.10	1.00 ± 0.14	1.16 ± 0.14	0.82 ± 0.08	1.04 ± 0.14
330	0.34 ± 0.05	0.40 ± 0.07	0.50 ± 0.04	0.82 ± 0.08	0.42 ± 0.09	0.70 ± 0.09
360	0.42 ± 0.06	0.36 ± 0.08	0.44 ± 0.05	0.70 ± 0.07	0.44 ± 0.05	0.64 ± 0.07

Appendix 2: **Mean (±SEM) serum LH concentrations in Freisian X Bunaji cows following treatment on d 0 and d 9 with 50 µg lecirelin (group 1) or 100 µg lecirelin (group 2) or 2 ml saline (control) over a 6 h period.**

Serum LH concentrations (mlU/ml)

Time (mins)	Control		Group 1		Group 2	
	Day 0	Day 9	Day 0	Day 9	Day 0	Day 9
-30	0.28 ± 0.04	0.28 ± 0.04	0.56 ± 0.04	0.58 ± 0.04	0.60 ± 0.07	0.62 ± 0.04
0	0.34 ± 0.05	0.34 ± 0.05	0.56 ± 0.05	0.50 ± 0.07	0.70 ± 0.10	0.58 ± 0.07
30	0.62 ± 0.06	0.50 ± 0.06	6.00 ± 0.71	4.60 ± 0.51	4.60 ± 0.40	4.20 ± 0.37
60	0.52 ± 0.13	0.38 ± 0.06	8.20 ± 0.37	8.20 ± 0.80	8.40 ± 0.51	8.20 ± 0.80
90	0.42 ± 0.04	0.38 ± 0.07	10.00 ± 0.63	10.40 ± 0.51	12.60 ± 0.75	11.80 ± 0.92
120	0.46 ± 0.05	0.54 ± 0.10	13.00 ± 0.27	**14.80 ± 0.37**	**16.00 ± 0.63**	**22.00 ± 3.39**
150	**0.60 ± 0.07**	**0.56 ± 0.10**	**14.00 ± 0.55**	14.60 ± 1.54	13.20 ± 0.58	19.40 ± 3.79
180	0.54 ± 0.10	0.4 ± 0.08	6.20 ± 0.66	10.60 ± 0.87	7.60 ± 0.51	10.00 ± 2.02
210	0.46 ± 0.11	0.42 ± 0.06	3.40 ± 0.51	5.80 ± 0.37	4.60 ± 0.51	5.80 ± 1.24
240	0.50 ± 0.07	0.38 ± 0.10	1.80 ± 0.25	4.00 ± 0.32	2.20 ± 0.25	3.80 ± 0.97
270	0.38 ± 0.04	0.48 ± 0.10	0.76 ± 0.05	2.80 ± 0.37	1.10 ± 0.12	2.20 ± 0.58
300	0.44 ± 0.12	0.46 ± 0.05	0.96 ± 0.16	1.50 ± 0.16	1.00 ± 0.14	1.20 ± 0.34
330	0.32 ± 0.04	0.42 ± 0.12	0.48 ± 0.04	0.68 ± 0.13	0.50 ± 0.07	0.74 ± 0.22
360	0.44 ± 0.04	0.28 ± 0.04	0.44 ± 0.05	0.50 ± 0.07	0.40 ± 0.07	0.44 ± 0.04

Appendix 3: **Mean (±SEM) serum FSH concentrations in Bunaji Cows following treatment on d 0 and d 9 with 50 µg lecirelin (group 1) or 100 µg lecirelin (group 2) or 2 ml saline (control) over a 6 h period.**

Serum FSH concentrations (mlU/ml)

Time (mins)	Control		Group 1		Group 2	
	Day 0	Day 9	Day 0	Day 9	Day 0	Day 9
-30	0.66 ± 0.05	0.76 ± 0.05	0.54 ± 0.10	5.70 ± 0.25	0.46 ± 0.09	6.40 ± 0.43
0	0.46 ± 0.12	0.70 ± 0.09	0.42 ± 0.05	6.30 ± 0.37	0.58 ± 0.06	7.50 ± 0.35
30	0.46 ± 0.05	0.62 ± 0.06	0.90 ± 0.04	8.70 ± 0.44	0.88 ± 0.06	9.00 ± 0.79
60	0.46 ± 0.05	0.72 ± 0.07	1.16 ± 0.12	7.40 ± 0.43	1.22 ± 0.10	8.70 ± 0.37
90	0.50 ± 0.03	0.72 ± 0.09	1.68 ± 0.11	18.20 ± 1.07	1.90 ± 0.19	17.80 ± 0.66
120	0.44 ± 0.09	0.66 ± 0.08	**2.64 ± 0.21**	25.60 ± 1.69	**2.56 ± 0.21**	26.40 ± 2.73
150	0.54 ± 0.10	0.72 ± 0.09	1.50 ± 0.08	30.00 ± 1.58	1.70 ± 0.10	**31.00 ± 1.18**
180	0.44 ± 0.09	**1.74 ± 1.07**	1.60 ± 0.07	24.60 ± 1.17	1.60 ± 0.09	26.20 ± 1.07
210	0.42 ± 0.06	0.74 ± 0.06	1.32 ± 0.06	22.80 ± 0.86	1.28 ± 0.06	23.00 ± 0.84
240	0.52 ± 0.11	0.66 ± 0.05	1.22 ± 0.07	25.00 ± 0.84	1.08 ± 0.05	24.40 ± 1.21
270	0.50 ± 0.07	0.58 ± 0.07	0.56 ± 0.12	**30.80 ± 2.96**	0.62 ± 0.08	30.00 ± 1.38
300	0.38 ± 0.04	0.68 ± 0.06	0.48 ± 0.04	28.00 ± 1.22	0.62 ± 0.06	30.40 ± 2.04
330	**0.58 ± 0.08**	0.70 ± 0.07	0.64 ± 0.09	28.00 ± 0.95	0.60 ± 0.07	30.00 ± 1.58
360	0.40 ± 0.05	0.62 ± 0.06	0.64 ± 0.09	27.00 ± 0.95	0.58 ± 0.09	28.80 ± 1.07

Appendix 4: Mean (±SEM) serum FSH concentrations in Freisian X Bunaji cows following treatment on d 0 and d 9 with 50 µg lecirelin (group 1) or 100 µg lecirelin (group 2) or 2 ml saline (control) over a 6 h period.

Time (mins)	Control		Group 1		Group 2	
	Day 0	Day 9	Day 0	Day 9	Day 0	Day 9
-30	0.62 ± 0.06	0.72 ± 0.07	0.78 ± 0.07	7.10 ± 0.56	0.62 ± 0.06	7.70 ± 0.46
0	0.82 ± 0.06	0.72 ± 0.09	0.88 ± 0.10	9.60 ± 0.43	0.72 ± 0.07	10.40 ± 0.51
30	0.72 ± 0.09	0.58 ± 0.06	1.42 ± 0.10	11.40 ± 0.60	1.40 ± 0.18	13.00 ± 0.55
60	0.74 ± 0.14	0.70 ± 0.08	2.24 ± 0.11	14.40 ± 1.03	2.78 ± 0.24	17.20 ± 2.06
90	0.72 ± 0.09	0.66 ± 0.07	2.62 ± 0.06	16.80 ± 1.36	2.96 ± 0.16	20.80 ± 1.66
120	0.66 ± 0.07	0.74 ± 0.06	**3.28 ± 0.12**	23.80 ± 1.36	**4.10 ± 0.29**	30.80 ± 2.29
150	0.74 ± 0.06	0.70 ± 0.08	3.12 ± 0.12	**29.40 ± 1.69**	3.50 ± 0.22	**37.40 ± 2.18**
180	0.72 ± 0.09	0.58 ± 0.07	2.46 ± 0.14	26.40 ± 2.73	2.76 ± 0.11	35.00 ± 2.74
210	**0.84 ± 0.12**	**0.82 ± 0.10**	1.80 ± 0.11	26.40 ± 1.57	2.14 ± 0.10	29.00 ± 1.87
240	0.74 ± 0.07	0.70 ± 0.07	1.40 ± 0.13	19.00 ± 1.00	1.64 ± 0.27	34.60 ± 3.76
270	0.60 ± 0.07	0.62 ± 0.06	0.98 ± 0.07	20.60 ± 1.33	0.90 ± 0.14	27.60 ± 1.12
300	0.62 ± 0.06	0.72 ± 0.06	0.72 ± 0.06	21.00 ± 0.45	0.72 ± 0.07	23.00 ± 1.55
330	0.76 ± 0.05	0.68 ± 0.06	0.68 ± 0.04	22.40 ± 0.81	0.74 ± 0.11	21.00 ± 1.18
360	0.70 ± 0.07	0.60 ± 0.07	0.64 ± 0.07	22.00 ± 1.05	0.74 ± 0.06	20.40 ± 1.21

Appendix 5: Peak LH concentrations for Bunaji and Friesian X Bunaji cows (mlU/ml)

		Day 0	Day 9
Bunaji	Control	0.66 ± 0.07^{a}	0.72 ± 0.06^{a}
	Group 1	8.50 ± 0.50^{b}	17.90 ± 0.90^{d}
	Group 2	10.20 ± 0.80^{b}	20.20 ± 1.56^{e}
Freisian X Bunaji	Control	0.72 ± 0.07^{a}	0.72 ± 0.06^{a}
	Group 1	14.10 ± 0.46^{c}	16.20 ± 0.66^{cd}
	Group 2	16.00 ± 0.63^{cd}	26.00 ± 2.92^{f}

Appendix 6: Peak FSHconcentrations for Bunaji and Friesian x Bunaji cows (mlU/ml)

		Day 0	Day 9
Bunaji	Control	0.68 ± 0.0^{a}	0.72 ± 0.06^{a}
	Group 1	2.64 ± 0.21^{b}	15.80 ± 0.66^{c}
	Group 2	2.70 ± 0.09^{b}	26.00 ± 2.92^{d}
Freisian X Bunaji	Control	0.92 ± 0.09^{a}	0.96 ± 0.07^{a}
	Group 1	3.38 ± 0.10^{b}	31.00 ± 1.87^{d}
	Group 2	4.20 ± 0.25^{b}	39.40 ± 1.96^{e}

Appendix 7: Interval to peak LH concentrations for Bunaji and Friesian x Bunaji cows (mins)

		Day 0	Day 9
Bunaji	Control	126.00 ± 30.59[ab]	156.00 ± 27.50[ab]
	Group 1	132.00 ± 7.35[a]	138.00 ± 7.35[a]
	Group 2	132.00 ± 7.35[a]	132.00 ± 7.35[a]
Friesian X Bunaji	Control	156.00 ± 27.50[ab]	180.00 ± 16.43[b]
	Group 1	144.00 ± 6.00[a]	138.00 ± 7.35[a]
	Group 2	120.00 ± 0.00[a]	132.00 ± 7.35[a]

Appendix 8: Interval to peak FSHconcentrations for Bunajiand Friesian x Bunaji cows (mins)

		Day 0	Day 9
Bunaji	Control	156.00 ± 11.22^{ab}	132.00 ± 15.30^{b}
	Group 1	120.00 ± 0.00^{b}	132.00 ± 7.35^{b}
	Group 2	114.00 ± 6.00^{b}	132.00 ± 7.35^{b}
Friesian X Bunaji	Control	180.00 ± 18.97^{a}	164.00 ± 20.15^{ab}
	Group 1	126.00 ± 6.00 ^{b}	156.00 ± 6.00^{a}
	Group 2	126.00 ± 6.00^{b}	162.00 ± 7.35^{a}

Appendix 9: Progesterone concentration

Days	Bunaji			Friesian X Bunaji		
	Control	Group 1	Group 2	Control	Group 1	Group 2
0	0.62 ± 0.06	1.70 ± 0.34	1.90 ± 0.29	0.74 ± 0.10	1.70 ± 0.12	2.30 ± 0.25
1	0.80 ± 0.09	2.40 ± 0.26	2.90 ± 0.33	1.14 ± 0.09	2.16 ± 0.14	2.70 ± 0.21
2	1.20 ± 0.09	2.68 ± 0.36	3.06 ± 0.24	1.50 ± 0.03	3.00 ± 0.32	3.50 ± 0.22
3	1.90 ± 0.19	3.28 ± 0.35	3.82 ± 0.13	1.84 ± 0.07	3.42 ± 0.25	4.08 ± 0.20
4	2.30 ± 0.25	4.04 ± 0.16	4.54 ± 0.16	2.60 ± 0.19	4.00 ± 0.16	4.70 ± 0.12
5	3.10 ± 0.24	4.96 ± 0.19	5.66 ± 0.41	3.40 ± 0.19	4.94 ± 0.17	5.80 ± 0.25
6	4.60 ± 0.12	5.60 ± 0.07	6.24 ± 0.28	4.30 ± 0.20	5.36 ± 0.16	6.20 ± 0.25
7	5.14 ± 0.37	6.62 ± 0.27	7.02 ± 0.21	4.50 ± 0.67	6.42 ± 0.12	6.70 ± 0.58
8	6.06 ± 0.41	3.32 ± 0.69	3.52 ± 0.91	4.92 ± 0.90	4.00 ± 0.82	3.00 ± 0.52
9	5.56 ± 1.02	2.94 ± 0.84	3.18 ± 1.08	5.26 ± 1.08	3.72 ± 1.04	2.70 ± 0.72
10	5.96 ± 1.18	2.88 ± 1.09	3.36 ± 1.41	6.70 ± 0.14	3.44 ± 1.45	2.04 ± 0.99
11	6.16 ± 1.35	3.06 ± 1.26	3.14 ± 1.38	4.96 ± 1.76	3.58 ± 1.30	2.44 ± 1.14
12	6.56 ± 1.47	3.30 ± 1.41	3.16 ± 1.47	6.40 ± 1.37	3.30 ± 1.32	2.36 ± 1.29

Appendix10: A Herd of Bunajiand Friesian x Bunajicows located at Livestock section, Samaru College of Agriculture, Samaru - Zaria

Appendix 11: With Livestock attendant's atSamaru College of Agriculture, Samaru - Zaria

Appendix 12: Hormonal analysis in the laboratory

Appendix 13: Various ELISA hormonal assay kits

Appendix 14: Accubind ELISA microwells packaging

Appendix 15: Typical content of Accubind ELISA microwell kit (Progesterone)

Appendix 16: Ethical Clearance

Committee On Animal Use And Care

Directorate of Academic Planning & Monitoring
Ahmadu Bello University, Zaria

Appl No: ABUCAUC/2021/Pharmacology & Toxicology/114 7th September, 2021
Approval No: ABUCAUC/2021/114

Prof. M. Mamman,
Department of Pharmacology & Toxicology,
Faculty of Veterinary Medicine,
Ahmadu Bello University,
Zaria.

Dear Sir,

APPROVAL OF RESEARCH STUDY 'EVALUATION OF TWO DOSES OF GONATROPIN-RELEASING HORMONE WITH PROSTAGLANDIN F2 ALPHA ON FOLLICULAR DYNAMICS OF BUNAJI AND FRIESIAN x BUNAJI COWS'

This is to convey the approval of the ABUCAUC to you for the aforestated study domiciled in the Department of Pharmacology & Toxicology. The approval is predicated on the assumption that you shall maintain and care for the Experimental Animals as approved after the visitation of the Committee.

Monitoring of the Research by spot checks, invitations or any other means the Committee deems fit shall be undertaken at the convenience of the Committee.

This approval can and shall be revoked should a significant breach in the terms and condition of the approval occur. It is hence your responsibility to ensure that the agreed terms are maintained to the end of the Study.

The said approval shall be posted on the ABUCAUC Page on the University's website.
Note upon completion of the research, ethical clearance certificate will be issued.

A.B. Abdulkadir
For: Chairman, ABUCAUC.

Cc. Director, IAIICT
Dean, Faculty of Veterinary Medicine
HOD, Pharmacology & Toxicology
Prof. C. A. Kudi, Chairman, ABUCAUC

Chairman: Prof C. A. Kudi DVM, M.Sc, PhD (ABU), PhD (Lel.) PGDE (PLY) 08065978003