# A STUDY OF FOLLICULAR DYNAMICS IN THE KENYAN BORAN COW

**Dr. Muraya Joan BVM (UoN)**

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**DEPARTMENT OF CLINICAL STUDIES**

**FACULTY OF VETERINARY MEDICINE**

**UNIVERSITY OF NAIROBI**

## DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Signature…………………………………….Date…………………..

Dr. Muraya Joan, BVM (University of Nairobi).

This thesis has been submitted for examination with our approval as the university supervisors

Dr. Mutembei Henry M. (BVM, MSc, Dip, PhD, CertAWEL)

Date: ……………………….. Signature: …………………………..

Dr. Tsuma Victor. T. (BVM, PhD, FRVCS)

Date: …………………….... Signature: …………………………...

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## DEDICATION

This work is dedicated to my parents and siblings for believing in me and their unfailing encouragement, my friends who stood by me even when the going got tough and to my son whom I hope this work will inspire to great heights.

## LIST OF ABBREVIATIONS

**AI**: Artificial Insemination

**ARTs**: Assisted Reproductive Techniques

**ASALs**: Arid and Semi-Arid Lands

**BCS**: Body Condition Score

**CL**: *Corpus Luteum*

**DF**: Dominant Follicle

**ECL**: Estrous Cycle Length

**ET**: Embryo Transfer

**FSH**: Follicle Stimulating Hormone

**IVEP**: In-vitro Embryo Production

**LH**: Luteinizing Hormone

**MHz**: Mega Hertz

**mm**: Millimeters

**OPU**: Ovum Pick Up

**P**: Probability

**$PGF_{2\alpha}$**: Prostaglandin F2 alpha

**SEAZ**: Small East African Zebu

**SEM**: Standard Error of Means

**SF1**: Subordinate Follicle One

**SF2**: Subordinate Follicle Two

**SPSS**: Statistical Package for the Social Sciences

**SIFET**: Sexed Semen In-vitro Fertilization and Embryo Transfer

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## ABSTRACT

Although ovarian follicular dynamics has been documented in some Zebu cows, literature on the same for the Boran cow is scarce. The current study aimed at providing this information so as to optimize capacity of this breed through utilization of some assisted reproductive technologies like estrous synchronization, *in-vitro* embryo production and embryo transfer.

Follicular development characteristics during estrous cycle were evaluated using a 5.0-7.5 Megahertz linear array portable ultrasound device from 15 randomly selected cows that were open but cycling and of a 2-2.5body condition score. The cows were induced into estrous using 500µg (2ml) of Cloprostenol (Estroplan®). Daily scanning of the ovaries was done and the follicles counted, measured for diameter and recorded. These activities were carried out daily for two inter-estrous cycle lengths. Also the location of the three largest follicles and the *corpus luteum* were sketched for each ovary on a daily basis. The three sketched follicles were used to track and evaluate for daily follicular dynamics. Follicular parameters such as follicle numbers and sizes were utilized to determine wave emergence, selection and deviation, dominance and atresia characteristics that constituted the dynamics. Changes in the size and growth of *corpus luteum* were also tracked.

The cows exhibited two, three and four follicular waves during the estrous cycles. Most of the cows (66.67%) exhibited the three wave cycles, while 26.67% had the two wave cycles and 6.67% had the four wave cycles. The pre-ovulatory follicle attained a mean maximum diameter of13.56 ± 1.73 millimeters, which was significantly ($P \leq 0.05$) higher than the diameter of all the other dominant follicles. Cows with two wave cycles had an estrous cycle length of about 18 days while those with three and four wave cycles had extended estrous cycle lengths of about 25

days. In the cows with fewer wave cycles, the duration of dominance phase of first wave was approximately three days longer than their 3-wave and 4-wave counterparts. There was no significant difference in both the growth and atresia rates for dominant and subordinate follicles among waves within the estrous cycles. Although there was no significant difference in maximum diameter of the dominant follicle between the first and the second wave in cows exhibiting the two wave cycles, three-wave and four-wave cycle diameters of the dominant follicle of the ovulatory wave was significantly larger than those of the other waves. There was no significant difference indiameter of *corpus luteum* noted among the cows. The *corpus luteum* from four wave cyclespersisted the longest period.

It was concluded that the follicular dynamics of Boran cow compares well with other Zebu breeds studied so far and three ovum pick up sessions can be done for utilization of this breed in *in-vitro* Embryo Production and Embryo Transfer.

# CHAPTER 1

## 1.0 INTRODUCTION

Up to 80% of Kenya's landmass is arid or semi-arid land (ASAL). Since crops do not grow easily in these areas, the inhabitants who are mainly pastoralists depend on livestock as their most viable source of livelihood. Zebu cattle (*Bos indicus*) are the predominant cattle kept in these ASAL areas.The Zebu cattle are better adapted to survive in the harsh and unfavorable climatic conditions of the ASALs that are characterized by high ambient temperature, poor feed quality and high disease challenges (Herlocker 1999). In spite of its adaptive superiority to the ASALs, the Zebu cattle have low production and reproductive potential compared to *Bos taurus* cattle (Lamothe-Zavaleta, *et al.,* 1991; Mukasa-Mugerwa 1989).

Among the Zebu breeds, the most commercially viable in Kenya is the Boran. It's mainly kept by pastoralist communities as a source of milk and by commercial beef ranches as the main indigenous beef breed (Okeyo e*t al*., 1998; Rewe *et al*., 2006). Although this seemingly low valued Boran can be uplifted to its optimum reproductive potential using available reproductive technologies (ARTs), there is a scarcity of information on reproductive physiology of this breed. In the cow, ovarian function is part of the reproductive axis that needs to be well understood in order to effectively utilize ARTs to enhance the reproductive potential. The ovum, the female gamete, develops in follicles within the ovary. Understanding how follicular growth occurs and the mechanisms of its control is important for the optimization and successful utilization of ARTs to control and improve fertility in animals.

To this extent, ultrasound imaging has enabled sequential visualization of the ovary on a day-to-day basis without compromising the ovarian and endocrine status of the cow. From such studies, it has been shown that ovarian follicles develop in waves during the bovine estrous cycle and different number of waves exists among different breeds of cows (Ginther *et al*., 1989a). Each follicular wave is comprised of successive phases referred to as emergence, selection, deviation, dominance, atresia and/or ovulation. Subsequently, a *Corpus Luteum* (CL) develops at the site of the ovulatedgraafian follicle and through its glandular secretions it is responsible for the regulation of the estrous cycle. It has been well established in *Bos taurus* cattle that more than 95% of all estrous cycles consist of two or three follicular waves (Adams 1999; Evans 2003). Similarly, a few studies on follicular characterization have been done in some Zebu breeds of cattle e.g. Nelore, (Mollo *et al*., 2007) and Gyr, (Gambini *et al*., 1998), with some showing up to four-wave cycles (Rhodes *et al*., 1995; Bo' *et al*., 1995) and occasionally five-wave cycles (Viana *et al.,* 2000).

Since no information is available on the peculiarities of follicular dynamics and luteal development of the Kenyan Boran cow, this study was conceived to fill in these existing information gaps by studying the follicular dynamics and luteal developmental trends during the estrous cycle in the Kenyan Boran cow.

**Objectives of the Study**

The aim of the study was to generate information that would enhance the understanding of the ovarian physiology in this breed and in turn enable effective and efficient utilization of various assisted reproductive technologies like estrous synchronization, oocyte recovery, embryo production in the laboratory and transfer into recipient.

**The study's specific objectives were:**

1. To determine the number of follicular waves developing during the estrous cycle in the Kenyan Boran cow.
2. To establish the dynamics of the follicles; (number, size and growth patterns) developing within follicular waves of the Kenyan Boran cow.
3. To establish the influence of the *corpus luteum* on follicle development in the Kenyan Boran cow.

## CHAPTER 2

## 2.0 LITERATURE REVIEW

### 2.1 LIVESTOCK LIVELIHOODS IN KENYA

Livestock make their most important contribution to total food availability when they are produced in places where crops cannot be grown easily, such as marginal areas (Okeyo *et al*., 1998). In Kenya, about 70% of the over 14 million cattle found in this country are found in the ASALs (GoK 2007), and they are predominantly *Bos indicus* (Zebu) or their crosses. The communities in the ASALs depend on livestock as their most important source of livelihood and food security. The ASAL areas which are the vast majority of the Kenyan landmass are characterized by harsh and unpredictable climatic conditions among them low, unreliable and poorly distributed rainfall, high temperatures of around 30-45$^0$C in hot months, nutritive stress and hostile disease-parasite environments. In this country, livestock production is estimated to contribute between 50 and 95 per cent of the income of pastoralist families (Aklilu and Catley 2009). The zebu cattle has continued to be the most commercially used breed of beef cattle as it is highly adaptable to harsh climatic conditions found in most tropical range lands as well as its reasonably fair productivity and reproduction. Among the Zebu cattle the largest commercial beef breed kept in Kenya is the Kenyan Boran.

### 2.2 THE KENYAN BORAN

According to Rewe et al. (2006), the KenyanBoran cattle constitute the largest proportion of the indigenous breeds kept in the semi-arid Kenya for beef production. However, other beef cattle genotypes e.g. Small East African Zebu (SEAZ), *Bos taurus b*reeds such as the Hereford, Simmental, Charolais, Angus, their crosses and culled stock from the dairy cattle herds have served as sources of beef in the country (Indetie *et al*., 2000). The Kenyan Boran cattle are preferred to the *Bos Taurus* breeds by commercial ranchers due to their relative adaptability to the tropical production environment, which facilitates their greater ability to survive, grow and reproduce in the semi-arid conditions. These characteristics have been achieved following generations of natural and artificial selection in conditions of high ambient temperature, poor feed quality and high pathogen incidences that typify the ASALs of Kenya and low input production systems (Davis 1993; Herlocker 1999; Mpofu 2002; Okomo-Adhiambo 2002).

Breed comparisons involving the Boran and its crosses have shown the superiority of Boran cattle for survival under tropical conditions (Said *et al*., 2001). Due to these potentials, this breed is recommended for use in performance improvement of other indigenous and exotic cattle for beef production in the tropics (Trail *et al*., 1984; Indetie*et al*., 2000).

The reproductive performance of the KenyanBoran cow like other *Bos indicus* beef breeds is influenced by productive performance, survival ability and environmental conditions. The Kenyan Boran is hardy, well adapted and under marginal conditions it produces a calf every year (Okeyo*et al*., 1998), and has extremely good mothering abilities (Wasike *et al*., 2007).

In the recent past, some zebu breeds have been well characterized, especially with respect to their physiological reproductive parameters, follicular dynamics and oocyte manipulations (Camargo *et al.,* 2006, Viana *et al.,* 2000). Locally the Boran cow has been scantily studied and among the information available is its response to superovulation (Mukasa-Mugerwa 1989; Zerbini *et al.,* 1992; Tegegne *et al.,* 1994; Tegegne and Franceschini 1993) and its viability for *in-vitro* embryo production system (Mutembei *et al.,* 2008; Muasa 2010). As one of the viable breeds that can be reproductively exploited to its optimum potential through technologies such as

ET, IVEP and estrous synchronization, this information on ovarian physiology then becomes vital towards this undertaking, a need for which this study addresses.

## 2.3 ESTROUS CYCLE IN THE COW

The estrous cycle is the duration from one estrus (heat) to the next. In cows, the estrous cycle is about 21 days long, but it can range from 17 to 24 (Sartori *et al*., 2004).The estrous cycle in cows is divided into four stages: proestrus, estrus, metestrus, and diestrus (Kojima 2003). Proestrus is the period of folliculogenesis and last for three days. During this period, there is a Dominant Follicle (DF) that establishes itself and it produces 17β-estradiol in the granulosa cells. This estrogen causes the expression of estrus signs. The estrogen peaks before estrus causing the preovulatory surge of LH Hormone. Inhibin is also produced by the growing follicle and it prevents the growth of other smaller follicles. This inhibition of smaller follicles assures selection of a single follicle to ovulate.

This is then followed by estrus phase which is the period of sexual receptivity when a cow is receptive to the bull. It lasts for 24-36 hours after which ovulation occurs in the next 11-14 hours (Hansel *et al*., 1961).The thecacells start producing progesterone, which inhibits LH and Follicle Stimulating Hormone (FSH) release (Noakes *et al.,* 2009).

After ovulation, a *corpus hemorrhagicum* develops and this occurs in metestrus phase that lasts for three days. Since the CL is not mature yet, there are no prostaglandin receptors on it, thus making luteolysis via prostaglandin $F_{2\alpha}$ impossible. The FSH surge that occurs at this time may choose the first follicular wave for the next cycle. After this, a diestrus phase follows which lasts for 14 days. By this time there is a fully functional *corpus luteum* which has glandular function and it produces progesterone hormone (Hansel et al., 1961). Metestrous and diestrousstagesmake up the luteal phase, within which there are two, three or four waves of follicular growth depending on the wave whose dominant follicle ovulates. The follicles grow, become static for about 2 days and then regress. At the end of this phase, if no pregnancy signal is received by the CL, the luteolytic cascade starts (Fajersson *et al*., 1991). The prostaglandin $F_{2\alpha}$ synthesized by the uterus is transferred to the ipsilateral *corpus luteum* by a local utero-ovarian countercurrent exchange mechanism. The $PGF_{2\alpha}$ then binds to the large luteal cells and causes luteal death via either direct action or vascular constriction. The prostaglandin also causes release of oxytocin from the large luteal cells, which causes the uterus to release more prostaglandin (Noakes *et al.,* 2009). This gives a 'fail-safe' mechanism to ensure luteal death and return to estrus. Estrogen from the dominant follicle is important in that it induces the uterine prostaglandin synthesis and the increased number of uterine oxytocin receptors.

The average inter-estrous interval is 21 day, for both *Bos taurus* and *Bos indicus* cattle (Bó *et al*., 2003), and two-wave cycles have been shown to be shorter than three-wave cycles, 19 versus 23 days, (Alvarez *et al*., 2000; Townson *et al*., 2002; Sartori *et al*., 2004). There are, however, exceptions, in whichhigh-producing lactating Holstein cows have been shown to have a longer estrous cycle length (23 days), due to a prolonged time between luteolysis and ovulation (Sartori*et al*., 2004).

### 2.4 FOLLICULOGENESIS

#### 2.4.1 Follicular Dynamics in Cows

A bovine female is born with a pool of approximately 133,000 primordial follicles which is not renewable and is gradually depleted until it reaches near zero when the cow is 15 to 20 years of age (Hansel *et al*., 1961).

Since the advent of bovine ovarian ultrasonography in 1984, enormous progress has been made in the understanding of folliculogenesis and the development of the bovine *corpus luteum* (Durocher *et al*., 2005; Ginther 1998; Pierson and Ginther 1988; Sirois and Fortune 1988). Ultrasound enables the description of the dynamics of follicular growth in follicles greater than 1 mm in diameter (Durocher *et al.,* 2005). In cows, follicles larger than 1 mm can be found throughout the estrous cycle.

Many authors have documented that during an estrous cycle, follicles develop in wavelike manner (Savio *et al*., 1988; Ginther *et al*., 1989a; Knopf *et al*., 1989; Taylor and Rajamahendran 1991). A follicular wave is characterized by the synchronous growth of a cohort/group of follicles, one of which continues growing to become the dominant follicle while the others regress at variable intervals. The fate of the DF is either ovulation when it's in the last wave of a cycle or regression when it is among the preceding waves.

In cattle, the wave-like pattern of follicle development has been studied (Roche *et al.,* 1998; Adams 1999; Ireland *et al.,* 2000; Fortune *et al.,* 2001). Studies in *Bos Taurus* cattle have indicated occurrence of two to four follicular waves during the estrous cycle, with predominance of two waves, and very rarely four waves (Sirois and Fortune 1988; Townson *et al*., 2002; Sartori *et al*., 2004). Studies in the Zebu cows; Nelore heifers (Mollo *et al*., 2007), Gir cows (Gambini *et al*., 1998;Viana *et al.,*2000), and Brahman cows (Zeitoun*et al*., 1996) showed a predominance of three waves with some observation of two, four, and even a few five waves per cycle (Viana *et al.,*2000). It is however a normal finding in *Bos indicus* cattle to have four follicular waves during estrous cycles (Viana *et al*., 2000) but in *Bos taurus* cattle, four or more follicular waves per cycle are usually associated with delayed luteolysis or failure to ovulate

(Adams 1999). Short estrous cycles with one follicular wave have been reported around the time of puberty (Evans *et al.,* 1994a) and *post-partum* after first ovulation (Savio *et al.,* 1990).

There is an indication that cows that have two follicular waves per cycle tend to have shorter cycles, ovulate larger and older follicles and are less fertile than cows with three waves per cycle (Townson *et al.,* 2002).Stages of follicular waves have been studied extensively in cattle and consist of three stages: recruitment, selection, and dominance (Binelli *et al*., 2006, Viana *et al*., 2000).

**2.4.4.1 Recruitment**

The concept of recruitment is used to define the entrance of follicles into the growing pool.Follicle recruitment is often used synonymously with wave emergence (Ginther *et al.,* 1996) but is more accurately defined as the growth of follicles that have become gonadotropin dependent (Driancourt 2001). The day of follicle wave emergence is the first day of a follicular wave when a growing cohort of follicles is first detectable using ultrasonography (Bo *et al.,* 1995). Recruitment of a cohort of follicles, 8 to 41 (average, 24) small follicles of 1 to 3 mm (Jaiswal *et al*., 2004) in diameter, is stimulated on each ovary by a transient rise in FSH. The peak concentration of FSH occurs when the future dominant follicle attains a mean diameter of approximately 4 mm, after which the concentrations declines, and is at basal concentrations by the time follicular selection occurs (Ginther *et al.,* 2000a). The mechanism responsible for the initial decline in FSH concentration is unknown; however, 17β estradiol and inhibin are follicular products that probably play a major role in the decline of FSH. This process takes approximately 2-4 days and ends around day four of the estrous cycle for the first wave in bovines (Adams 1999). Differences in antral follicle population between breeds exist. At the onset of each follicular wave, approximately 24 small (2 to 5 mm) viable antral follicles have been detected in

*Bos taurus* cattle (Ginther *et al*., 1996). However, in *Bos indicus* cattle, greater numbers of small follicles during wave emergence have been reported.Buratini Jr. et al. (2000) described the occurrence of approximately 50 small follicles in the ovaries of Nelore heifers. By directly comparing number of small follicles (3 to 5 mm) in the ovaries between *Bos taurus* and *Bos indicus* cattle, Alvarez et al. (2000) observed a greater number of follicles at wave emergence in Brahman (39 ± 4) compared to Senepol (33 ± 4), or Angus (21 ± 4) multiparous lactating cows. Additionally, recent studies performed with both breeds of cattle in contemporary environmental and nutritional conditions showed that *Bos indicus* had a greater number of follicles at wave emergence compared to *Bos taurus* cattle (Carvalho *et al*., 2008; Gimenes *et al*., 2008).

#### 2.4.4.2 Selection and Deviation

Follicular *s*election is the process by which a single follicle from the recruited cohort is selected to continue to grow and become dominant, while the remaining follicles of the cohort undergo atresia. With the decline in circulating FSH concentrations, small follicles are presumably unable to continue growth and the selected follicle (dominant follicle) may shift its dependency from FSH to LH (Ginther *et al.,* 1999b). The decreased circulating concentration of FSH at the time of selection is important for the selection of a single dominant follicle. The decline in circulating concentrations of FSH is presumably driven by increasing concentrations of 17β-estradiol (and perhaps inhibin) produced by the cohort of recruited follicles (Ginther *et al.,* 2000b). Increased concentrations of 17β-estradiol and inhibin may feedback on the hypothalamic-pituitary axis to selectively suppress FSH secretion (Martin *et al.,* 1988). Follicle selection results in a decrease in the number of growing follicles in a wave to one that will ovulate if it's the final wave of the cycle or will regress if in the pre-ovulatory waves. This ill-defined process probably occurs over a period of time and is thought to end when the dominant follicle(s) has been selected from the subordinate follicles as seen by a difference in follicle size. This point in time, where there is a divergence in growth rates is referred to as deviation (Ginther *et al.,* 1996).

At follicular deviation, the selected follicle continues to grow while the subordinate follicles enter atresia (Ginther *et al.,* 1999b). In cows, deviation usually occurs when the largest follicle reaches a diameter of approximately 8 mm in about 2.7 days after the initiation of a follicular wave (Ginther *et al.,* 1997; Ginther *et al.,* 1999b) or 61 hours after the LH surge (Kulick *et al.,* 1999).

In *Bos Taurus* breeds, follicular deviation occurs when the largest developing follicle reaches 8.5 to 9.0 mm in diameter (Ginther *et al*., 1996; Sartori *et al*., 2001), whereas, in Zebu cattle, deviation occurs when the largest growing follicle reaches 5 to 7 mm (Castilho *et al*., 2007; Figueiredo *et al*., 1997; Sartorelli *et al*., 2005; Gimenes *et al*., 2008). Bastos *et al*. (2010) observed that deviation occurred, on average, 2.3 d after ovulation, independent of breed.

**2.4.4.3 Dominance**

The number of follicles recruited is usually greater than the typical number of ovulatory follicles for a given species. However, only a species-specific number of ovulatory follicles continues to grow for more than a few days and reaches ovulatory size. These follicles are called "dominant" follicles because it is believed that once they are selected, they in some way prevent further growth and differentiation of their sister, subordinate follicles and prevent further follicular recruitment (Fortune 1994).

The dominance phase of the follicular wave occurs when a follicle has been selected and continues to grow at a faster rate than the largest subordinate follicle, and inhibits the emergence of a new follicular wave (Ginther*et al.,* 1996). Following selection and establishment of a dominant follicle, follicular recruitment is inhibited until dominance is lost or ovulation occurs.

Inhibition of follicular recruitment may be mediated by inhibiting the transient rise in circulating concentrations of FSH (Adams *et al*., 1993b). Alternatively, it has been said that the dominant follicle directly inhibits growth of small follicles through the secretion of a factor(s) that acts directly on other follicles in the ovary. Regardless of the mechanism, destruction or ovulation of a dominant follicle results in a transient rise in circulating concentrations of FSH and subsequent initiation of a new follicular wave (Adams *et al.,* 1992).

During estrous cycles in taurine cows, the dominant follicles has been shown to reach a maximum diameter of approximately 10–20 mm (Fortune *et al.,* 1988; Savio et al., 1988; Ginther*et al.,* 1989c) and the largest subordinate follicles to reach maximum diameters of approximately 8 mm (Ginther *et al.,* 1989a).*Bos indicus* cows however have been shown to have smaller dominant follicles diameters (12.3 and 11.3mm, respectively) in cows with two-wave cycles, (Figueiredo *et al*., 1997; Sartorelli *et al*., 2005.)

**2.4.4.4 Luteal Phase**

The luteal phase spans the time of *corpus luteum* formation and maintenance which begins with ovulation and ends with luteolysis. Progesterone is the primary secretory product of the *corpus luteum* and is regulated by secretions of the anterior pituitary, uterus, ovary, and embryo. The regulation of progesterone secretion is likely controlled by a balance of luteotropic (stimulate progesterone) and luteolytic (inhibit progesterone) stimuli, given that both types of stimuli are secreted concurrently during the estrous cycle. In ruminants, the effects of differing circulating concentrations of progesterone on the development of the dominant follicle in cattle have been attributed to changes in LH secretion.

Progesterone has a central role in the regulation of the estrous cycle as it determines estrous cycle length. It suppressed the growing phase of the dominant follicle in a dose-dependent manner (Adams *et al*., 1992); is associated with suppression of LH pulse-frequency (Ireland and Roche 1982; Roberson *et al*., 1989; Stock and Fortune 1993) and subsequently yielding a shorter inter-wave interval (Adams *et al*., 1992; Lucy *et al*., 1992). LH is considered to be the primary luteotropic hormone and concentration of luteal LH receptors is positively correlated with changes in progesterone and luteal growth (Niswender *et al.,* 2000). A positive relationship may exist between the CL and follicular development. Indeed, Savio et al. (1988) observed that approximately 63% of dominant follicles developed in the ovary ipsilateral to the CL, and Driancourt et al. (1991) showed that the ovary not bearing a CL had lower follicular activity than the CL bearing one. On the other hand, other authors found an inhibitory effect of the CL, whereby the size and number of medium to large follicles were greater in the ovary contralateral to the CL (Matton e*t al*., 1981; Pierson and Ginther 1989b). However, Ginther et al. (1989a) reported no significant intra-ovarian effects of the CL on characteristics of the dominant follicle, such as growth rate and maximum diameter. Thus, the nature of the interplay between the CL and the follicle is complex and may depend in part on the type of follicle and pregnancy status (Rexroad and Casida, 1975).

## 2.5. OVARIAN ULTRASONOGRAPHY IN BOVINE

Ultrasonography is the imaging of deep structures of the body by recording the echoes of pulses of ultrasonic waves directed into the tissues and reflected by tissue planes where there is a change in density. The basic principle of ultrasonography is that the ultrasonic waves are confined to a narrow beam that may be transmitted through or refracted, absorbed, or reflected by the medium toward which they are directed; depending on the nature of the surface they strike (Ginther *et al*., 1989d).

In diagnostic ultrasonography the ultrasonic waves are produced by electrically stimulating a crystal called a transducer. As the beam strikes an interface or boundary between tissues of varying density (e.g., muscle and blood) some of the sound waves are reflected back to the transducer as echoes. The echoes are then converted into electrical impulses that are displayed on an oscilloscope, presenting a “picture” of the tissues under examination.

As 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, corpus hemorrhagic, and corpus luteum all contain varying degrees of dense cells, which reflect the ultrasound waves and result in a gray image on the screen.

## 2.6 ASSISTED REPRODUCTIVE TECHNOLOGIES

Reproductive biotechnologies in farm animal are greatly responsible for the significant progress made in breeding and genetics in cattle (Thibier 2005). Various techniques have been developed and refined to obtain a large number of offspring from genetically superior animals and within minimum time possible. Artificial Insemination (AI) and Embryo Transfer (ET) alone have resulted in an almost complete transformation of animal breeding, especially in the dairy industry. Today, assisted reproduction and biotechnology allow breeders to design and direct the reproductive course, disseminate desired traits and hasten genetic improvement. Generation interval can be greatly reduced by combining AI, which is the oldest and most widely used Assisted Reproductive Technology (ARTs), with the more recent techniques, such as estrus synchronization, superovulation, semen sexing and ovum pick up from immature females even out of breeding season, and in vitro embryo production and transfer.

Estrus synchronization is an artificial manipulation of the estrous cycle to bring a group of cows to exhibit estrus at a closely controlled time period unlike as would occur in nature. Among the many developed protocols, luteolysis is the most commonly used with Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) as the main luteolytic agent (Macmillan and Burke 1996). A variation in onset of estrus, distributed over a 6-day period has been reported in cattle following estrus synchronization using $PGF_{2\alpha}$. In crossbred Boran, Mukasa-Mugerwa (1989) reported heat occurring two to three days following $PGF_{2\alpha}$ administration.

*In vitro* embryo production is one of the relatively new assisted reproductive technologies (ARTs) in cattle breeding and husbandry (Camargo *et al.,* 2006). It utilizes oocytes from either live (obtained through Ovum Pick-Up: OPU) or from ovaries of slaughtered animals. Through the years, the number of transferable embryos provided by OPU has significantly increased mainly due to the technological improvement of IVEP (Viana *et al.,* 2010). Partly, the variation observed in OPU results is related to developmental status of ovarian follicular populations when they were aspirated. The selection of dominant follicles in successive follicular waves determines fluctuation in the number and development status of follicles available for aspiration at any given time during the estrous cycle.

Due to the greater antral follicle population in *Bos indicus* cattle in relation to *Bos taurus* cattle, *in-vitro* embryo production is much more successful in *Bos indicus* cattle (Viana and Camargo 2007). In Kenya, development of non-sexed-IVEP protocols using slaughter house sourced Boran oocytes has already been undertaken (Muasa 2010). The next more efficient phase would be to obtain gametes from live Boran cows using OPU. For this to be effectively done, a deep understanding of the ovarian physiology is required an undertaking that can be achieved through sequential ovarian ultrasonography. While numerous of these studies have been carried out in the

*Bos taurus* cows (Ginther *et al*., 1989a; Savio *et al.,*1988; Sirois and Fortune 1988; Ginther *et al.,* 1989c) this information is lacking for the Kenyan Boran cow. This study aimed at providing this vital information on follicular dynamics in the Kenyan Boran that would in turn ease in the application of some of these ARTs.

# CHAPTER 3

## 3.0 MATERIALS AND METHODS

### 3.1 Selection and Restraint of Cows

Data were collected between the months of February and April 2011. The Boran animals were herded within the Kapiti plains ranch located in Machakos County of Kenya (latitude: 01$^{o}$ 30' 00" and longitude of 37$^{o}$ 00' 00").Data collection was done daily between 0900 and 1200 hours.

Thirty cows were selected from a herd of 100 Boran cows so as to ensure that data was collected only from cows that were between five and six years of age (with history of previous calving), were also open and cycling at the time of recruitment and not nursing. All the selected cows were of Body Condition Score (BCS) of 2-2.5, as recommended previously by Westendorf et al. (1988) in a scale of 1-5. Of the 30 cows, 15 were purposively selected for good health, temperament and presence of a *corpus luteum* within the ovary. This is the group that was used in this study.

The selected cows were mixed with heifers to graze together under uniform conditions of feeding and management during the entire study period. Animals were allowed to graze in the fields where kikuyu grass and natural shrub was in plenty. They were also supplied with mineral licks at the boma where they spent the night. Watering was done once a day at the dam in the grazing field where animals were allowed access to the water for a period of ninety minutes. The cows were also sprayed once a week using Triatix® accaricide against external parasites and no disease vaccination was carried out within the study period. Prior to examination and ovarian evaluations, each animal was restrained in a crush for ease of rectal palpation and ovarian ultrasonographic scanning procedures. Once all the cows were examined and data collected, they were released to graze in the open pastures.

### 3.2 Ovarian Evaluation

The fifteen selected cows were synchronized for estrous on the same day so as evaluate follicular waves under uniform conditions of folliculogenesis. Estrous synchronization was done using intramuscular injection of 500µg (2ml) of cloprostenol (Estroplan®) at 0900 hours, making this day 0 of the study.

From day one of the study (24hrs) after the injection mentioned above, each of the cows was evaluated for a period of two Estrous Cycle Lengths (ECLs). Cows were kept in an open boma at night with a watchman keeping guard and noting any heat activity. During the day, heat activity was observed and recorded at six o'clock in the morning and six o'clock in the evening for a period of 30 minutes each.

On a daily basis, the following activities were carried out for each animal: - (1) General body health assessment (2) Rectal palpation of the uterus and ovaries (3) Ultrasonographic scanning of the ovaries (4) Drawing of sketches of the three largest follicles within the scanned ovary (5)Measurement of follicle diameters (6) Counting and recording follicular populations within the scanned ovary (7) Recording of all ovarian structures observed (7) Measuring and recording the CL diameters (8) Entering and storing all static images obtained during scanning.

A 7.5 Mega Hertz dual frequency linear array probe was used to evaluate the ovarian structures. For each cow, palpation per rectum was done and the probe used to obtain daily ovarian pictures that were recorded and stored on a memory stick attached to the scanner. Daily sketches of the ovaries were made to give an indication as to where in the ovary the three largest follicles and/ or CL were located.

The ovaries were first palpated by hand to identify any follicles and/or CL. Thereafter, a linear probe set at 7.5 MHz and guided by the hand was inserted into the rectum after being enclosed in a disposable sleeve filled with the acoustic jelly to cover the surface of the transducer. The transducer was then moved cranially and placed dorsal to the ovary. The transducer was then moved slowly back and forth across the ovary to scan the entire surface. Scanning of both ovaries was done and the images of the ovarian structures stored within the scanner and all other noted parameters recorded in a research book. All palpated and scanned structures, together with their relative locations within the ovary, were sketched on a note book and the sketches were used to trace the same structures within the same ovary the following day during scanning. All examinations were made by the same operator.

Ovarian assessment and the ultrasonic identification of the ovarian structures were done as described previously (Ginther1995). Briefly, the ovary was traced as a globular irregular hypo-echoic structural area with a surrounding hyper-echoic tunica. The clear and diffuse area in the inferior part was indicative of the ovarian stroma while the white area was the tunica albuginea (Fig 1). The medullar layer of the ovary was seen as hyper-echoic diffuse pattern, while the cortical layer was normo-echoic or moderately hypo-echoic. The hilus was traced as a hyper-echogenic line that penetrated the ovary.

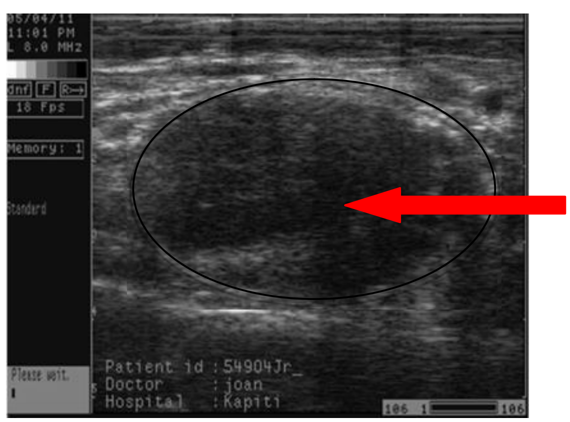


**Figure 1: Ultrasonic image of the bovine ovary; the echogenicity of the stroma and surrounding tissues is shown (ovary area within the circle shown by arrow)**

During scanning, all follicles were counted and their numbers recorded under three categories as previously reported by Muasa (2010) in slaughter house material. Group one: those with diameter of 1-3mm, group two: those with a diameter of 4-6mm and group three: those with diameters 7mm and above. The diameters of the three largest follicles on every ovary were measured and recorded and their location within the ovary sketched daily on a Research Reference book. The sketches of the three biggest follicles were used to easily trace them for evaluation of their daily changes in diameter as well as daily follow up within the ovary until ovulation and/or atresia occurred. This was done by first obtaining static images (freezing the images) and then measuring the diameter using software within the Aquilla Veterinary Ultrasound Scanner as previously described (Burke *et al*., 2000). Briefly, the scanning was made in such a way that the upper and lower lines of the greater lengths of the diameters were visible as thin white lines within the image of the structure being assessed. These diameters were then determined using inbuilt electronic calipers of the scanner. These activities were repeated daily for two estrous cycle lengths. After ovulation, a subsequent CL was noted and its daily diameter was measured and noted.

The daily sketches of the three largest follicles were used to track and evaluate the wave dynamics. The various follicular parameters visible on the ovary were determined as previously described (Alvarez *et al.,* 2000; Burke *et al.,* 2000). Follicular populations were obtained through counts of all visible follicles within the image showing the entire ovary. Diameters of the follicles were then measured and grouped according to their sizes as described previously by Muasa (2010; see above under section 4.2.1).

The follicle was described as dominant follicle (DF) of a wave when its diameter measured at least 7mm and above, and clearly exceedingthe diameter of all other follicles in the wave (Ginther*et al.,* 1996). A subordinate follicle (SF) was described as the one that originated from the same follicular pool as the dominant follicle but then terminated growth after the deviation period. These follicles were noted as the second and the third largest follicles subsequently seen and labeled the Subordinate Follicles One (SF1) and Two (SF2) respectively. Ovulation was described whenever disappearance of the dominant follicle was noted and subsequently a CL formed at the same location on the ovary.

Description of number of waves was done as previously documented by Ginther et al. (1989a). Briefly, cycles were described as having two, three or four waves of follicular development depending on number of waves that formed before a dominant follicle ovulated. For instance, a cow in which the DF of the first wave regressed and that of the second wave ovulated was noted to behaving two follicle waves during that estrous cycle while Three and four wave cycles had their respective ovulatory DF within the third and fourth waves of follicle development. The day of wave onset/wave emergence was defined as the day on which the dominant follicle was retrospectively traced to be 1 to 3 mm in diameter (Burke *et al.,* 2000).Such definitions were made possible by obtaining the growth rate for each particular follicle so as to trace back the day of the cycle when that follicle emerged. Atresia onset was considered as the day from which follicles began reducing in size.

Maximum diameter and growth rate of the dominant follicle within all the follicular waves were also measured and estimated. The growth rate of the dominant follicle was determined from the day the dominant follicle was first identified to the day when its diameter no longer increased more than 1 mm (Alvarez *et al.,* 2000). Growth rate (mm/day) of follicles was calculated by subtracting the diameter on the day of detection from the maximum diameter and dividing this by the interval in days (Rhodes *et al.,* 1995).

### 3.3 Data Management and Analysis

A summary sheet for all information obtained during rectal palpations, daily scans and sketches was prepared in a Research Book for ease of reference. All images of the scans were stored within the memory space of the scanning machine and also within an external memory disk. Entries for diameters of various follicles, *corpus luteum* and counts of follicular populations were then entered into a computer excel sheet. The excel sheets were then exported to Statistical Package for the Social Sciences (SPSS) edition 12.0 for analysis. The excel sheets data were used to make plots and charts for ease of reporting. Descriptive statistics was used to calculate the mean, variance and standard error of means. Comparisons between the groups (two-wave, three-wave and four-wave cycles) were done using analysis of variances. Correlations for parameters of follicle growth rate, estrous cycle length, wavelength and dominance of the first wave within and among the groups was done using SPSS statistical software and reported as tables.

## CHAPTER 4

## 4.0 RESULTS AND DISCUSSION

### 4.1 Palpation per Rectum

Relatively soft (fleshy) structure on the surface of the ovary in the location of a preceding graafian follicle was palpated and identified as a *corpus hemorrhagicum*. The *corpus luteum* was felt as a hard structure protruding over the surface of the ovary, with a distinct crown and a neck. Soft blister-like pitting areas (follicles) of different sizes were palpable whenever they appeared. The structures were sketched on a research book (fig 2 and 3) for ease of location during scanning as previously described (Pieterse *et al.,* 1990).

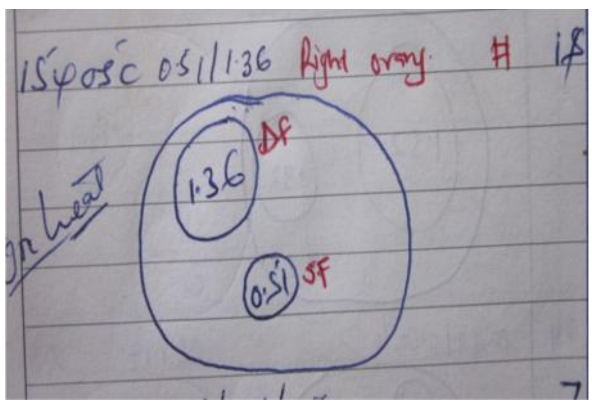


**Figure 2: Sketch of the Right Ovary as Felt during Palpation per Rectum (animal number 15405C)**

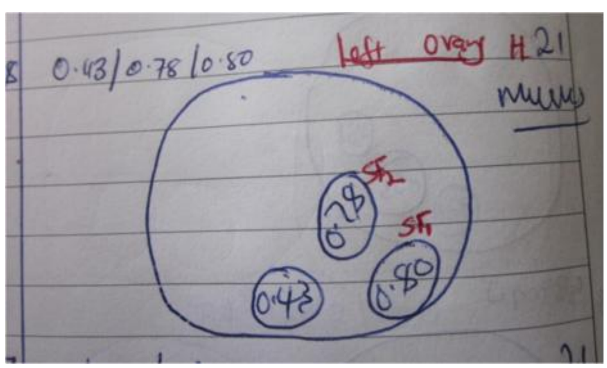


**Figure 3: Sketch of the Left Ovary as Felt during Palpation per Rectum (animal number 15405C)**

### 4.1.2 Appearance of Ovarian Structures during Ultrasonography

Different sizes of follicles appeared as black circumscribed (an-echoic) zones during scanning. The images of the follicles were sometimes irregularly shaped whenever neighboring structures impinged onto them. Small follicles of 1-3 mm in diameter appearing in a group were the initial structures to be identified (Fig 4, see arrow).


**Figure 4: Sonogram of an ovary with small sized follicles (≤3mm in diameter and less)**

The initial group then developed into a group of larger but fewer follicles (4-6 mm in diameter) as folliculogenesis progressed (Fig 5, see arrow).

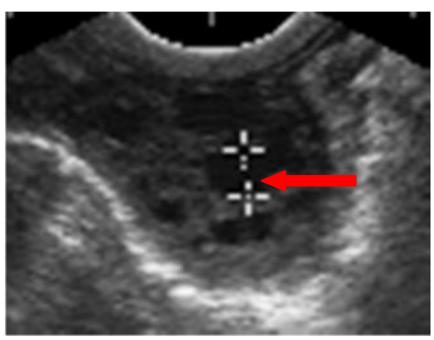

**Figure 5: Sonogram of ovary with Medium Sized Follicles (4-6mm in diameter)**

Dominant follicles (DFs) appeared as follicles measuring over 7mm in diameter (Fig6, see arrow). The ovulatory dominant follicle (DF) was the largest in diameter and had an-echogenic antrum surrounded by an external hyper-echogenic layer. The image of the ovulatory DF disappeared from the ovary the following day of scanning due to loss after the ovulation process.

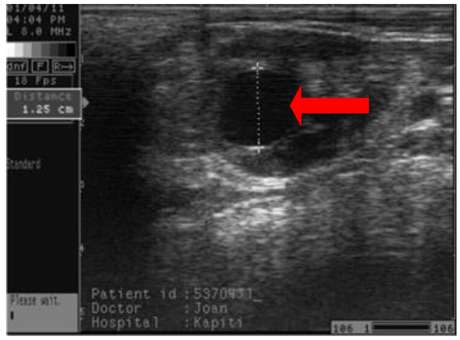


**Figure 6: Sonogram of the ovary with a Dominant Follicle measuring 12.5mm in diameter**

A cystic follicle appeared similar to a DF but it persisted in the ovary for over 10 days. It also had distinctly thin walls and measured over 20mm in diameter (Fig 7, see arrow). Only one cow had a cystic follicle

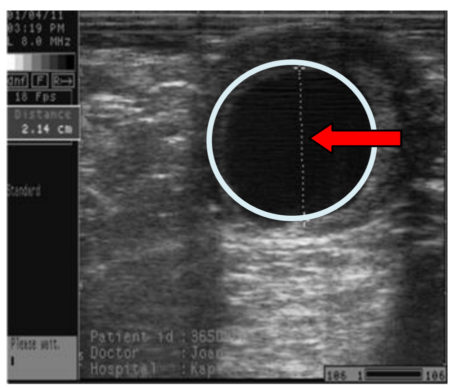


**Figure 7: Sonogram of a Follicular Cyst Measuring 21.4mm in Diameter**

The corpus luteum (CL) was seen as a granular echogenic structure with distinct borders (Fig 8, see white circle) and it was noted three to four days after day of ovulation. It attained maximum diameter 12 to 14 days post ovulation.

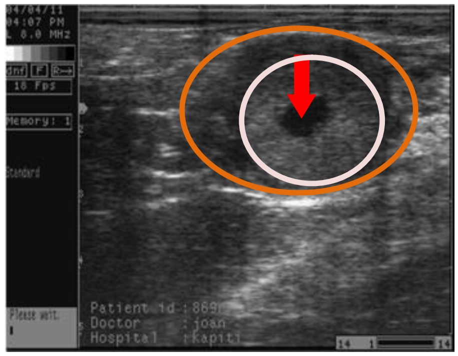

**Figure 8: Sonogram of an Ovary with a *Corpus Luteum* (CL; white circle)**

The images detected and described in this study for follicles are similar to those previously described (Ginther*et al.,* 1989a). Although a pathological condition of a follicular cyst was detected in only one cow, such pathologies are normal occurrences. The most frequently encountered pathological conditions of the bovine ovaries are follicular and luteal cysts. Follicular cysts are follicles that fail to ovulate, exceeds 25mm in diameter and persists for more than 10 days (Al-Dahash and David 1977b). A cyst is usually a symptom of disturbance of ovarian function (Sakaguchi *et al.,* 2006). A follicular cyst can be differentiated from a luteal cyst by its thin wall and uniformity in an-echogenicity of its follicular fluid (Ginther 1998).

The images described for the CL in this study agrees with those described by Kastelic *et al*. (1990b). These CL sometimes contain cavities measuring up to 2-10 mm in diameter, as also noted in four Boran cows in the study (Fig 6; red arrow). The cavities in these CL last less than 10 days.

### 4.2 Number of Follicular Waves per Estrous Cycle

The mean estrous cycle length (n= 15) for the cows was 22.7 ± 4.64 days. Two, three and four follicle waves per estrous cycle were noted (Fig 9-11). Four cows (26.67%) had two follicular waves per cycle (Fig. 9), 10 cows (66.67%) had three follicular waves per cycle (Fig. 10) and one animal (6.67%) had four follicular waves per cycle (Fig 11).

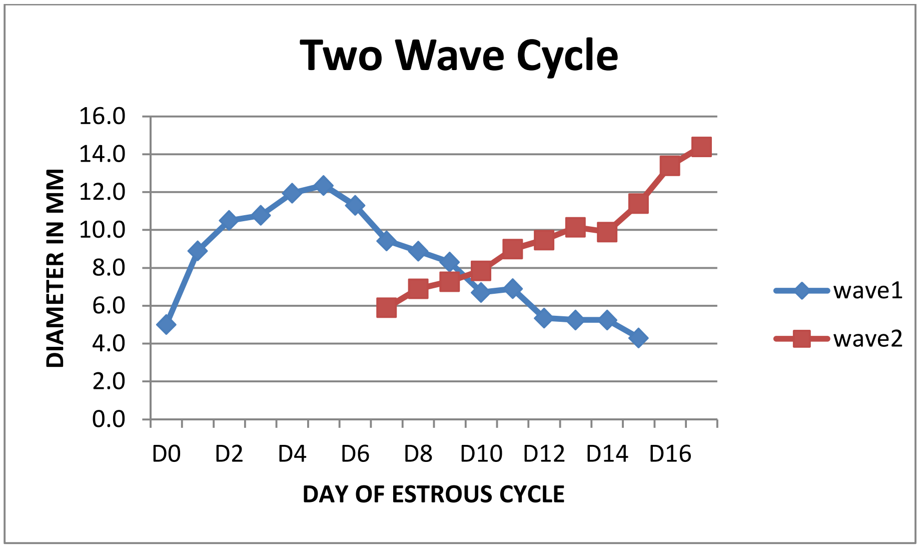


**Figure 9: Two Wave Cycle Dominant Follicles**

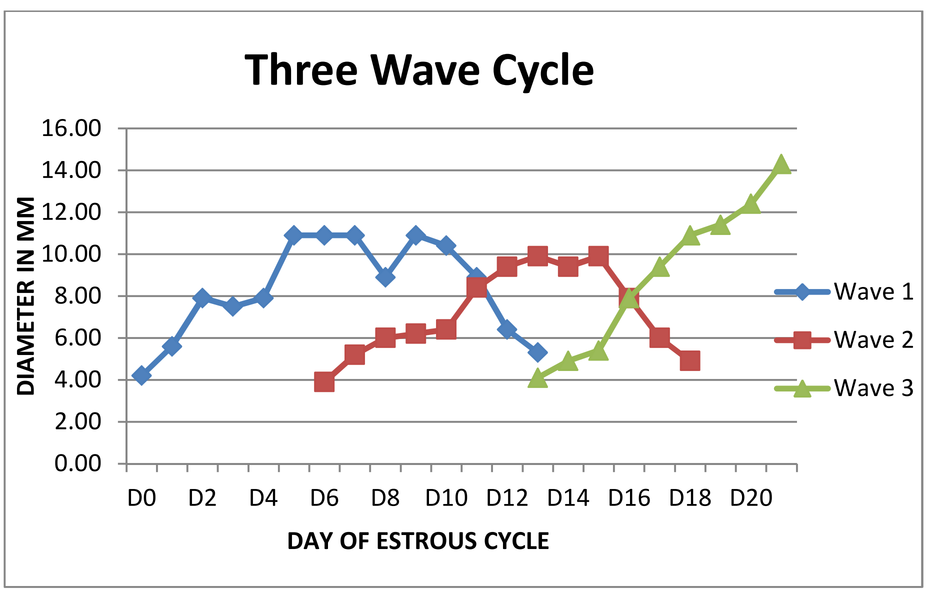


**Figure 10: Three Wave Cycle Dominant Follicles**

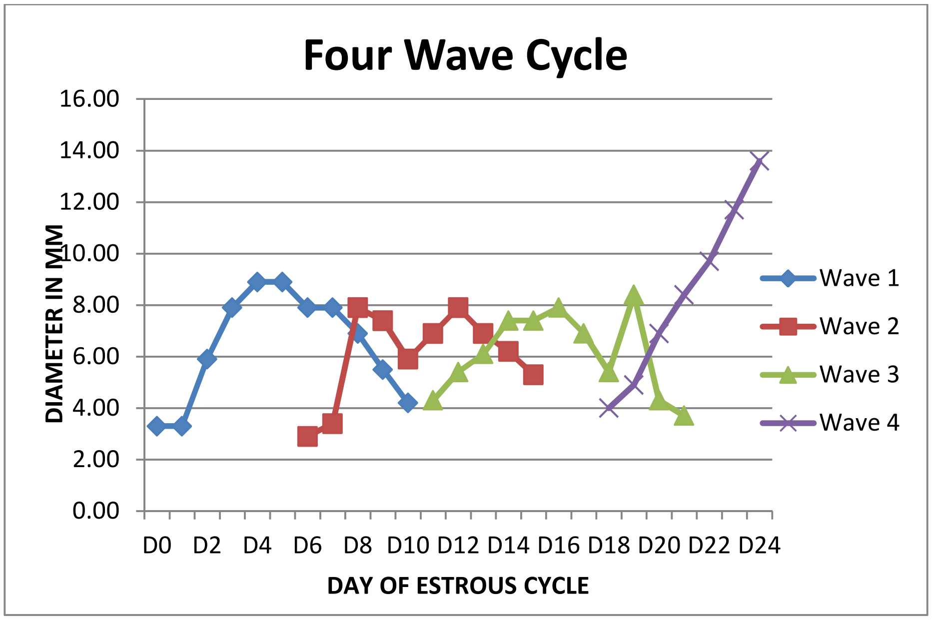


**Figure 11: Four Wave Cycle Dominant Follicles**

The occurrences of follicular waves in the two consecutive estrous cycle periods in a cow revealed 86.7% repeatability of wave distribution (Fig 12). Two cows (13.3%) had different follicular waves per cycle; one cow had two waves followed by three waves per cycle while the other cow had three waves followed by four waves per cycle. These two animals were grouped according to the wave pattern of the first estrous cycle.

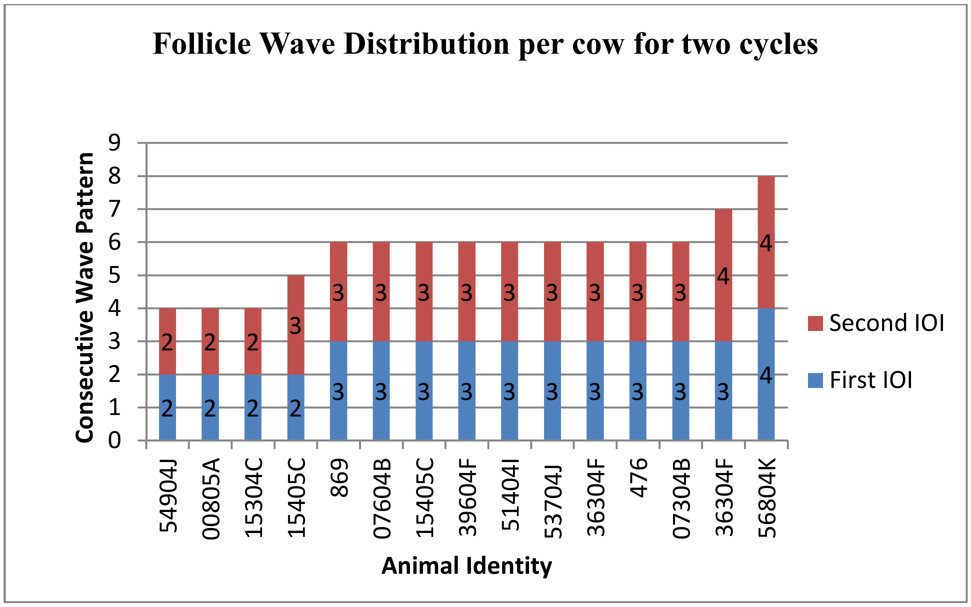


**Figure 12: Distribution of Follicle Waves in Cows for Two Consecutive Estrous Cycle Lengths**

There was a positive correlation (P>0.05) between the number of follicle waves in a cycle and the estrous cycle length (ECL) (Fig 13); ECL increased with increasing number of follicular waves per cycle. The mean ECL was 25.0 ± 4.0 days for four-wave cycles, 23.6 ± 1.05 days for three-wave cycles and 18.6 ± 1.9 days for two-wave cycles.

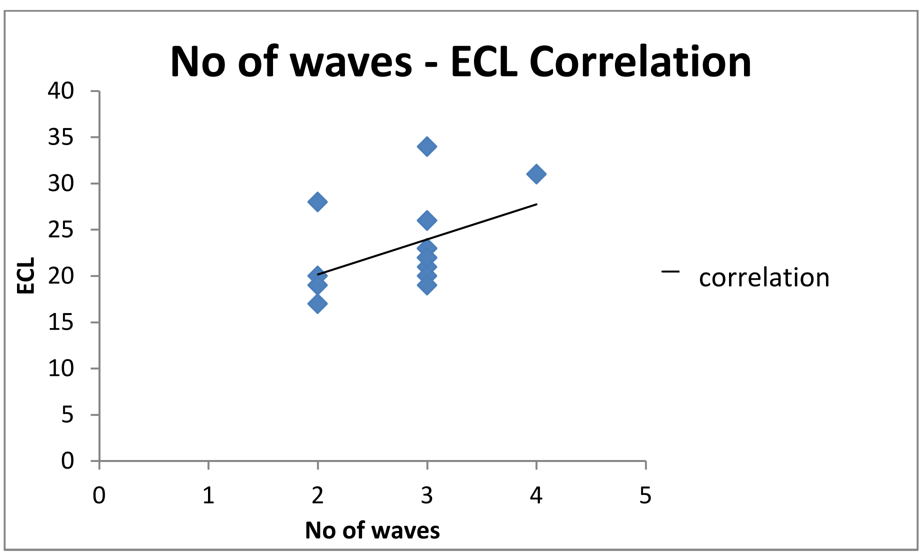


**Figure 13: Correlation of Number of Waves and Estrous Cycle Length**

Minor differences in follicle development can be noted for the Boran cows when compared to other breeds. However, generally the dynamics of follicle development noted for the Boran cow is similar to those described for other breeds. The Gir (Gambini *et al.,* 1998; Viana *et al.,* 2000) and Brahman cows (Zeitoun *et al.,* 1996) have similar follicle waves per cycle like Boran cow. The European cows have also been shown to have either two or three follicular waves per cycle with a higher prevalence of the three follicular waves per cycle (Savio *et al.,* 1988; Sirois and Fortune 1988; Adams 1999). Although the adult Nelore cows (Zebu) had two- and three follicular waves percycle and a higher incidence of the two follicular wave cycles, their heifers had three follicular wave cycles like the Boran cows (Figueiredo *et al.,* 1997).

The mean ECL of the Boran is similar to one observed across breeds (Sartori *et al.,* 2004; Bó *et al.,* 2003). In this study there was a tendency of an increase in number of follicular waves with the lengthening of the cycle (r = 0.449; P = 0.053). This observation agrees with similar observations by other authors (Alvarez *et al*., 2000; Viana *et al.,* 2000; Townson *et al*., 2002; Sartori *et al*., 2004). There have been, however, exceptions in which high-producing lactating Holstein cows had longer estrous cycle due to a prolonged time between luteolysis and ovulation (Sartori *et al*., 2004).

Previously, as it is the case in this study, a higher occurrence of distribution of two- and three-wave patterns has been reported for cows (Sirois and Fortune 1988; Ginther et *al.,* 1989a; Taylor and Rajamahendran 1991; Savio *et al.,* 1990a; Adams 1994; Evans *et al.,* 1994; Celik *et al.,* 2005). Though factors predictive of a specific pattern have not been identified and the repeatability of a given wave pattern within individuals is not yet known, low plane of nutrition (Rhodes *et al.,* 1995) and heat stress (Wolfenson *et al.,* 1995) have been reported to cause increase in occurrences of three-wave patterns in Taurine cows. However wave patterns in Zebu cows are shown to be influenced by parity (Figueiredo *et al.,* 1997).

### 4.3 Dynamics of Follicles; size, number and growth patterns of follicles within a wave

#### 4.3.1 Sizes of Follicles within a Wave

The growth of follicles was in phases according to the sizes of follicles noted. Three groups of follicles were detected (Fig 14).

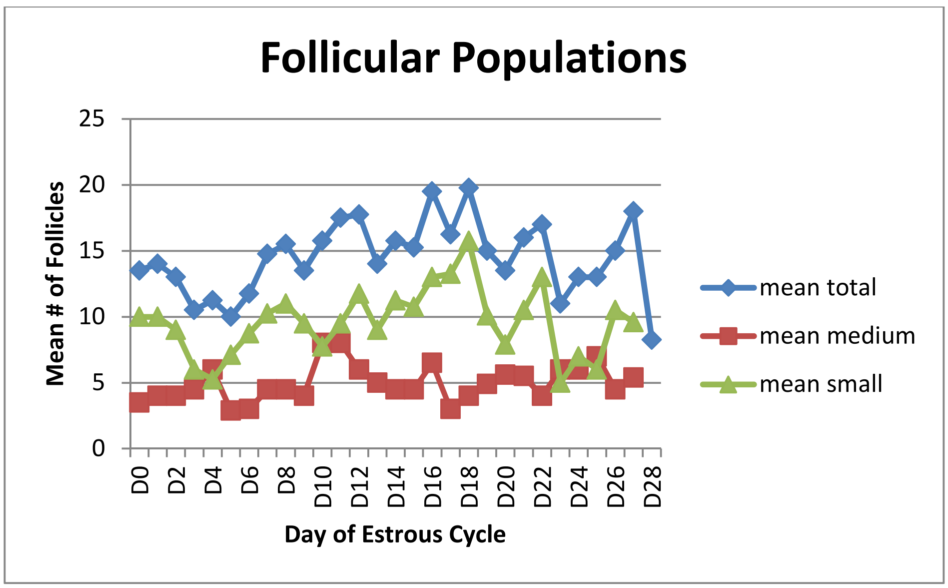


**Figure 14: Population of Follicles in a Cycle**

Group one of follicles (1-3 mm) were observed during the phase of wave emergence and the follicles. Group two follicles (4-6mm) were observed during the phase of selection while group three follicles were in dominance phase and their diameters were above seven millimeters (mm). The mean follicle population was 7.12 ± 1.12 for emergence phase (Group one) and 4.12 ± 0.46 for selection phase (Group two). A significantly larger number of follicle population of group one and two follicles ($P \leq 0.05$) developed in the left ovary but more of the group three follicles developed in the right ovary.

### 4.3.2 Numbers and Growth Patterns of Follicles within Waves

Three stages of follicle development were identified; the growing stage when the follicle diameter was increasing, the static stage when the follicle diameter was not changing, and the regressing stage when the follicle diameter was reducing (Fig 15). The mean period of the growth stage for the DF of wave one in cows with two-wave cycles was 7.8± 0.6 days while the mean period of regression for same follicles was 4.0±1.61 days. Non-ovulatory DFs of cows with three follicular waves had a short growth period of 5.50±1.5 days but were of long regression period of 7.0±1.0 days. The period of DF growth stage in three-wave and four-wave cycle cows was similar ($P \leq 0.05$).

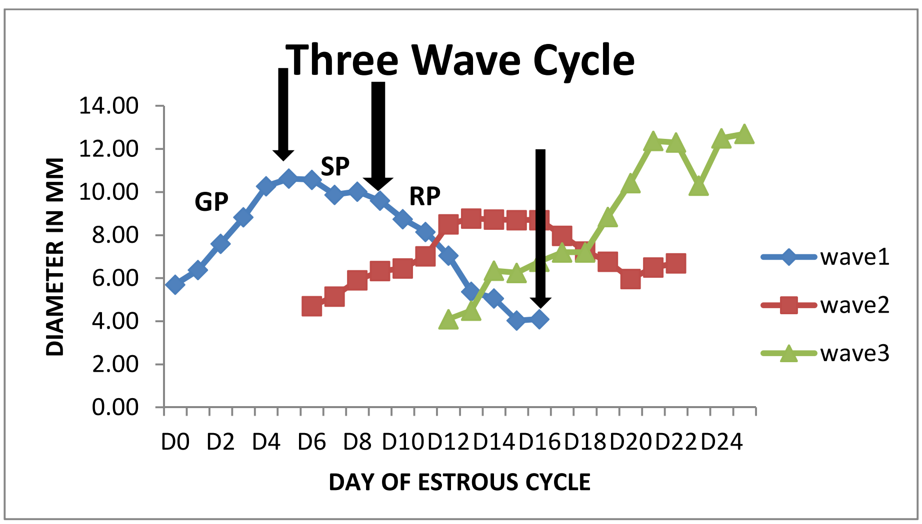


**Figure 15: Stages of Follicle Development**

***KEY: GP** –Growth Stage, **SP**- Static Stage, **RP**- Regression Stage*

The first wave in two-wave cycles emerged on day 0.61 ± 0.06 while the second wave emerged on day 10.40 ±1.17 (Table 1). At emergence phase the mean number of follicles was 17.5 ± 2.67. Inthree-wave cycles the waves emerged on day 0.42 ± 0.16 for wave one and the number of follicles was 15.2 ± 1.98. The second and third waves in these cows emerged on day 8.15±0.56 and 15.00±0.98, respectively. Four wave cycle animals had their waves emerge on day 0.5 ± 0.5, 7.0 ± 2.0, 14.5 ±3.5 and 17.0 ±3.0 for waves one, two, three and four, respectively and the number of follicles was 16.4 ± 1.66. Four-wave cycle cows had significantly longer period of second wave while two-wave and three-wave cycle cows had longer periods of first wave (P≤0.05).

**Table 1: Wave Onset and Wavelengths in different Follicle Wave Cycles**

| | | **First wave** | **Second wave** | **Third wave** | **Fourth wave** |
|---|---|---|---|---|---|
| ***Wave onset*** | 2-wave cycle | $0.61{\pm}0.06^{a}$ | $10.40{\pm}1.17^{b}$ | | |
| | 3-wave cycle | $0.42{\pm}0.61^{a}$ | $8.15{\pm}0.56^{b}$ | $15.00{\pm}0.98^{c}$ | |
| | 4-wavecycles | $0.50{\pm}0.50^{a}$ | $7.00{\pm}2.00^{b}$ | $14.50{\pm}3.50^{c}$ | $17.00{\pm}3.00^{d}$ |
| ***Wavelength*** | 2-wave cycle | $16.20{\pm}1.24^{1}$ | $11.60{\pm}1.20^{2}$ | | |
| | 3-wave cycle | $15.77{\pm}1.03^{1}$ | $13.15{\pm}1.48^{1}$ | $8.77{\pm}0.74^{2}$ | |
| | 4-wavecycles | $10.00 \pm 2.00^{1}$ | $13.50 \pm 4.50^{1}$ | $8.50 \pm 1.50^{1}$ | $7.00 \pm 1.00^{2}$ |

$^{ab;12:}$ ***values followed by different superscript letters in the same row differed significantly P≤0.05; values in means ± SEM***

Follicle selection (Figure 16) in cows with two-wave cycles occurred in day 3.16±0.56 when the dominant follicle had diameter of 5.21 ± 0.56 mm for the wave one and on day 10.23±1.98 with DF diameter of 5.97 ± 0.40 mm for wave two. The follicle selection for cows with three wave cycles occurred on day 4.13±0.99 with DF diameter of 6.01 ± 0.67mm, day 12.53± 1.9 with DF diameter of 4.12 ± 0.34mm and day 19.10 ± 2.03 with DF diameter of and 5.20 ± 0.39mm for waves one, two and three, respectively. In four wave cycle cows selection occurred on day 2.9 ± 0.17 with DF diameter of 3.7 ± 0.26mm for wave one, day 9.8 ± 0.5 with DF diameter of 5.6 ± 0.45mm for wave two, day 17.3 ± 0.7 with DF diameter of 4.9 ± 0.34mm for wave three and day 20.1 ± 0.87 with DF diameter of 5.9 ± 0.33 mm diameter for wave four.

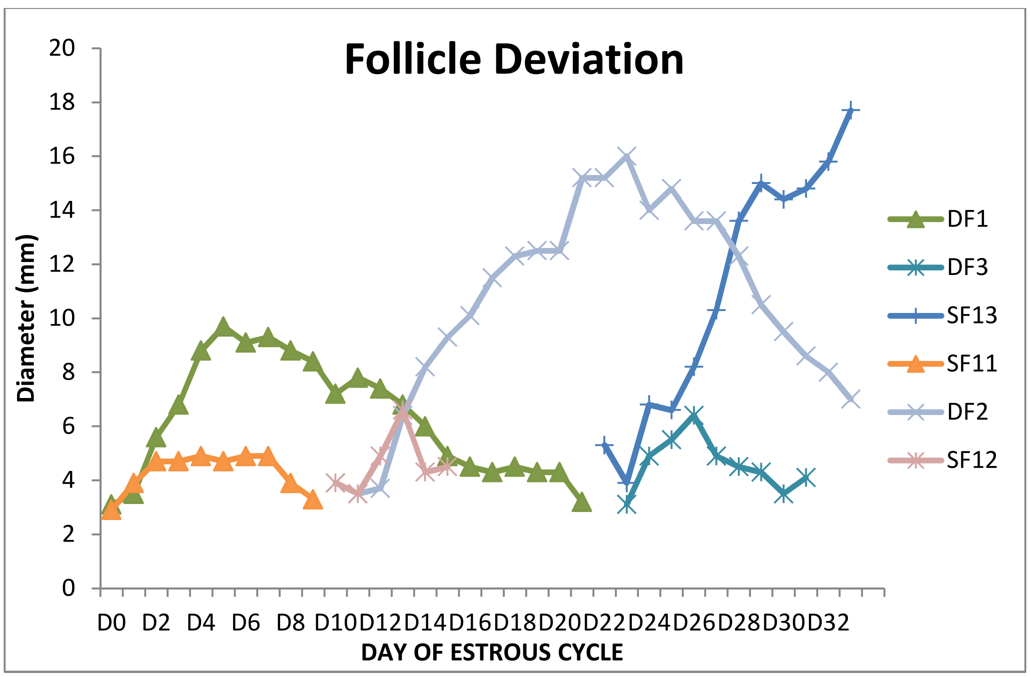


**Figure 16: Follicle Deviation for Cows with Three Follicle Wave Cycles**

***The arrows show the day and diameter at divergence (occurrence of observed deviation) of the Dominant Follicle (DF)***

Two types of dominant follicles were observed; non-ovulatory and ovulatory follicles. In every wave of follicle development, it was the ovulatory follicle that attained the maximum diameter when compared to the other selected dominant follicles (Table 2). The mean maximum diameter of the ovulatory follicle for the Boran cow was 13.56 ± 1.73mm.

**Table 2: Diameters of Dominant follicles in Two-, Three-, and Four-Wave Cycles**

| **PARAMETERS** | **Inter-Ovulatory Intervals** | | |
|---|---|---|---|
| | **2-wave cycles** | **3-wave cycles** | **4-wave cycles** |
| Maximum diameter of non-ovulatory DF 1 (mm) | 13.7 ± 0.92$^{a,1}$ | 10.95 ± 0.34$^{a,1}$ | 10.6 ± 1.75$^{b,1}$ |
| Maximum Diameter of non-ovulatory DF2 | | 10.63± 0.58$^{a,1}$ | 8.00 ± 0.60$^{b,1}$ |
| Maximum Diameter of non-ovulatory DF3 | | | 8.80 ± 0.54$^{1}$ |
| Maximum Diameter of ovulatory dominant follicle | 14.0 ± 0.85$^{a,1}$ | 13.52 ± 0.50$^{b,2}$ | 12.85 ± 0.75$^{c,2}$ |

***$^{abc}$values in superscript within the same row differ significantly (p≤0.05). $^{123}$ values in superscript in the same column differ significantly P≤0.05*****.Values given as mean mm ± SEM**

Differences in sizes of dominant follicle within waves were observed (see superscript values in Table 2). Dominant follicles were detected more in the right ovary; mean frequencies of occurrences of 51.3% - 80% in all follicle waves. One cow had two equally dominant follicles (double dominance) in two follicular waves (Fig 17).

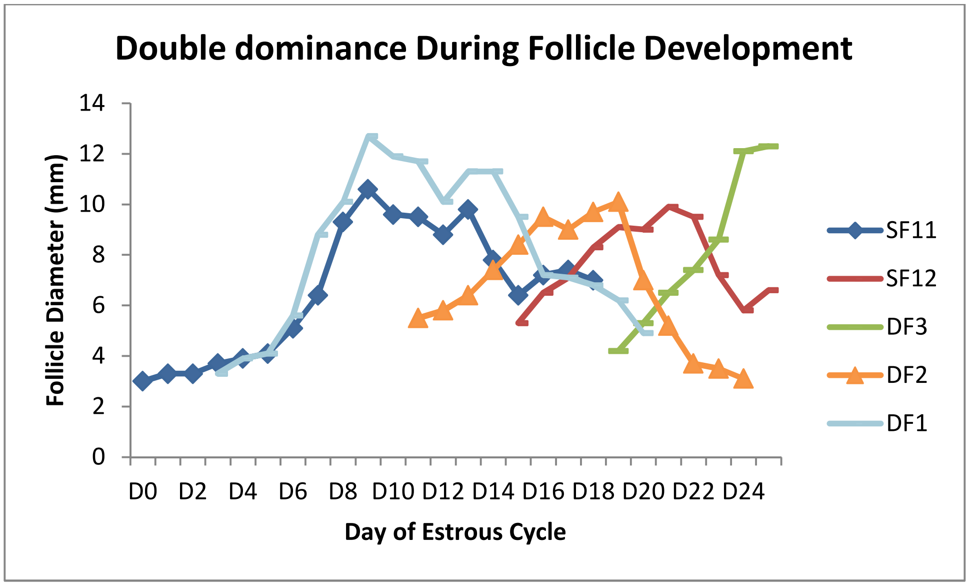


**Figure 17: Double Dominance of Follicles during Folliculogenesis**

The dominant follicle developed significantly at faster rates than the other follicles (Table 3). The two closest follicles (subordinate follicles in the wave) did not show any difference in their growth and atresia rates (P≤0.05).

**Table 3: Mean Growth and Atresia Rates (mm/day) of Three Largest Follicles in Cows in different Wave Cycles**

| Parameter | Dominant Follicle | | Subordinate Follicle 1 | | Subordinate follicle 2 | |
|---|---|---|---|---|---|---|
| | Growth rate | Atresia rate | Growth rate | Atresia rate | Growth rate | Atresia rate |
| Two-wave Cycles | $0.85 \pm 0.03^{a}$ | 1.01 ± 0.29 | $0.56 \pm 0.77^{b}$ | 0.58 ± 0.11 | $0.36 \pm 0.27^{b}$ | 0.50 ±0.51 |
| Three-wave Cycles | $0.83 \pm 0.04^{a}$ | 0.97 ± 0.07 | $0.55 \pm 0.48^{b}$ | 0.62 ± 0.06 | $0.29 \pm 0.26^{b}$ | 0.49±0.47 |
| Four-wave Cycles | $0.95 \pm 0.13^{a}$ | 1.32 ± 0.27 | $0.50 \pm 0.05^{b}$ | 0.93 ± 0.28 | $0.27 \pm 0.20^{b}$ | 0.52 ±0.30 |

$^{a\,b}$: *Data with different superscript within the same row differ significantly at (p≤0.05)*

No significant difference was observed in the duration of dominance of the DFs in two and three wave cycle cows but cows with four-wave cycles, the first and the second wave DFs had dominance durations that were significantly different than those of third and fourth waves ($P < 0.05$). The main characteristics observed for follicle development for cows with two follicular wave patterns are shown in Table 4. Although the maximum diameter of the dominant follicles did not differ significantly, the day dominant follicles achieved maximum diameter, as well as growth rate and lengths of their growth phases showed significant differences between the two waves ($P \leq 0.05$).

**Table 4: Dynamics of Follicle growth in Cows with Two Follicle Waves per Cycle (Mean ± SEM)**

| CHARACTERISTIC | FOLLICULAR WAVE | |
|---|---|---|
| | FIRST | SECOND |
| **DOMINANT FOLLICLE** | | |
| Maximum diameter (mm) | 13.6± 0.92 [a] | 14.00 ± 0.85 [a] |
| Day of maximum diameter | 7.20 ± 0.37 [a] | 19.40 ± 2.01 [b] |
| Growth rate (mm/day) | 0.92 ± 0.04 [a] | 0.78 ± 0.04 [b] |
| Length of growth phase | 7.8 ± 0.60 [a] | 11.60 ± 1.21 [b] |
| Duration of dominance | 12.40 ± 1.12 [a] | 9.80 ± 1.16 [a] |
| Onset of atresia(days) | 12.20 ± 1.11 [a] | |
| Atresia rate (mm/day) | 1.01 ± 0.29 [a] | |
| Length of atresia (days) | 4.00 ± 1.61 [a] | |
| **LARGEST SUBORDINATE FOLLICLE** | | |
| Maximum diameter (mm) | 7.40 ± 0.82 [a] | 7.12 ± 0.42 [a] |
| Growth rate (mm/day) | 0.60 ± 0.11 [a] | 0.52 ± 0.12 [a] |
| Atresia rate (mm/day) | 0.74 ± 0.19 [a] | 0.43 ± 0.92 [a] |

***[a b] values with superscript letters in the same row differed significantly $P\leq0.05$; Values in means*** *±SEM*

The dominant follicles differed in terms of their growth onset, wave length, maximum diameter and atresia onset (Table 5; P≤0.05) only in cows with three follicle waves per cycle.

Table 5: Dynamics of Follicle Growth in Cows with Three Follicle Waves per Cycles(mean ± SEM)

| CHARACTERISTIC | FOLLICULAR WAVE | | |
|---|---|---|---|
| | **FIRST** | **SECOND** | **THIRD** |
| **DOMINANT FOLLICLE** | | | |
| Maximum diameter (mm) | 10.95 ±0.34 [a] | 10.63 ± 0.58 [a] | 13.52 ± 0.50 [b] |
| Day of maximum diameter | 6.31 ± 0.44 | 14.54 ± 1.22 | 22.31 ± 1.04 |
| Growth rate (mm/day) | 0.87 ± 0.07 [a] | 0.73 ± 0.52 [a] | 0.87 ± 0.81 [a] |
| Length of growth phase | 7.08 ± 0.40 [a] | 7.77 ± 0.77 [a] | 8.62 ± 0.76 [a] |
| Duration of dominance | 9.31 ± 0.60 [a] | 8.38 ± 0.99 [a] | 8.77 ± 0.74 [a] |
| Onset of atresia | 10.15 ± 0.58 [a] | 16.23 ± 1.18 [b] | |
| Atresia rate (mm/day) | 0.91 ± 0.10 [a] | .01± 0.10 [a] | |
| Length of atresia (days) | 5.69 ± 0.90 [a] | 4.46 ± 0.63 [a] | |
| **LARGEST SUBORDINATE FOLLICLE** | | | |
| Maximum diameter (mm) | 6.74±0.42[a] | 6.89 ± 0.37 [a] | 7.28 ± 0.40 [a] |
| Growth rate (mm/day) | 0.60 ± 0.90 [a] | 0.49 ± 0.07 [a] | 0.57 ± 0.10 [a] |
| Atresia rate mm/day) | 0.63 ± 0.10 [a] | 0.69 ± 0.08 [a] | 0.58 ± 0.11 [a] |

***abc values*** *followed by different letters in subscript in the same row differ significantly P≤0.05; Values presented in means ±SEM*

The findings for the Boran cow on wave emergence compared favorably to what was observed in European cows (Savio *et al*., 1988; Sirois and Fortune 1988; Gambini *et al*., 1998) and also for other Zebu cows (Sartori et al., 2010; Viana *et al*., 2000). Follicular emergence is usually in reference to the day of ovulation and it is characterized by the sudden growth of 8 to 41 small follicles (Jaiswal *et al*., 2004; Sartori *et al*.,2004). In other breeds wave emergence for two-wave cycle cows occur on day 2-4 and 10-11, which is in the same range with the Boran cows. As during recruitment phase the follicles are highly responsive to FSH, this forms the best timing for superovulatory activities as these responsiveness is lost as the follicles get to the next phase of their development.

Similar to the other studied Zebu cows, follicle selection in the Boran cow occurred when the DF was somewhat smaller than in the Taurine breeds (Ginther *et al.,* 1996; Sartori *et al.,* 2001; Sartorelli *et al.,* 2005; Castilho *et al.,* 2007; Bastos *et al*., 2010). Follicle deviation is characterized by a decrease in the growth rate of the largest SF and an increase in the growth rate of the DF (Ginther *et al*., 2001).

The maximum diameters of both the non-ovulatory and ovulatory follicles of the Boran cow seem to be smaller than those reported for Taurine cows (Ginther *et al.,* 1989b). However, the Boran cow is similar to other Zebu cows, whose DF diameters have been reported (Figueiredo *et al*., 1997; Sartorelli *et al*., 2005).

This study notes that in the Boran cows the largestdiameter was attained by the ovulatory DF ($P<0.05$). This was also noted and explained by Viana et al. (2000) for Gir cows and it is postulated that the status of the ovulatory DF provides the FSH stimulus for stronger growth so as to provide for larger diameter (Ginther *et al*., 1989b; Adams *et al*., 1992; Sunderland *et al*., 1994; Perez *et al*., 2003). The higher incidence of ovulatory DFs in the right ovary is attributed to the fact that the right ovary receives more blood supply compared to the left one and it is clinically observed to be more active than the right ovary.

The occurrence of more than one dominant follicle during a follicle wave in monovular species can either be a defect in the deviation mechanism (Fricke and Wiltbank 1999; Wiltbank *et al*., 2000) or a case in which twining would normally occur (Fricke and Wiltbank 1999).

All the characteristics observed for the DFs of the various waves for the Boran cow compare well with others documented for other breeds of zebu cows (Savio *et al*., 1988; Sirois and Fortune 1988; Taylor and Rajamahendran 1991;Viana *et al*., 2000).

### 4.4 Trends on Luteal Development

The *corpus luteum* in the Boran cow developed in a pattern that was similar throughout the cycle (Table 6, Fig 18).

**Table 6: Luteal Development Characteristics (mean ± SEM)**

| | **2-Wave Cycle** | **3-Wave Cycle** | **4-wave Cycle** | **P-value** |
|---|---|---|---|---|
| **Inter-ovulatory interval (days)** | 18.6 ± 1.9 | 23.6 ± 1.05 | 25.0 ± 4.0 | 0.03 |
| **Maximum diameter of CL (mm)** | 17.0 ± 1.29 | 15.9 ± 0.64 | 15.1± 0.9 | 0.44 |
| **Day* of onset of regression of CL** | 10.8 ± 1.74 | 14.4 ± 0.92 | 15.4±0.87 | 0.05 |
| **Day of maximum diameter of CL** | 11.2 ± 2.18 | 12.7 ± 0.61 | 15.5±4.50 | 0.21 |

**Day 0 = ovulation*

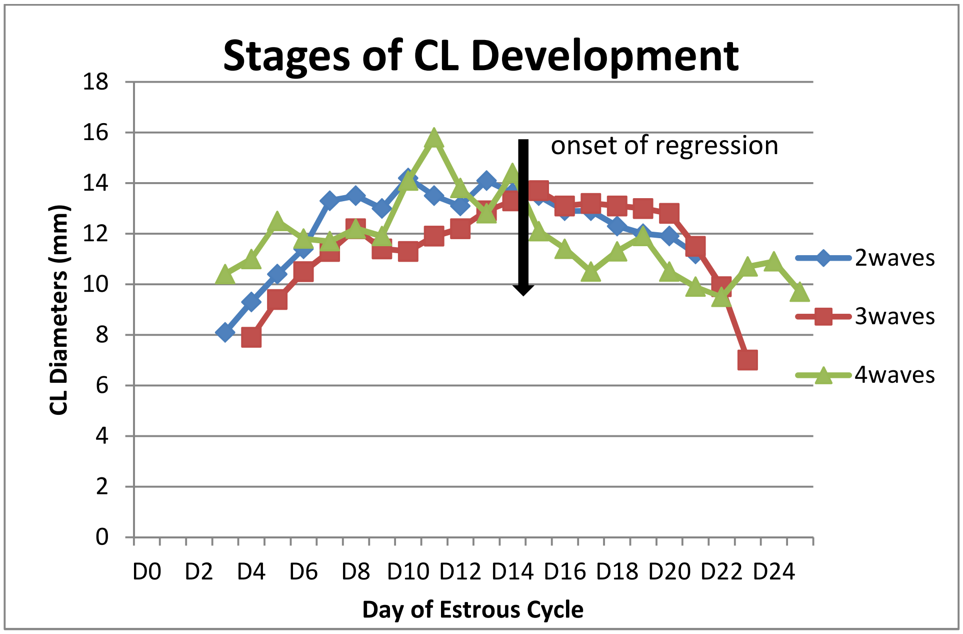


**Figure 18: Stages of *Corpus Luteum* Development**

Three phases of CL development were noted; a growth phase characterized by steady increase in the diameter of CL, a plateau or lag phase characterized by no increase in diameter of the CL and the luteal regression phase where there was a decrease in the diameter of the CL. There was no significant difference between the maximum diameters of the CL among cows with different waves per cycle. The CL seemed to regulate the dominance period of the DF in the Boran cow (Table 7).

**Table 7: Correlation between CL and Follicular Development**

| CORRELATE | r value | p value |
|---|---|---|
| Duration of dominance of Wave 1 versus day when Wave 2 dominant follicle became largest | 0.71* | 0.00 |
| Duration of dominance of Wave 1 versus day of onset of CL regression | -0.052 | 0.83 |
| Duration of dominance of Wave 1 versus number of waves (2 or 3) per cycle | -0.544* | 0.01 |
| Duration of dominance of wave 1 versus number of waves (3 or 4) per cycle | -0.427 | 0.11 |
| Duration of dominance of Wave 1 versus duration of the cycle (2 or 3 waves) | -0.125 | 0.62 |
| Duration of dominance of wave 1 versus duration of the cycle (3 or 4 waves) | 0.001 | 0.99 |
| Day of onset of CL regression versus estrous cycle length | 0.78* | 0.00 |
| Day of onset of CL regression versus number of waves per cycle (2 or 3 waves) | 0.45 | 0.06 |
| Day of onset of CL regression versus number of waves per cycle (3 or 4 waves) | 0.19 | 0.49 |

***where the value is significantly different ($P \leq 0.05$)***

The duration of dominance of Wave 1 was inversely related to the day of onset of luteal regression, the number of waves per cycle, and the length of cycle. A longer duration of dominance of the first wave lead to a delay in the attainment of maximum diameter of the subsequent wave DF ($P < 0.05$; Table 7). The day of onset of luteal regression was directly related to the number of waves per cycle and the duration of cycle for all cows.

In the Boran cow the diameter of the CL is as documented for other breeds (Ginther *et al*., 1989a; Rhodes *et al*., 1995; Figueiredo *et al*., 1997).

The CL has been previously shown to influence follicle development. The difference in DF sizes and dominance periods of DFs are attributed to the fact that DFs develop in the periods of different influences of progesterone, which has a significant effect on DF development by regulating LH pulse frequency and estradiol synthesis (Savio *et al*., 1993a). These factors eventually influence the length of dominance of the DF (Roche and Boland 1991; Cerri *et al*., 2009). It is speculated that acquisition of ovulatory capacity on any DF involves an increased expression of LH receptors on its granulosa cells to trigger further growth and ovulation (Sartori *et al*., 2001). The fate of the dominant follicle is also dependent on function of the *corpus luteum*, whose progesterone production triggers negative feedback effects on circulating FSH to initiate atresia of the DF (Ireland *et al*., 2000). Under such circumstances the functional dominance of the DF is lost to lead to a plateau in growth stage.

## CHAPTER 5

## 5.0 CONCLUSIONS AND RECOMMENDATIONS

### 5.1 CONCLUSIONS

The following are the main conclusions about follicular and luteal dynamics of the Boran cow

- The estrous cycle length is similar to those of other breeds (19-24 days).
- There are predominantly three follicular waves within the estrous cycle but a fourth wave may also occur.
- The inter-ovulatory interval (estrous cycle length) was positively correlated to number of waves exhibited per cycle.
- There were three phases of follicular development; Emergence, Selection and Dominance.
- Three groups of follicles were noted; Group one (≤3 mm in diameter), Group two (4-6mm in diameter and Group three (7mm or larger in diameter).
- The CL influenced the duration of dominance and ovulation of dominant follicle.

### 5.2 RECOMMEDATIONS

- To maximize on superovulatory response using FSH, this study recommends the timing of the first FSH treatment should coincide with first three days of the waves just before selection phase of folliculogenesis occurs.
- The three groups of follicles in the Boran cow are potential sources of viable oocytes for *in-vitro* embryo production at slaughter or during live animal ovum-pick up (OPU).
- That during Ovum Pick Up for embryo development for the Borancow three pick-up sessions per cycle would be a viable option for the enterprise.

# CHAPTER 6

## 6.0 REFERENCES

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