# Characterization of Ovarian Follicular Dynamics and Estrus Behavior in Fogera and Jersey Cattle Breeds

Dereje Yadeta Geleta

A thesis submitted to The Department Of-Applied Biology,

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Biotechnology

Office of Graduate Studies

Adama Science and Technology University

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Adama, Ethiopia

# Characterization of Ovarian Follicular Dynamics and Estrus Behavior in Fogera and Jersey Cattle Breeds

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First, I hereby declare that this Master of Science Thesis entitled "Characterization of Ovarian Follicular Dynamics and Estrus Behavior in Fogera and Jersey Cattle Breeds" is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

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We, the advisors of this thesis, hereby certify that we have read the revised version of the thesis ”Characterization of Ovarian Follicular Dynamics and Estrus Behavior in Fogera and Jersey Cattle Breeds” prepared under our guidance by Dereje Yadeta Geleta submitted in partial fulfillment of the requirements for the degree of Mater’s of Science in Biotechnology. Therefore, we recommend the submission of the revised version of the thesis to the department following the applicable procedures.

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**APPROVAL PAGE**

we, the advisors of the thesis entitled "Characterization of Ovarian Follicular Dynamics and Estrus Behavior in Fogera and Jersey Cattle Breeds" and developed by Dereje Yadeta Geleta hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

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We, the undersigned, members of the Board of Examiners of the thesis by Dereje Yadeta Geleta have read and evaluated the thesis entitled "Characterization of Ovarian Follicular Dynamics and Estrus Behavior in Fogera and Jersey Cattle Breeds" and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Biotechnology.

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# LIST OF ABBREVIATIONS

AI- Artificial Insemination

ANOVA- Analysis of Variance

ART- Assisted reproductive technology

BCS- Body Condition Score

BW- Body Weight

CI- Calving interval

CSA- Central statistical agency

CL- Carpus luteum

DAGRIS- Domestic Animal Genotype Resource Information System

DD- Date of Divergence

DF- Dominant Follicle

FSH- Follicle Stimulating Hormone

HS- Heat Stress

HARC- Holeta agricultural research center

IGF-I- Insulin-like Growth Factor-I

IOI- Inter Ovulatory Interval

IVEP- In Vitro Embryo Production

LH - Luteinizing Hormone

MFBCMR- Metekel Fogera breed conservation multiplication ranch

MGH- Megahertz

mm- Millimeter

NEB- Negative Energy Balance

NAIC- National artificial insemination center

| | |
|---|---|
| OPU- | Ovum Pick Up |
| PGF2α - | Prostaglandin F2α |
| SF- | Subordinate Follicle |

# ABSTRACT

*Ovarian follicles grow in a wave-like fashion during the estrus cycle of female animals. Understanding the reproductive physiology of female animals is used to optimize reproductive management. The current study was conducted from January to march 2022 at Ada'a Barga dairy farm to characterize the follicular dynamics and estrus behavior of Fogera and Jersey breeds. For this purpose, a total of 15 experimental animals (9 Fogera heifers and 6 Jersey cows) were used. A single intramuscular injection of 2ml PGF2α (Estrumate, Holland) hormone was used to synchronize and bring all animals to the same physiological status. For each cow, estrus behavior signs were visually observed and recorded. Follicular population, day of emergency, growth and atresia of the dominant follicle, a diameter of follicle, and ovary size during estrous cycles were evaluated using a trans-rectal real-time B-mode ultrasound system for three consecutive estrus cycles. Descriptive statistics was used to calculate the mean, variance, and standard deviation of means, and a comparison between the groups was done by t-test. Fogera heifer responded well to the hormonal synchronization. Fogara heifers (n=9) manifested two (29.62%), three (55.55%), and four (14%) follicular waves whereas Jersey cows (n=6) showed two (38.9%) and three (61.11%) follicular waves. Cycle length or inter ovulatory interval was 20.35 ± 2.8 and 20.67 ± 2.6 days for Fogera heifers and Jersey cows, respectively. The mean number of follicular population per animal was 13.9 ± 5.9 for Fogera heifers and 17.9 ± 7.0 for Jersey cows, where a statistically significant (p=*0.000*) difference was observed between the two breeds. The mean maximum diameter of the ovulatory follicle for Jersey cows was 17.2 ± 0.86 mm and for Fogera heifers 15.22 ± 1.3 mm. There was no statistical difference between the breed in terms of inter-ovulatory interval (IOI), however, the difference is statistically significant (p=*0.000*) in terms of follicular population and dominant ovulatory follicle size. It was concluded that the follicular dynamics of fogera heifers and jersey cows were characterized by a higher incidence of cycles with two or three follicular waves and jersey cows proved to have better for follicular population than fogera heifers.*

***Keywords:*** *- Dominant follicle, Estrus behavior, Follicular dynamics, Follicular wave, Number of follicular waves*

# CHAPTER ONE

# INTRODUCTION

## 1.1. Background of the study

Ethiopia has the largest livestock population in Africa, with around 60 million animals (CSA, 2017). The majority of these animals are indigenous breeds or populations that live in a variety of geographic and climatic situations throughout the country (Meseret *et al*., 2020) and Ethiopia is regarded as one of the original locations of Africa's indigenous cattle.

The indigenous cattle breeds are used for multipurpose traits particularly for milk, meat and draft as well as manure production however, the reproductive performance of the local cattle is low and inadequate (Tucho *et al*., 2021). Our current inability to obtain large numbers of gametes from genetically valuable females limits cattle genetic potential. Artificial insemination enabled the widespread dissemination of genes carried by valuable males (Fortune, 1993). The development of estrous synchronization, superovulation, and embryo transfer increased the number of offspring that can be obtained from superior females, but these methods are still limited in comparison to the rapid changes that can be effected through artificial insemination.. The recent application of ultrasonographic techniques to the study of bovine follicles has elucidated patterns of follicular development, and the use of ultrasonography in conjunction with various experimental manipulations has begun to provide new insight into the regulation of follicular growth, development and regression (Fortune, 1993).

Follicular dynamics is the growth of ovarian follicle in wave fashion. The ovarian follicle of a cow grows in waves during the cow's estrus cycle, which is not the same in all cows, it varies from cow to cow (Ginther *et al*., 1989). During the estrous cycle of heifers, two or three and sometimes four or five different follicular waves emerge. The waves emerge (follicles first identified by ultrasound at 3 to 4 mm) after ovulation. Each wave consists of numerous follicles that grow in synchrony and then diverge in diameter after a few days to form a dominant follicle and subordinate follicles. Depending on the stage of the estrous cycle, the dominant follicle grows large and becomes anovulatory or ovulatory. Trans rectal ultrasonic imaging is an effective tool for characterizing follicular waves since it is non-invasive and allows for repeated examinations of ovarian follicles (Ginther, 1993).

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The selection of breeding females is critical for reproductive efficiency, owing to the fact that several ovarian physiological features might have a direct impact on the amount and quality of oocytes (Lonergan *et al*., 1994). Numbers of follicles in the ovarian reserve vary greatly among individual adult cattle and the ovarian antral follicle population is linked to the reproductive potential of females (Mossa *et al*., 2012). Fogera cattle breed is one of Ethiopia's recognized indigenous cattle breeds. They are kept for multipurpose functions for farmers. The daily milk yield and lactation length performance of Fogera cattle type is high as compared to other indigenous breeds under the unsuitable and exceptionally challenging environment (Kebede *et al*., 2015). Of the population of animals found in this country's exotic breeds account for 0.1% (Adisu & Zewdu, 2021). Jersey breed originated from the Island of Jersey (Porter *et al.,* 2016) and is one of the exotic breeds found in Ethiopia.

Although Ethiopia has a lot of livestock population, the profits the country is earning are low (Hamid *et al*., 2021).In addition to other factors, the main factor that has been impeded the productivity of indigenous cattle is the low genetic potential of the breed. This can only be overcome by understanding reproductive physiology of these animals. In the cow, ovarian function is part of the reproductive axis that needs to be well understood. Ovarian physiology during estrous cycles in Fogera and Jersey breeds needs to be understood to optimize reproductive management techniques and to use appropriate reproductive biotechnology tools in order to uplift to its optimum reproductive potential. To do this there is a paucity of information on reproductive physiology of Fogera and Jersey breeds at *ex-situ* situation. Therefore, this study was intended to characterize the ovarian follicular dynamics, ovarian follicular population and estrus behavior for Fogera and Jersey cattle.

## 1.2. Statement of the Problem

Reproductive physiology of zebu cattle is different from European breeds of cattle by size of dominant follicle, ovulation moment and number of follicular wave. On ovarian physiology follicular dynamics is one of the most important subject and was largely studied in European breeds (*Bos taurus*) (Taylor and Rajamahendran, 1991). In Ethiopia to date only for one breed (Boran) follicular dynamics has been characterized. Fogera cattle breed is known by their good milk producer among indigenous cattle breed. However, no information generated yet concerning their follicular dynamics, follicular population and estrus behavior at *ex-situ* situation in Fogera cattle and also for Jersey cattle breed since their introduction to Ethiopia. The aim of this study was to characterize estrus behavior, ovarian follicular dynamics and follicle population in Fogera and Jersey cattle breeds under *ex-situ* situation.

## 1.3. Objectives

- To characterize follicular dynamics in Fogara and Jersey cattle breeds.
- To assess estrus behavior characteristics of Fogara and Jersey cattle breed.

## 1.4. Significance of Study

Knowing of estrus behavior, follicular population and follicular dynamics of indigenous and exotic cattle breeds would improve reproductive performance through various biotechnological interventions. Understanding ovarian follicular dynamics would assist to:

- Synchronizing estrus with more precision.
- For selection of best dam to be parent of next generation.

# CHAPTER TWO

# LITERATURE REVIEW

Africa is home to a wide range of ruminant livestock and animal species, all of which are genetically distinct. Africa, as a center of cattle domestication, is home to genetically varied cattle, the result of generations of co-evolution with a diverse range of people, each choosing for different traits in various production systems and circumstances (Okeyo *et al.*, 2010). Most African cattle are phenotypically humped cattle, often known as zebu cattle (Bos indicus) (Hanotte *et al.*, 2000). The zebu is Africa's largest single cattle breed. Zebu types or strains are plentiful throughout the continent and have a high level of tolerance to difficult environmental conditions, making them the only form of cattle that can survive over much of Africa.

They've adapted to local environmental conditions that aren't ideal for exotic European breeds of origin (e.g. high temperatures, long periods of drought, vector-borne disease). African indigenous taurine cattle *Bos taurus* (humpless cattle) are now found nearly exclusively in West Africa, while commercial taurine breeds and their crossbreed are found almost in every part of the continent. Besides these two main types of cattle, the continent is home to sanga and Zenga, which are crossbreds between the indigenous taurine and zebu (sanga), and zebu and sanga (Zenga) (Mwai *et al.*, 2015) .The first zebu bulls introduced to Africa were crossed with longhorn humpless cattle and produced Sanga cattle, whereas the second wave of introductions resulted in the formation of zenga (sanga×zebu). Present-day African cattle can be classified into four broad categories: the humpless Bos taurus, widely distributed in West and Central Africa; the humped Bos indicus (zebu), distributed widely in eastern and the dry parts of West Africa; the sanga, found mainly in eastern and southern Africa; and sanga x zebu types (zenga) found in eastern Africa (Rege & Tawah, 1999) .

Ethiopia has a wide range of environments and a large cattle population of roughly 60 million animals (CSA, 2017). The majority of these animals are indigenous breeds or populations that live in a variety of topographic and climatic situations throughout the country (Meseret *et al.*, 2020) . Ethiopia's closeness to the main livestock entry routes into Africa makes it a good candidate for this indigenous cattle (the Horn and East Coast), and its diverse agro-ecology (ranging from extreme lowlands to Afro-alpine zones), this country is regarded as a secondary hybridization zone and as one of the original locations of Africa’s indigenous cattle. Semitic immigrants from Arabia are said to have brought the first zebu cattle to Ethiopia via Somalia and its subsequent interbreeding with the taurine Longhorns are said to have developed the present-day sanga cattle (Rege & Tawah, 1999) .

## 2.1. Fogera cattle breed

Several breeds are said to have arisen from crossbreeding between zebu and sanga populations in the East African highlands, where huge concentrations of zebu (arriving from Asia) initially occurred, allowing for mixing with sanga cattle, which were already present. The resulting breeds were given their own classification which is called zenga and exclusively found in east Africa. Fogera cattle breed is among eight members of zenga breed (Hanotte *et al*., 2000) .

Fogera cattle breed is one of the recognized indigenous cattle breeds found in Ethiopia. The breed is raised in the districts surrounding Lake Tana, and it is one of the most populous and productive breeds in the Amhara region and throughout the country. The breed is known for its tolerance to high altitudes, parasite infestation and disease, fly burden, wet soils or swampy areas, low-quality feed, and other unfavorable environmental conditions. Fogera breed is characterized and well known by its pied coat of black-and-white or black-and-grey; short, stumpy, pointed horns; hump ranges from thoracic to cervicothoracic; dewlap is folded and moderate to large; docile temperament; used for draught, milk, and meat (DAGRIS, 2007). Fogera cattle breed, which is inhabited in the Amhara region, is renowned for its milk production that offers1500.0 liter of milk in one lactation season. However, due to various issues such as genetic admixture with uncharacterized local breeds and inbreeding, shift in the production system (shift of grazing lands to croplands and crop diversification) leading to shrinkage of grazing land, poor management practices (such as feed scarcity and disease prevalence), lack of well-defined breeding programs, poor selection strategy, and genetic gain, its productivity is currently declining (Tesfa *et al*., 2016). Farmers describe and pick the Fogera breed because of its lower age at first calving, capacity to plow swampy areas, and superior dowry (trade value), and social benefits and prefer a swampy area where it obtains better feed (Tesfa *et al*., 2016) .

Cattle productivity is largely determined by their ability to reproduce. In dairy cattle, reproductive performance is an extremely important trait (Gabriel *et al*., 1983) .Among the characteristics of reproductive performance; Weaning weight, Age at first service, Age at first calving**,** calving interval are bases for dairy production. The reproductive performance of Fogera cattle was assessed by different scholars and their results were variable (table 2.1). The variances across writers for the same breed could be attributable to differences in data collection site and structure, year differences (which could also be due to differences in feed availability and related managerial differences), and differences in data cleaning methods (Tesfa *et al*., 2016).

Table 2.1: Summary of reproductive performance of Fogera cattle

| **Birth Weight (Kg)** | Reference |
|---|---|
| 21.4±0.09 | (Tesfa *et al*., 2016) |
| 23.1±0.27 | Asheber (1991) |
| 22.5±0.17 | Addisu (1999) |
| 21.1±0.1 | (Giday, 2001) |
| 21.2 | (Gebremariam & Chakravarty, 2003) |
| 22 | (Bitew *et al*., 2010) |
| 21±0.03 | Almaz (2012) |
| 21.5 | MFBCMR 2013 |
| **Weaning weight (kg)** | |
| 102.2±0.77 | Tesfa et al.,2016 |
| 99.9±3.92 | Asheber 1991 |
| 100.9±0.8 | Giday 2001 |
| 97 | Alemseged and Chakra arty (2003) |
| 88.6±0.33 | Almaz (2012) |
| 114±1.9 | Addisu (1999) |
| 122.8 | MFBCMR (2013) |
| ***Age at first service (months)*** | |
| 38.9±0.72 | Tesfa et al.,2016 |

| | |
|---|---|
| 44.8 | Giday 2001 |
| 45.4±1.2 | Gebeyehu et al 2005 |
| **Age at first calving (months)** | |
| 51.8±0.72 | Tesfa et al.,2016 |
| 47.6 | Addisu (1999) |
| 49.3 | Addisu et al (2007) |
| 39 | MFBCMR (2013) |
| 50.8±0.36 | Melaku et al 2011b |
| 53.8 | Asheber 1992 |
| 54.6±0.4 | Giday 2001 |
| 52.4±0.17 | Almaz (2012) |
| 52.3±1.77 | Belay 2014 |
| **Calving interval(month)** | |
| 19.5±0.32 | Tesfa et al.,2016 |
| 19.37 | Almaz (2012) |
| 19.6 | Melaku et al 2011b |
| 19.9 | MFBCMR (2013) |
| 20.1 | Addisu et al (2007) |
| 18.7 | Giday 2001 |

## 2.2. Jersey cattle breed

Jersey cattle are a British breed of small dairy cattle native to the British Channel Islands of Jersey. Jersey cows adapt well to a variety of temperatures and conditions, and unlike many other breeds that originated in temperate regions, they can withstand extreme heat. It has been exported to a variety of nations around the world (Porter *et al.*, 2016) . Jersey cattle are now found in at least 82 countries around the world, demonstrating their adaptability to a wide range of climatic and geographical conditions. The breed has a relatively small frame with an average weight of 410 kg (900 lb) and produces more milk per unit of body weight than any dairy breed. Jersey cows outperform other dairy breeds by living an average of 18 percent longer. Jerseys also possess increased reproductive efficiency when compared to Holsteins for traits including calving interval, days open, age at first calving, and calves per lifetime (Huson *et al*., 2020) .Jersey cows have been selected for tropical research and development programs because of their small body size, hardiness and adaptability, low maintenance requirements, high feed conversion efficiency, and high milk fat content, according to research reports in the tropics (Thakur *et al*., 2019).The breed has also good reproductive performance (table 2.2). As a result, they could be a useful option in the Ethiopian highlands as a supplement to intensive and large-scale dairy farms, as well as a genetic pool for genetic improvement operations (Hunde *et al*., 2015).

Table 2.2: Summary of reproductive performance of jersey cattle in Ethiopia

| Reproductive performance | Reference |
|---|---|
| **Age at first calving (in months)** | |
| 29.9 ± 0.17 | Direba, *et al*..2015 |
| **Calving interval (CI) (in days)** | |
| 497± 3.69 | |
| **Number of services per conception (NSC)** | |
| 2.02±0.02 | |
| **Calving interval (CI) (in days)** | (Lemma *et al*., 2010) |
| 450.09 | |
| **Birth weight (kg)** | |
| 22.87 | |
| **Weaning weight (kg)** | |
| 108.88 | |

## 2.3. Factors Affecting ovarian physiology of Cattle

### 2.3.1. Nutrition Effect

Nutrition has an important impact on the reproductive performance of cattle. Malnutrition causes weight loss and poor body condition, delays puberty, lengthens the post-partum period before conception, disrupts normal ovarian cyclicity by lowering gonadotropin release, and raises the risk of infertility (EunKyung *et al*., 2015). An animal needs energy for its maintenance, growth, reproduction, production, and fetal growth. Normally during early lactation, animal milk production is greater but feed intake is lower. This combination creates NEB and the animal starts to use body reserves to overcome the energy deficit. NEB during the first3 weeks of lactation is highly correlated with the interval of the first ovulation. Poor nutrition, particularly insufficient calorie intake, can cause prolonged postpartum anestrus, resulting in significant body weight loss and a reduction in estrus expression (Boland *et al*., 2001; Overton & Waldron, 2004). Chronic NEB induces a linear decline in the maximum diameter of successive dominant follicles, finally leading to anestrus in the final estrus cycle before anovulation due to decreased LH pulse frequency (Staples *et al*., 1998) .The ovary's reactivity to hormones and circulating energy sources is another relationship between reproduction and nutrition. The variability in energy intake affects the dominant follicle's daily and overall growth rates. A low-energy diet slows growth, owing to a drop in IGF-I levels, which are necessary for follicular development (Adashi *et al*., 1985) .

### 2.3.2. Uterine disease

Recognized uterine pathogens, prospective uterine pathogens, and opportunistic contaminant bacteria are all types of bacteria that can infect the uterine lumen. damaging the endometrium, uterine infection also affects ovarian function (Sheldon *et al*., 2002; Williams *et al.*, 2005). These microorganisms are linked to more severe clinical uterine illness and endometrial inflammation (Bonnett *et al.*, 1991) . In cattle, uterine contamination during the postpartum period interferes with the return of ovarian follicular growth and function, ultimately disrupting ovulation (Opsomer *et al*., 2000) . Infection and inflammation in the uterus are linked to changes in the pattern of follicle growth on the ovaries ipsilateral to the previous pregnancy, as well as shorter luteal phase lengths.(L Mateus *et al*., 2002; Luísa Mateus *et al*., 2002; Sheldon *et al*., 2002) . It's unclear what effect uterine infection has on the corpus luteum (CL). Infections are linked to both early corpus luteum regression and luteolysis failure, resulting in a prolonged luteal phase (Opsomer *et al.*,

2000) . Animals with a higher bacterial growth density in the uterine lumen have smaller ovarian dominant follicles and lower peripheral plasma estradiol concentrations compared with normal postpartum cows. Greater postpartum proliferation of uterine bacterial pathogens resulted in smaller initial dominant follicles, which, if ovulated, resulted in smaller corpora lutea and lower circulating progesterone concentrations (Williams *et al*., 2007) . Postpartum dairy cows' delayed resumption of ovarian cyclicity contributes to severe economic losses for dairy farmers (Dourey *et al*., 2011) .

### 2.3.3. Effect of Season

Heat stress alters the follicular development pattern in cattle. Exposing cows to heat stress during a complete estrous cycle altered the dynamics of the turnover of the first and second follicular waves by reducing the size of the dominant follicles (Roth *et al*., 2000) .The initial response of heat stress was dominance depression, as seen by a significant drop in plasma immune reactive inhibin and, as a result, elevated FSH levels. The early development of the second (preovulatory) follicular wave and an increase in the number of large follicles throughout the follicular phase resulted from this altered hormone output. A significant rise in FSH secretion and a decrease in the number of medium-sized follicles were seen as a delayed response to heat stress. During the summer and autumn, both the immediate and delayed responses to heat stress have been found to play a role in lowering cattle fertility (Roth *et al*., 2000) .The temperature of the follicular antrum in ovarian tissues can control ovulation during the pre-ovulatory stage (Ronald H F Hunter & López-Gatius, 2020) . Pre-ovulatory follicles may be over 1 _C cooler than neighboring tissues and both compartments are cooler than rectal temperatures in cows. Most follicles of pre-ovulatory size in lactating dairy cows that were cooler than deep rectal temperature ovulated, but follicles that did not display such a temperature disparity did not (López-Gatius & Hunter, 2019; López-Gatius & Hunter, 2017) . To maintain their functioning, the ovaries may demand a lower temperature than adjacent organs and Graafian follicles are always colder than stroma as they grow (Ronald Henry Fraser Hunter *et al*., 2006; Morita *et al*., 2020) . Small follicles are sensitive to physiological changes that occur during heat stress (Torres-Júnior *et al*., 2008) . Temperature gradients can be created by counter-current heat exchange systems between veins and arteries. Many organs have been found to transport heat locally, and it is well-established in the reproductive tissues of female animals (Cicinelli et al., 2004; N Einer-Jensen & Hunter, 2000;

Niels Einer-Jensen & Hunter, 2005) . Local counter-current transfer can be thought of as an organ function regulatory mechanism that allows for thermal and endocrine interaction between the ovary, oviduct, and uterus (Ronald H F Hunter & López-Gatius, 2020) . Seasonal effects on reproduction could include not just climatological variations linked with each season, but also seasonal changes in nutritional and managerial aspects. The rainy season had the highest conception rates, which were linked to feeding availability(Weaver & Meijerhof, 1991) .

### 2.3.4. Types of Breeds

Gonadotropin and steroid secretion, follicular growth and luteal development, and/or the secretion of metabolites and metabolic hormones that play important roles in reproduction all contribute to breeding differences in various reproductive traits (Alvarez *et al*., 2000) .Inevitably, the dose of FSH required to produce superovulation in Bos taurus cattle is 30 to 50 percent more than that required in Bos indicus cattle (Barati *et al*., 2006; Silva-Santos *et al*., 2014) . The smaller size of the ovarian structures (follicles and corpora lutea), which is a breed characteristic, was thought to be related to the lower quantity of FSH required to produce superovulation in Zebu cattle (Figueiredo *et al*., 1997) .Follicle population also depends on types of breeds as reported by (Dorice *et al*., 2019) .

### 2.3.5. Age of animal

In comparison to other model animals, such as rats, cows have a longer reproductive life (about 13 years)(Hull *et al*., 1996) . They show similar follicular wave, follicle selection, ovulation patterns as well as age-associated endocrinal changes to humans (Adams *et al*., 2008) .Age-associated decline in fertility is common in mammals (Spandorfer *et al*., 2004) . The decline in oocyte reserves occurs on the premise that the follicle pool is established before birth and that no de novo oocyte synthesis occurs during a cow's life (Kerr *et al*., 2006) . The number of tiny antral follicles with a diameter of 4–5 mm declined with age in cows, while the plasm concentration of follicle stimulating hormone (FSH) increases (Iwata, 2016) , and the negative relationship between the reduction in antral follicle number and the age of cows has also been reported in other studies (Cushman *et al*., 2009; Yamamoto *et al*., 2010) .

## 2.4. Follicular Dynamics in Cattle

From the first accounts of the female gonad, there has been controversy in the study of ovarian structure and function (Huhtaniemi, 2007) . Hippocrates did not attribute any reproductive role to the ovary in the 5th century BC, instead suggesting that the production of a new life was the consequence of the action of two types of semen, one from the male (ejaculate) and one from the female (ovarian) (menstrual blood). It was not until the mid- 1600s that the ovary was recognized as an egg-producing organ. Regnier de Graaf, a Dutch physician, is credited with being the first to recognize the ovary's proper function in his 1672 book, "New treatise touching the generative organs of women." However, all of these early modern biologists believed that the follicle itself was the egg—much like a little bird's egg without a shell. Karl Ernst von Baer, an Estonian physician, published the first description of a mammalian egg in 1827, based on his microscopic research of ovarian vesicles (follicles) in a dog's ovary. However, it would be another 100 years before the first investigations of follicle growth dynamics were conducted(Adams *et al*., 2008) .

### 2.4.1. Oogenesis and folliculogenesis

Oocytes begin as primordial germ cells in the endoderm of the embryonic yolk sac and migrate to the gonadal ridge by amoeboid migration through the dorsal mesentery of the gonadal ridge by Day 35 of gestation in cattle. Primordial germ cells undergo a limited number of mitotic divisions during migration and upon arrival at the gonadal ridge. The primordial germ cells stop mitotic division during internalization, becoming trapped in germ cell cords (ovigerous cords) made up of epithelial cells that are separated from the surrounding mesenchymal cells by a basal lamina, and are referred to as oogonia. In cattle, meiosis (transition to primary oocytes) occurs around Day 75–80 of gestation, and the first meiotic division does not continue through the pachytene stage of Prophase-I. The great majority of surviving oocytes are encircled by a single layer of flattened epithelial cells from the germ cell cords, forming primordial follicles. Oocytes that fail to be surrounded by epithelial cells degenerate(Smitz & Cortvrindt, 2002) .The change of the primordial follicle's flattened pre-granulosa cells into a single layer of cuboidal granulosa(follicular) cells—a primary follicle—starts follicular growth (activation) (Braw-Tal & Yossefi, 1997).

### 2.4.2. Wave emergence and follicular dominance

With the advent of new ultrasound scanners capable of resolving structures as small as 1 mm, researchers were able to conduct a study to characterize the developmental pattern of 1–3 mm follicles in cattle and determine the stage at which the future dominant follicle achieves a size advantage over its cohorts. Follicular wave emergence in cattle is characterized by the sudden (within 2–3 days) growth of 8–41 small follicles that are initially detected by ultrasonography at a diameter of 3–4 mm. The growth rate of the wave's follicles is similar for about two days, then one follicle is chosen to continue growing (dominant follicle), while the others become atretic and regress (subordinate follicles). The first follicular wave emerges on the day of ovulation (day 0) in both two-wave and three-wave oestrous cycles.). Emergence of the second wave occurs on day 9 or 10 for two-wave cycles, and on day 8 or 9 for three-wave cycles. In three-wave cycles, a third wave emerges on day 15 or 16 (Fig 2.1). Progesterone (for example, dioestrus) causes atresia in the dominant follicles of subsequent waves. The ovulatory follicle emerges from the dominant follicle present at the commencement of luteolysis, and the following wave does not arise until the day of the subsequent ovulation. In two-wave cycles (day 16), the corpus luteum begins to regress earlier than in three-wave cycles (day 19), resulting in a shorter oestrous cycle (20 days versus 23 days, respectively). As a result, the average interval of 21 days occurs only as a result of the average of two- and three-wave cycles (Adams, 1999) .

The periodic synchronous growth of a group of antral follicles is referred to as the wave pattern of follicular development. The mechanism involved with follicular wave dynamics is based on the ovary's differential responsiveness to FSH and LH(Jaiswal *et al*., 2004). Folliculogenesis is the transformation of small primordial follicles into large preovulatory follicles(Britt, 2008) . A bovine female is born with approximately 133,000 primordial follicles, which are not renewable and steadily decline until they are nearly gone by the time the cow is 15 to 20 years old (Muraya, 2013b) .

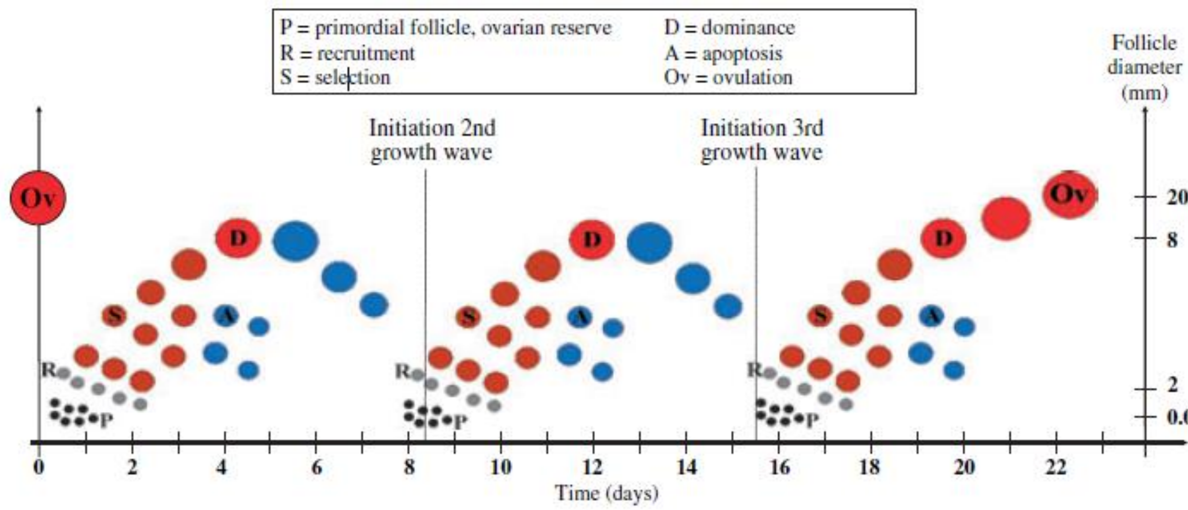


Figure 2.1: Dynamics of ovarian follicular development

(adapted from ( Aerts & Bols, 2010))

Follicles develop in a wave-like manner (Taylor & Rajamahendran, 1991). Primordial follicles may undergo progressive development shortly after formation or may lie dormant for years before resuming growth (Cushman *et al*., 2002; Yang & Fortune, 2008). Once progressive follicle growth begins, a variety of local and systemic regulatory factors, including insulin, IGF-I, steroids (estradiol, testosterone), gonadotropins, and a wide range of growth factors, are required to keep the follicle growing until it reaches the fully mature Graafian stage before ovulation (Britt, 2008). Emergence, selection, and dominance are the fundamental reproductive processes in farm animals, followed by either atresia or ovulation of the DF, which is mediated by the significant function of FSH and LH (Ginther *et al.*, 2002) .

The term "recruitment" refers to the process of follicles entering the developing pool (Fig 2.1). Wave emergence and follicle recruitment are often used interchangeably (Ginther *et al.*, 1996). The first day of a follicular wave, when a rising cohort of follicles is first observable by ultrasonography, is known as follicle wave emergence (Bo *et al*., 1995). A brief surge in FSH stimulates the recruitment of a cohort of follicles, 8 to 41 (average, 24) small follicles of 1 to 3 mm in diameter, on each ovary (Jaiswal *et al.*, 2004) . There are differences in the population of antral follicles between breeds. In Bos taurus cattle, approximately 24 tiny (2 to 5 mm) viable antral follicles have been found at the onset of each follicular wave (Ginther *et al*., 1996). However, in *Bos indicus* cattle, greater numbers of small follicles during wave emergence have been reported

(Buratini *et al*., 2000) .Small follicles are presumed unable to continue growing as circulating FSH concentrations fall, and the selected follicle (dominant follicle) may alter its reliance from FSH to LH (Ginther *et al*., 1999). For the selection of a single dominant follicle, the decreased circulating level of FSH at the moment of selection is critical. When a follicle is chosen and continues to grow at a greater pace than the largest subordinate follicle, the dominance phase of the follicular wave occurs(Fig 2.1), which prevents the creation of a new follicular wave (Ginther *et al*., 1996) . Following the selection and establishment of a dominant follicle, follicular recruitment is inhibited until dominance is lost or ovulation occurs. the number of the follicular wave per estrus cycle is different between breeds and within breeds, one follicular wave(Savio *et al.*, 1988) . , two follicular waves(Ginther *et al*., 1989), three follicular waves , and four (Yang and Fortune, 2008) have been reported.

## 2.5. Embryology of reproductive organ (ovary)

The development of the normal female reproductive tract is a complex process. The reproductive system of the bovine female includes several organs such as ovaries, genital pathways or tubular portion that surround the oviducts, uterus, vagina, and vulva, the attached glands, embryonic vestiges, blood vessels, and nerves (Valadão *et al.*, 2018). The word “ovary” is derived from the Latin word “ovum,” meaning egg .The ovary is a dynamic organ that undergoes some of the most dramatic changes in structure and function of any adult mammal’s tissue (Strauss III & Williams, 2019).. The mammalian ovary is not only the female gonad, containing the supply of germ cells to produce the next generation, but also the female reproductive gland, controlling many aspects of female development and physiology. The ovaries have two differentiated portions: a medullary zone formed by connective tissue, fibroelastic and vessels (arteries, veins, and lymphatics), and nerves, all together responsible for the conservation and nutrition of the organ. The other portion is the cortical zone, which is surrounded by the germinal epithelium and the tunica albuginea within it. The follicles and corpora lutea in their different stages of development and regression are located in this last portion (Valadão *et al*., 2018). After the union of an oocyte and a spermatozoon to become a zygote, all cells up to the eight-cell stage of embryogenesis appear to have similar totipotency (potential to become any lineage), because these cells all appear morphologically identical. However, with the formation of a 16-cell morula, the cells begin the.Process of differentiation with cells being allocated either to the inside or outside of the embryo

(Edson et al., 2009). The initial process of gonad formation is regulated by several transcription factors such as *Emx2* (Miyamoto *et al*., 1997), *Wilms tumor 1* (*Wt1)*, (Tilmann & Capel, 2002), *Lhx9* (Birk *et al*., 2000), and *steroidogenic factor 1* (*Sf1)*, (Ingraham *et al*., 1994). The finding of *Sry* for testis determination leads to the assumption that an ovarian counterpart of *Sry* must exist for ovarian determination (Yao, 2005). Ovarian development commences with ovarian medulla and germinal epithelium emergence from the genital ridge and mesonephros, with primordial germ cells (PGCs) arriving as yolk sac endoderm colonists. PGC migration to the gonadal anlage is controlled by stem cell factor (SCF), with dorsal mesentery SCF-positive autonomic nerve fibers guiding their ascent. The bone morphogenic protein (BMP) system is intimately involved both in murine PGC formation (Orsi *et al.*, 2017). The PGCs enter the indifferent gonad, and eventually the ovary forms and permits the PGCs to differentiate into oocytes (Edson *et al*., 2009). Primordial germ cells in the bovine female fetus differentiate into oogonia during the first trimester of gestation. Oogonia divide mitotically well into the second trimester of gestation, generating a peak of approximately 2.7 million germ cells around day 110 of gestation. During the second trimester, oogonia form egg nests in the ovarian cortex and enter the first prophase of meiosis where they become arrested in the diplotene stage. Somatic germinal epithelial cells from the surface of the ovary invade the egg nests and form a single flattened layer of epithelial cells around each oocyte. The primordial follicle comprises the arrested oocyte surrounded by the single layer of epithelial cells. Once the primordial follicle is formed, the follicular epithelial cells that are in direct contact with the oocyte interact through localized cellular signals to regulate oocyte maturation (Britt, 2008).

## 2.6. Endocrinology (hormones) of estrus cycle

Numerous hormones generated by specialized glands known as endocrine glands regulate female reproduction. Hormones are chemical substances produced by specialized tissues that act on specific tissue at a distance and in extremely low concentrations to achieve specific effects. The pituitary, the ovary and its anatomy, and the uterus all manufacture these hormones. The hormones are transported to certain sections of the body via the blood lymph system, where they perform their purpose. The hypothalamus, pituitary, ovary, uterus, and placenta are all potential sources of reproductive hormones. The bovine estrous cycle is governed by a number of hormones. The recruitment and expansion of follicular waves, the timing of ovulation, and the length of the estrous cycle are all influenced by changes in the concentrations of these hormones.

The involvement of the hypothalamus in the control of pituitary gland function was first suggested by (Green & Harris, 1949). The gonadotrophin-releasing hormone is the major hypothalamus hormone involved in gonadotrophin release. The main pituitary gonadotrophins in cows are LH and FSH. Both bovine LH and FSH are glycoproteins with a molecular weight of around 30 000 Daltons. They were first purified by (Reichert Jr, 1962) and (Reichert Jr & Jiang, 1965) respectively. Both are synthesized by the basophil cells of the anterior pituitary gland(Peters, 1985).

FSH induces the formation of LH-receptors in follicular cells and acts as a follicle growth initiator. The maturation and ovulation of the Graafian follicle, as well as the production and maintenance of the corpus luteum, are thought to be the primary roles of LH. The amount of Follicle Stimulating Hormone (FSH) in the blood increases after ovulation (Fig 2.1). At the start of each follicular wave, a rise in FSH leads a group of follicles (cohort) to be recruited. FSH concentrations in the blood diminish after the cohort has been enlisted. Luteinizing Hormone regulates the selected follicle's growth and development beginning around the time of selection. The dominant follicle's growth and development are likewise regulated by Luteinizing Hormone. A dominant follicle is a follicle that has a dominating position in the female reproductive system. Follicular dominance appears to be controlled by a number of mechanisms acting in concert. These include alterations in peripheral FSH concentrations by estradiol and inhibin secreted by the dominant (ovulatory) follicle, as well as the possible production of local ovarian factors, which can inhibit the development of subordinate follicles directly (BK *et al.*, 1995). Evidence that follicular dominance and inhibition of the growth of subordinate follicles are due to the production of follicle growth inhibitory factors (FGIFs) by dominant follicles has been increasing. It has been demonstrated that steroid- and inhibin-depleted follicular fluid can inhibit follicular development in cattle *(Wood et al.*, 1993).

Estrogens, oestradiol-17B is the most physiologically active estrogen in cows. The two-cell process is used to produce this steroid in ovarian follicles. Estradiol-concentrations are highest in the fluid of the biggest antral follicles. During the majority of the estrous cycle, plasma oestradiol-17f3 concentrations are low, but they rise during the preovulatory period, peaking at or shortly before the commencement of estrus. Estradiol levels are rising at this time because the dominant follicle is getting bigger. The preovulatory gonadotrophin surges are thought to be caused by a positive feedback process caused by increased estradiol concentrations. Although it is generally known that the preovulatory rise in estradiol stimulates the preovulatory surges of LH and FSH, the maximum display of estrous behavior is not well understood (Peters, 1985).

Plasma concentrations of progesterone, the most important steroid released by the corpus luteum, indicate its development, maintenance, and regression. Concentrations rise about day 4 of the cycle, peak between days 8 and 16, and then drop to baseline before the next estrus and ovulation. Progesterone reduces the frequency of LH pulses, resulting in a negative feedback effect on LH secretion. Progesterone prepares the uterus for pregnancy, keeps the pregnancy going if fertilization happens, and prevents cows from ovulating and standing estrus. Progesterone production often increases when the CL grows larger during the start of the estrous cycle. Around 5 days following standing estrus, elevated progesterone concentrations can be found. If a cow does not conceive, she is said to be barred from breeding.

Peripheral testosterone concentrations have been observed to increase at the time of luteal regression and immediately before estrus after androgens were extracted from the bovine ovary. As estrus approaches, the concentrations of androstenedione, dehydroepiandrostenedione, and testosterone in the ovarian vein grow. Androgens are intermediates in the manufacture of other steroids, such as estrogens, therefore their significance in the female body is uncertain. They may, however, play a function in the regulation of gonadotrophin secretion and/or the induction of estrus behavior.

Prostaglandins are hydroxylated unsaturated fatty acids that are all derivatives of prostanoic acid, the parent molecule. They are found throughout the mammalian body and play a variety of roles. In endometrial tissue, the precursor of PGF, arachidonic acid, was discovered. The luteolysis in the cow is uterine PGF 2a. When a pregnancy is not recognized by the mother, the uterus releases prostaglandin F2a (PG) to cause luteolysis (luteolysis means destroying the CL).  The cow will not be able to return to standing estrus if luteolysis does not occur, and so will not be able to become pregnant during the breeding season.

## 2.7. Estrous Cycle in Cattle

The estrous cycle refers to the time between one estrus (heat) and the next. The estrous cycle lasts roughly 21 days in cows, but it can range from 17 to 24 days (Sartori *et al.*, 2004) .Proestrus, estrus, metestrus, and diestrus are the four stages of the estrous cycle in cows (Kojima., 2003). Proestrus is a three-day period during which folliculogenesis occurs. During this time, a Dominant Follicle (DF) develops itself in the granulosa cells and begins to produce 17-estradiol. Estrus symptoms are brought on by this estrogen. The preovulatory surge of the LH Hormone occurs when estrogen rises before estrus. The expanding follicle also produces inhibin, which inhibits the formation of additional smaller follicles. The suppression of lesser follicles ensures that just one follicle is chosen to ovulate. The estrus phase, or the period of sexual receptivity when a cow is receptive to the bull, follows Proestrus (Fig 2.2).

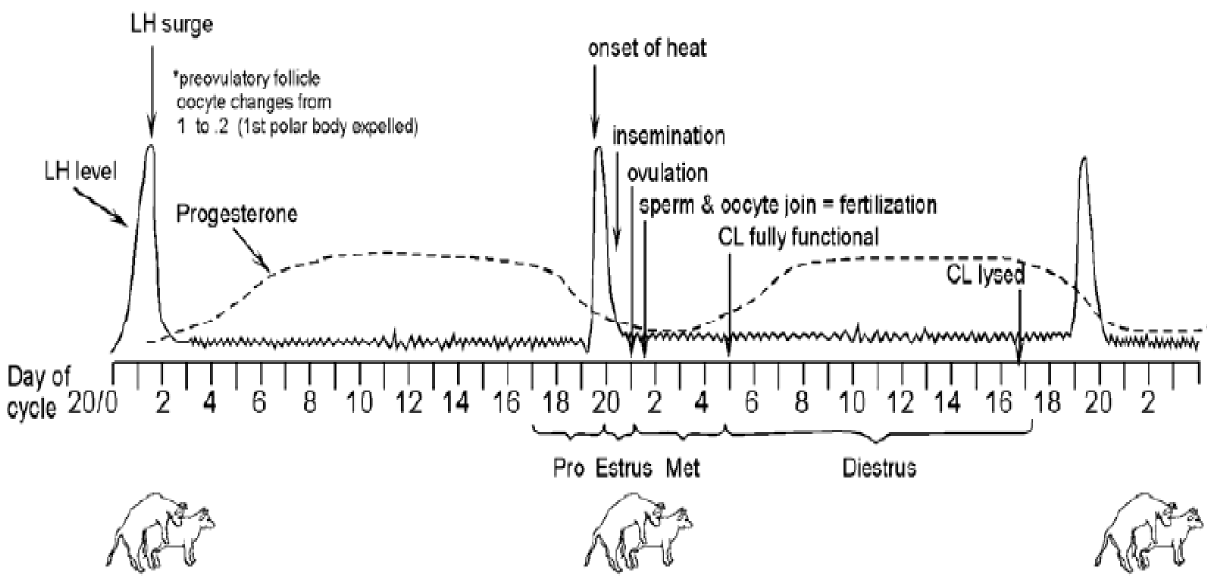


Figure 2.2: stages of estrus cycle with corresponding hormone
(adapted from R.L. Larson and R.F. Randle)

It lasts for 24-36 hours before ovulation takes place in 11-14 hours. After ovulation, a corpus hemorrhagic develops and this occurs in the metestrus phase that lasts for three days. Having four follicular waves during estrous cycles is a common occurrence in Bos indicus cattle (Torres-Júnior *et al*., 2008) . on the other hand, Four or more follicular waves per cycle in Bos taurus cattle, frequently associated with delayed luteolysis or failure to ovulate (Adams, 1999).

## 2.8. Estrus Behaviors

Cows have an estrus cycle throughout the year, from puberty to pregnancy every 20 to 21 days (standing estrus), and then during the non-pregnancy period. The standing heating time can be as short as 3 hours and as long as 28 hours, with an average of 12 to 16 hours. Standing estrus is the behavior of a cow or heifer standing still while the bull or herd of cows is riding. It is the best (but not absolute) indicator of pre-estrus when a pre-ovulatory follicle will form on one of the two ovaries. The main and most reliable sign of heat is a fixed response, that is when a heifer or cow is standing still by a companion or bull during the mount. These usually are caudal mounts, but cranial mounts sometimes are observed (Fig 2.3).

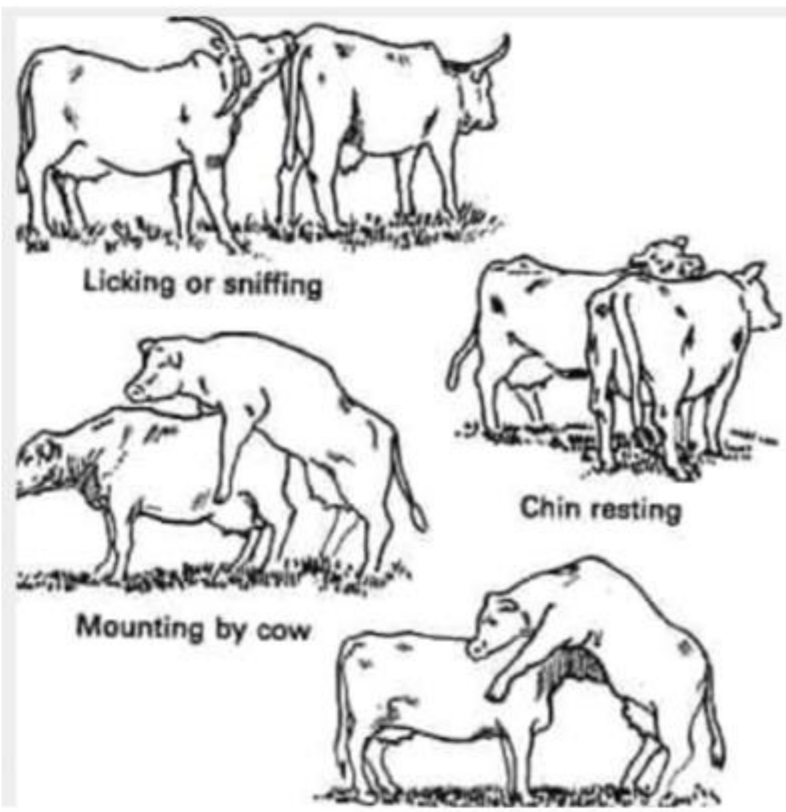


Figure 2.3: schematic representation of some of estrus behavior sign
(Adapted from veterinary science hub, 2018)

Other signs of estrus include circling, chin-resting, licking, sniffing, butting, vulva discharging, bellowing, decreased milk production, decreased feed consumption, and displaying the Flehmen posture (Zeitoun *et al.*, 1996) .

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1. Description of study area

This study was conducted at Ada'a Barga Dairy farm in West Shewa Zone of Oromia Regional State of Ethiopia. Adea Berga is located in Ethiopia's central highlands at $9^0$ 16' N latitude and $38^0$ 23' E longitude (Fig 3.1), 70 km West of Addis Ababa and 35 km North West of Holeta on the road to Muger. It lies at an altitude of 2500 meter above sea level. It has a subtropical climate that is cool, with the mean annual temperature and rainfall of $18^0$C and 1225 mm, respectively (HARC, 2010). The perennial grasses and sedges are the main vegetation of the area. The most common species dominating the pasture in the area are Clovers, Pennisetum and Andropogon. The farming system is semi intensive where the animals pass their time mostly by grazing and practice also an indoor feeding in their respective barn. The farm has a total of 400 hectare of land and the rest of land is being utilized for grazing and hey production. The whole pastureland is protected during rainy season for hey production and all animals were confined to the barn during this period.

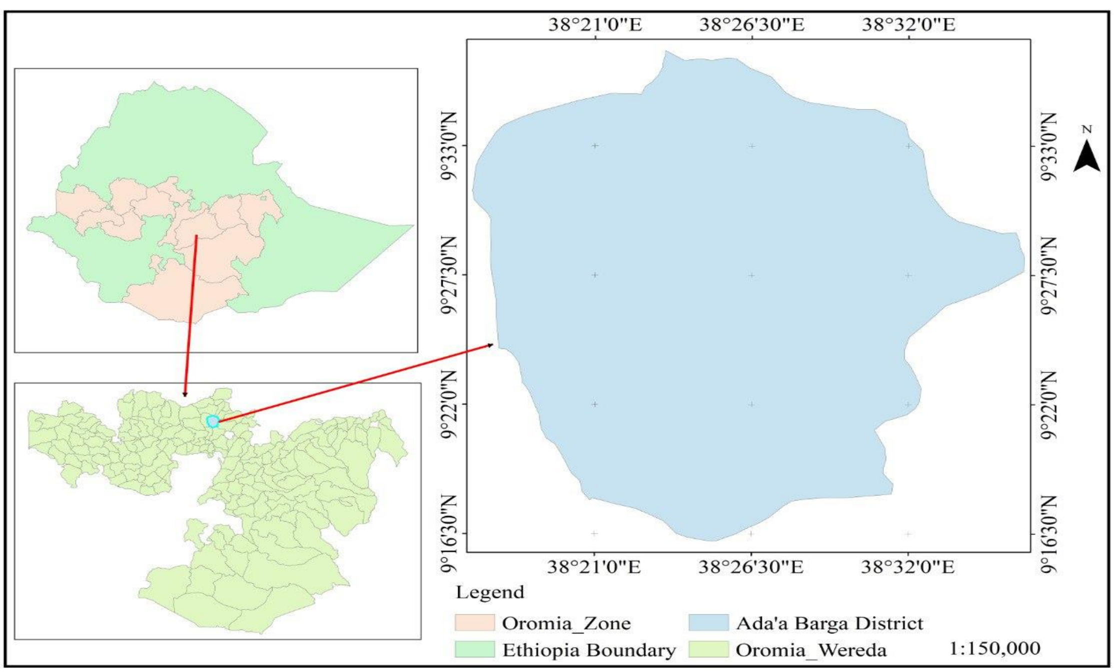


Figure 3.1:map of study area (Ada'a Barga)

## 3.2. study animals

About 38 Fogera heifers were brought to Ada'a Barga Dairy farm in June 2010 from Amhara region in order to multiply using reproductive biotechnologies. 400 pregnant Jersey heifers were imported in 1986 and brought to this farm. The farm had been producing and growing pure Jersey breed cattle from imported foundation stock for the dairy development enterprise's milk production, as well as acting as a bull dam station for the National Artificial Insemination Center (NAIC). Since 2007, it has been handed to Holeta Agricultural Studies Center for genetic improvement research (Hunde *et al*., 2015) .

## 3.3. study animal management

Hay made from grass (*Trifolium*, *Pennisetum* and *Andropogon)* that constituted the major proportion of the roughage supply was provided to experimental animals. Concentrate composed of 60% wheat bran, 38% noug seed cake (*Guizotia abyssinica*) and 2% salt were used as supplementary feed for cows . Water was provided on ad-libitum bases. Each experimental animal was held in a crush prior to ovarian evaluations to make rectal palpation and ovarian ultrasonographic scanning operations easier. Cows were let to graze in the open pasture after they had been evaluated and data collected

## 3.4. Study design

This study was conducted between the months of January to march 2022 on 15 selected multiparous non-lactating (dry-off) Jersey cows (n=6) and Fogera heifers (n=9). Study animals were purposively selected based on their good health and presence of a *corpus luteum* within the ovary. Cows with body condition score (BCS) between 3.5 and 4.5 in the scale of 1-5 were selected. The estimated age of Fogera heifers were about 5 years (have no birth record) whereas that of Jersey cows were in the range of 5 to 8 years old.

### 3.4.1. Estrus behavior assessment

Estrus detection was scheduled twice a day and estrus signs that cattle showed were recorded in each cycle using the developed record sheet form (Annex E)

### 3.4.2. Follicular dynamics characterization

Estrous synchronization was done using a single intramuscular injection of 2ml PGF2α (Estrumate, Holland) hormone to bring experimental animals to the same physiological status. After animals showed behavioral estrus signals, the day of ovulation (day 0) was established ultrasonically, and follicular dynamics monitoring was started. The nonidentity (mathematical) method was used to manage ultrasonic follicular data. Trans-rectal real-time B-mode ultrasound system with a 7.5 MHz linear array rectal transducer (SIUI, CTS-3300V; Shantou, China) was used for serial scanning of the ovaries(Jemal et al., 2020).

From day two of the study (48hrs) after the injection of hormone, each of the cows was evaluated for a period of three Estrous Cycle (63 days). Data collection was done daily between 7:00 - 12:00 AM. On a daily basis, the following actions were performed for each animal: Rectal palpation of the uterus and ovaries; Ultrasonographic scanning of the ovaries; Measurement of follicle diameters; Counting and recording follicular populations within the scanned ovary (to determine follicular populations, all visible follicles on each ovary were counted) (Annex A-3),the size of follicles was measured (taking the average of the two dimension) in both right and left ovaries. Date of wave emergence was determined retrospectively from the date of ovulation (Day 0). The date of divergence (DD) was defined as the time when the dominant follicle (DF) of the wave began to grow at a significantly different rate than the remainder of the wave's subordinate follicles (SF) (Muraya, 2013a).

Description of number of waves was done as previously documented by Ginther et al (1989a). Depending on the number of waves that occurred before a dominant follicle ovulated, cycles were classified as having two, three, or four waves of follicular development. For example, a female 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. 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). The number of days between two consecutive ovulations in the same female was taken as the inter-ovulatory interval (IOI).

## 3.5. Data Analysis

All recorded information during the experiment including the daily scans was filled in chart prepared for this purpose (Annex D). Records such as diameters of follicles, ovary size, and counts of follicular populations were entered into a computer on excel sheet. The excel sheets were then exported to Statistical Package for the Social Sciences (SPSS version 16) for analysis. Descriptive statistics was used to calculate the mean, variance and standard deviation of means where the results were presented in tabular, and graph. chi-square test were used to determine occurrence of ovulation. Comparisons between the groups interms of follicle population and ovary size were done using t-test.

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# CHAPTER FOUR

# RESULTS

## 4.1. Ovarian follicular dynamics

### 4.1.1. Estrus behavior characteristics

All experimental animals except one Jersey cow, exhibited heat signs within five consecutive days after 48 hours of 2ml $PGF_{2\alpha}$ hormone injections. The findings for Fogera cattle on behavioral heat manifestation like, mounting, stands to be mounted, chin resting, sniffing and restlessness were not identical with Jersey cows during estrus synchronization and subsequent heat signs for three consecutive estrus cycles. Among the physical sign, vaginal discharge and swelling of vulva were the most consistent (100%) estrous sign for both breed (Table 4.1). Estrus behaviors like mounting, attempting to mount, restlessness, chin resting on the rump, sniffing, standing to be mounted, swelling of vulva and vaginal discharge were registered (Fig 4.1).

Figure 4.1: Estrus behavior sign of fogera heifers and jersey cows

Table 4.1: Percentage and number of animals showing different signs of estrus behavior

| Estrus behavior sign | Types of breed | | | |
|---|---|---|---|---|
| | Fogera(N=9) | | Jersey(N=6) | |
| | number | percent | number | percent |
| Mounting | 8 | 88.89 | 6 | 100 |
| Stands to be mounted | 3 | 33.33 | 5 | 83.33 |
| Chin resting on the rump | 2 | 22.22 | 4 | 66.67 |
| Sniffing | 2 | 22.22 | 3 | 50 |
| Restlessness | 3 | 33.33 | 3 | 50 |
| Swelling of vulva | 9 | 100 | 6 | 100 |
| Vaginal discharge | 9 | 100 | 6 | 100 |

Fogera breed exhibited mainly mounting however, chin resting and sniffing are less exhibited but they exhibit on average heat signs such as standing to be mounted and restlessness. Jersey breed, however, outperformed the Fogera breed in exhibiting estrus behavior. Prolonged estrus signs (more than three days) were exhibited in both Fogera and Jersey cows

### 4.1.2. Follicular waves

Estrous cycles were evaluated for 63 days on Fogera and Jersey cows. Follicular dynamics with demonstrated characteristic pattern of follicular waves the recruitment of a group of 3 to 5 mm follicles followed by the selection, growth and atresia of anovulatory dominant follicle or ovulation of ovulatory dominant follicles were observed. Fogara heifers (n=9) manifested two (29.62%) (Fig 4.2A), three (55.55%) (Fig 4.3A) and four (14%) (Fig 4.4) follicular waves whereas Jersey cows (n=6) showed two (38.9%) (Fig 4.2B) and three (61.11%) (Fig 4.3B) follicular waves.

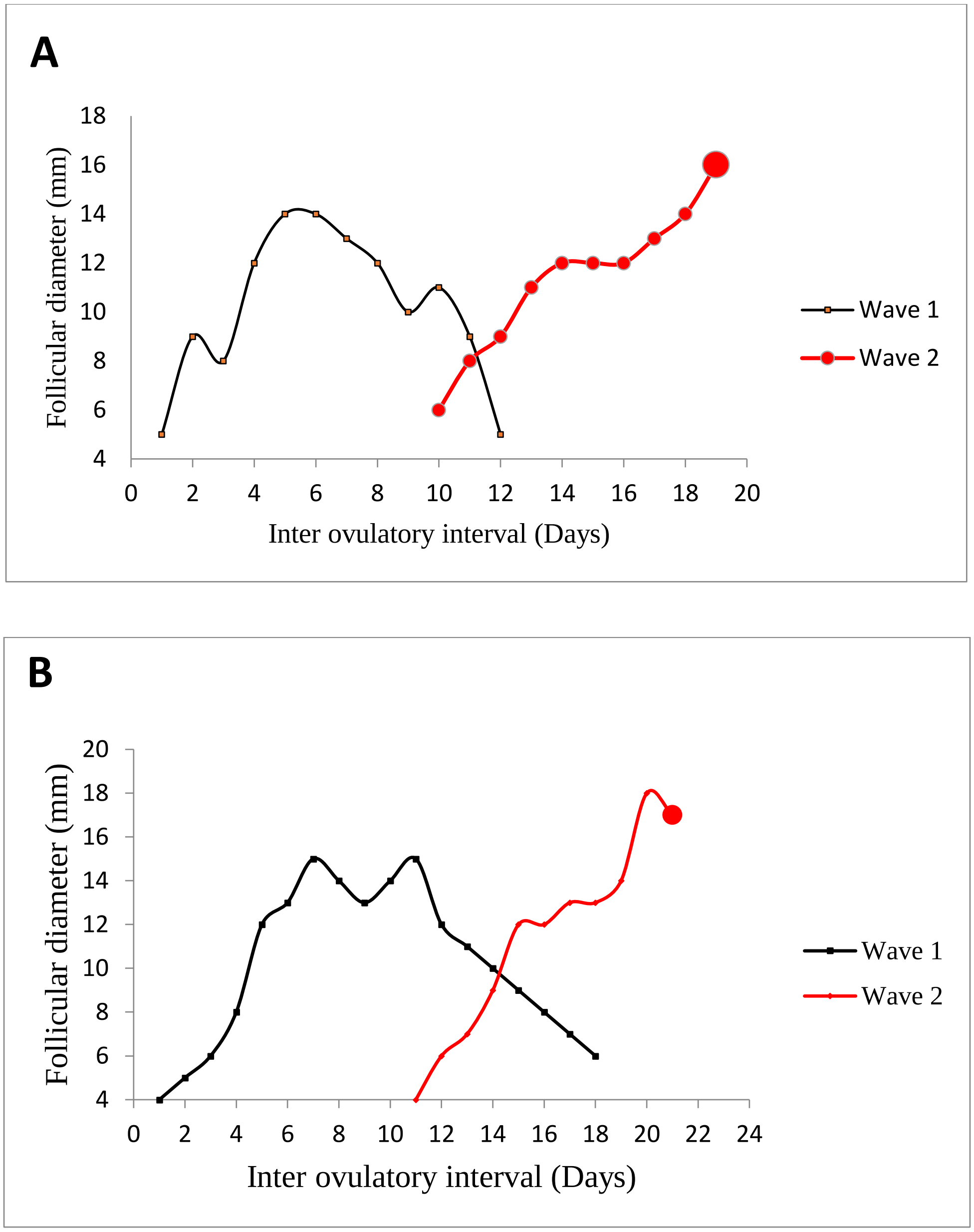


Figure 4.2: Dynamics of follicular development in a Fogera (A) and Jersey cows (B) during two-wave

The majority of Fogera cows (n=8, 88.89%) exhibited estrus cycles with two and three follicular waves. All Jersey cows (n=6, 100%) exhibited estrus cycles with two and three follicular waves. A Fogera cow (n=1, 11.11%) showed four follicular waves in the first and second estrus cycles and three follicular waves in the last (third) cycle.

Majority of the animals showed estrus cycle with three follicular waves and the below graph (Fig 4.2) shows animals those manifested three follicular wave.

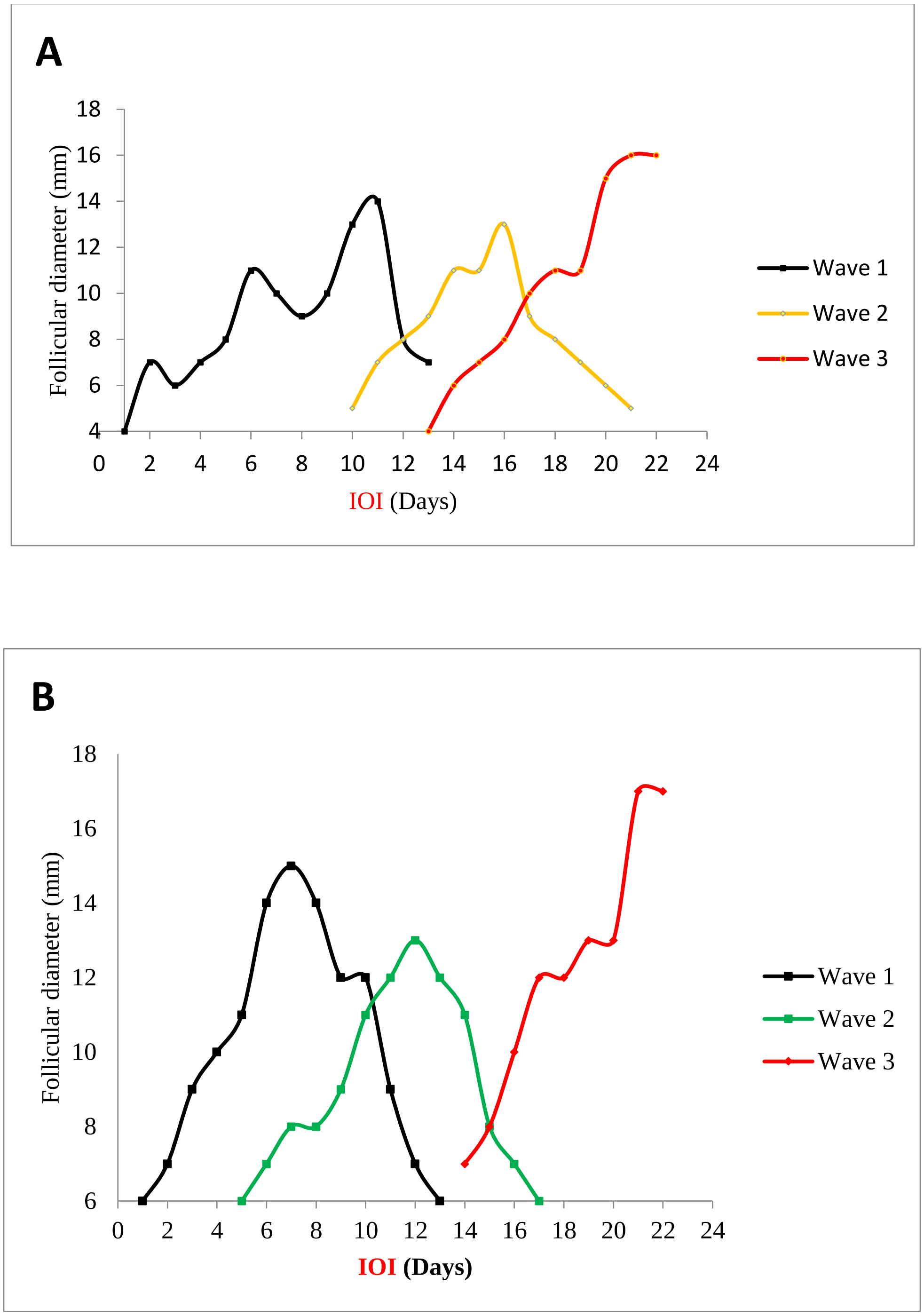


Figure 4.3: Dynamics of follicular development in a Fogara (A) and Jersey cows (B) during three-wave.

The growth pattern of second wave dominant follicle of Fogera heifer with four follicular wave emerged on fifth day, third wave on twelfth day and fourth wave on sixteenth day as shown on (Fig 4.3)

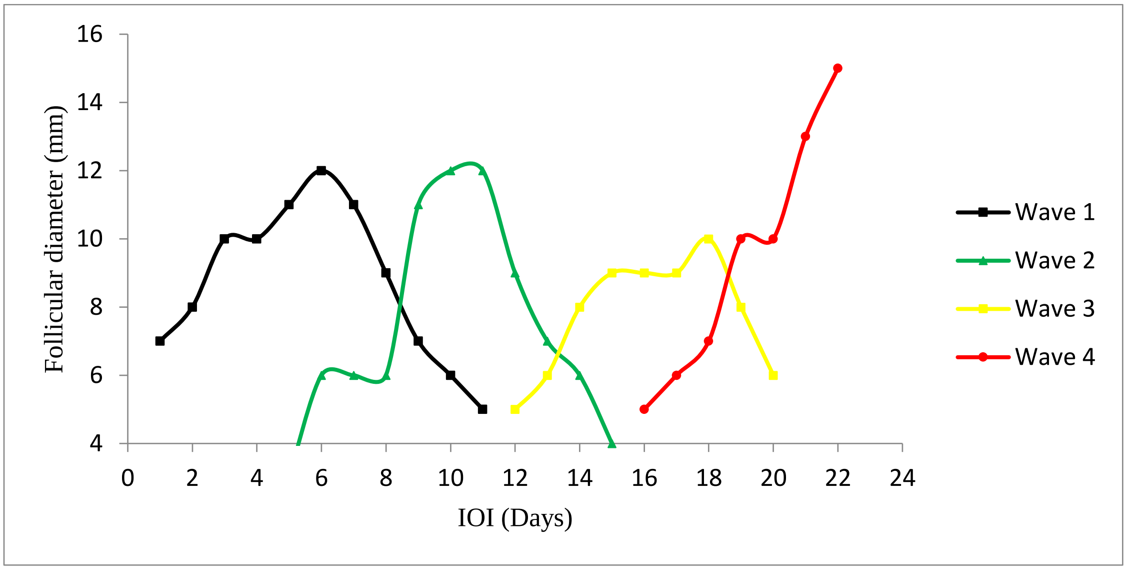


Figure 4.4: Dynamics of follicular development in a Fogera heifer during four-follicular waves

The estrous cycle length or inter ovulatory interval Fogera and Jersey is (20.35 ± 2.8 and 20.67 ± 2.6 days), respectively. The mean maximum diameter of the ovulatory follicle for Jersey cows (17.2 ± 0.86 mm) was higher than the diameter of the Fogera cows (15.22 ± 1.3 mm) ($p$<0.005). There was no statistically difference in dominant follicles growth or atresia rates between the breeds and between animals with different follicular waves. Table 4.2 shows the characteristics of follicular dynamics in the both breeds with two follicular waves during the estrous cycle.

The growth pattern for the first dominant follicle of Fogera and Jersey cows with two- waves emerged on day (0.25 ± 0.5 and 0.3 ± 0.5) and its regression occurred on day (9.13 ± 2.8 and 8.43 ± 2.8) of the estrus cycles, respectively (table 4.2). The dominant follicle reached its maximum diameter on day7.88 ± 2.9 for Fogera and 7.43 ± 2.7 for Jersey cows (table 4.2). The second wave emerged on day 10.0 ± 3.21 for Fogera and 10.29 ± 2.06 for Jersey cows, the dominant (ovulatory) follicle selected on day 13.25 ± 2.71 for Fogera, and 14.0 ± 1.91 for Jersey cows (table 4.2).

The maximum diameter of the dominant follicle for the first wave was smaller ($p<0.05$) than the diameter of the second wave dominant (ovulatory) follicle for Fogera breed. However, there was no difference ($p>0.05$) between the first and second waves of the dominant follicle maximum diameter in Jersey breed.

Table 4.2: Characteristics of follicular waves, growth and atresia rate of dominant follicle in Fogara heifers and Jersey cows with two follicular waves during estrus cycle (mean ±SD)*

| Characteristics | **Follicular Waves** | |
|---|---|---|
| | First | Second |
| | Fogara | |
| Wave onset (day) | 0.25 ± 0.46 | 10.0 ± 3.21 |
| Wave length (days) | 14.13 ± 3.09 | 9.13 ± 2.70 |
| Day of maximum diameter | 7.88 ± 2.90 | 18.13 ± 2.53 |
| Maximum diameter (mm) | $13.63 \pm 0.5^{a}$ | $15.13 \pm 0.99^{b}$ |
| Growth rate (mm/day) | 1.19 ± 0.46 | 1.23 ± 0.21 |
| Divergence day | 3.75 ± 1.04 | 13.25 ± 2.71 |
| Length of growth phase (days) | 8.13 ± 2.80 | 8.5 ± 2.45 |
| Onset of atresia (day) | 9.13 ± 2.80 | - |
| Atresia rate (mm/day) | 1.41 ± 0.13 | - |
| Length of atresia (days) | 7.13 ± 2.10 | - |
| | **Jersey** | |
| Wave onset (day) | 0.29 ± 0.49 | 10.29 ± 2.06 |
| Wave length (days) | 14.71 ± 3.30 | 11.00 ± 2.16 |
| Day of maximum diameter | 7.43 ± 2.76 | 20.29 ± 2.21 |
| Maximum diameter (mm) | 15.43 ± 1.27 | 16.57 ±1.13 |
| Growth rate (mm/day) | 1.39 ± 0.58 | 1.05 ± 0.15 |
| Divergence day | 4.29 ± 0.76 | 14.0 ± 1.91 |
| Length of growth phase (days) | 7.43 ± 2.76 | 10.29 ± 2.36 |
| Onset of atresia (day) | 8.43 ± 2.76 | - |
| Atresia rate (mm/day) | 1.32 ± 0.17 | - |
| Length of atresia (days) | 8.14 ± 1.07 | - |

** Means followed by different letters within rows differ (p< 0.05).*

The main characteristics of the follicular dynamics in the cows with three follicular waves are shown on Table 4.3.The first wave in Fogera and Jersey cows with three-follicular waves emerged on day (0.18 ± 0.4 and 0.25 ± 0.46) of the estrous cycle, respectively (table 4.3). The dominant follicle growth pattern of the first follicular wave for Fogera heifers and Jersey cows with three follicular waves reached maximum diameter on day 6.55 ± 1.7 and 5.8 ± 1.2 and began atresia on day 7.5 ± 1.9 and 6.75 ±1.2 respectively (table 4.3). The second wave showed up on day 7.5 ± 1.9 for Fogera heifers and 6.0 ± 1.1 for Jersey cows, and the dominant follicle had selected on day (11.10 ± 2.4 and 10.4 ± 2.3) reached maximum diameter on day (12.9 ± 3.0 and 12.3 ± 1.4) and began atresia on day (14.5 ± 2.7 and 13.6 ± 1.4) for Fogera heifers and Jersey cows, respectively (table 4.3).

The third wave (ovulatory) emerged on day (14.3 ± 2.4 and 12.8 ± 2.2), with the dominant follicle being selected on day (17.82 ± 2.2 and 16.4 ± 2.6) and reached its maximum diameter on day (21.3 ± 1.3 and 20 ± 1.9) of the estrus cycle, for Fogera and Jersey respectively (table 4.3).

Table 4.3: Characteristics of follicular waves, growth and atresia rate of dominant follicles in Fogara heifers and Jersey cows with three follicular waves during the estrus cycle (mean ± SD)*

| Characteristics | Follicular Waves | | |
|---|---|---|---|
| | First | Second | Third |
| | | **Fogara** | |
| Wave onset (day) | 0.18 ± 0.40 | 7.54 ± 1.86 | 14.27 ± 2.41 |
| Wave length (days) | 11.27 ± 1.35 | 12.0 ± 2.57 | 8.0 ± 1.41 |
| Day of maximum diameter | 6.55 ± 1.67 | 12.91 ± 3.02 | 21.27 ± 1.74 |
| Maximum diameter (mm) | 12.45 ± 1.44[a] | 12.09 ± 1.5[a] | 14.91 ± 1.5[b] |
| Growth rate (mm/day) | 1.21 ± 0.53 | 1.078 ± 0.43 | 1.32 ± 0.22 |
| Divergence day | 4.0 ± 1.0 | 11.09 ± 2.39 | 17.82 ± 2.23 |
| Length of growth phase (days) | 6.45 ± 1.86 | 6.45 ± 1.75 | 7.91 ± 1.2 |
| Onset of atresia (day) | 7.45 ± 1.86 | 14.45 ± 2.7 | - |
| Atresia rate (mm/day) | 1.29 ± 0.36 | 1.3 ± 0.25 | - |
| Length of atresia (days) | 5.36 ± 1.03 | 5.91 ± 1.04 | - |
| | | **Jersey** | |
| Wave onset (day) | 0.25 ± 0.46 | 6.0 ± 1.07 | 12.8 ± 2.2 |
| Wave length (days) | 11.75 ± 1.75 | 13.38 ± 1.9 | 8.8 ± 1.9 |
| Day of maximum diameter | 5.75 ± 1.17 | 12.25 ± 1.4 | 20 ± 1.9 |
| Maximum diameter (mm) | 14.63 ± 0.92[a] | 14.38 ± 1.8[a] | 17.4 ± 0.7[b] |
| Growth rate (mm/day) | 1.59 ± 0.32 | 1.17 ± 0.22 | 1.41 ± 0.24 |
| Divergence day | 3.63 ± 0.74 | 10.38 ± 2.3 | 16.4 ± 2.6 |
| Length of growth phase (days) | 5.75 ± 1.17 | 7.5 ± 1.2 | 8.13 ± 1.0 |
| Onset of atresia (day) | 6.75 ± 1.17 | 13.63 ± 1.4 | - |
| Atresia rate (mm/day) | 1.50 ±0.16 | 1.5 ± 0.4 | - |
| Length of atresia (days) | 6.75 ± 0.9 | 6.5 ± 1.1 | - |

** Means followed by different letters within rows differ (p< 0.05).*

There was a difference (*p*<0.001) between the size of both right and left ovaries of Fogera and Jersey cows. Table 4.4 shows the size (mm) of ovaries of both breeds (Fogera and Jersey)

Table 4.4: Comparison of the size (mm) of ovary in Fogara and Jersey cows

| Ovary type | Breed | N | Mean ± SD | t | *P-value* |
|---|---|---|---|---|---|
| Right ovary | Fogara | 549 | 30.08 ± 4.16[a] | -17.59 | 0.000 |
| | Jersey | 375 | 34.83 ± 3.9[b] | | |
| Left ovary | Fogara | 497 | 27.65 ± 5.34[a] | -14.36 | 0.000 |
| | Jersey | 376 | 32.27 ± 4.15[b] | | |

There were also difference ($p$<0.001) between the follicular count of right and left ovaries of Fogera and Jersey cows (table 4.5). The mean number of follicular population per animal was 13.9 ± 5.9 for Fogera heifers and 17.9 ± 7.0 for Jersey cows. There was significant difference ($p$<0.001) between Fogera and Jersey cows in mean number of follicular population per animal

Table 4.5: Comparison of follicular counts between Fogara and Jersey cows

| Follicular counts | Breed | N | Mean± SD | *t* | *p-value* |
|---|---|---|---|---|---|
| Right ovary | Fogara | 510 | 8.26 ± 2.37[a] | -9.5 | 0.000 |
| | Jersey | 349 | 10.02 ± 2.86[b] | | |
| Left ovary | Fogara | 446 | 8.25 ± 2.5[a] | -8.1 | 0.000 |
| | Jersey | 341 | 9.89 ± 3.1[b] | | |

# CHAPTER FIVE

# DISCUSSION

Afte 48hrs of hormonal injection all study animals except one Jersey cow, exhibited heat signs within five consecutive days. Behavioral signs of heat were weaker in Fogera heifers as compared to Jersey cows. Vaginal discharge, sniffing, mounting, stands to be mounted, swelling of the vulva, chin resting and restlessness are among estrus signs shown by both breed. This is inagreement with the report of Negussie *et al.*(2002). Orihuela (2000) had reported that estrus behavior expression in tropical breed is less than that of temperate. This in agreement with the present study where weaker estrus signs were observed in Fogera heifers compared to Jersey cows. The present finding is also in concordance with the previous findings of Tadesse *et al.*(2011) for boran and boran× holstein friesian crossbred cattle and Bó *et al*.(2003) for Brahman and Charolais cows. The relationship between cardinal signs of estrus is not common to all the cows and varies with breed (Lyimo *et al*., 2000; Van Eerdenburg *et al*., 2002). Even if Bos *indicus* and *Bos taurus* breeds share a common ancestor, there are disparity in various aspects of their reproductive physiology and behavior. These may be because of different natural and human selection pressures, compounded by strong genotype environment interactions Baruselli *et al*.(2006). Behavioral signs differ among individual cows in duration and intensity of estrus is also due to negative energy balance (NEB) which decreases expression of estrous behavior by suppressing hypothalamic production of GnRH and this in turn suppress LH pulsatile secretion which is responsible for maturation of follicle for production /secretion of estrogen hormone and environment is one factor for the difference of estrous behavioral sign (Reith and Hoy, 2018).

The three estrous cycles evaluated for 63 days on both breeds (Fogera and Jersey) manifested characteristic pattern of follicular waves. According to the present results the follicular dynamics of Fogera breed and jersey breed, their follicles have grown in a wave-like fashion, which confirms the previously known findings. In the present study, none of the cows had one or five follicular waves as reported by Viana et al.(2000). Two- and three- wave cycles occurred in the majority of animals (n=14, 93.3 %) during the estrus cycles. The incidence of higher estrus cycles with two and three follicular waves was also observed in European Taurus breeds Savio *et al*.(1988), Zebu cows Gambini *et al*.(1998), Gir breeds (Bos indicus) Viana *et al*.(2000) , Ethiopian Boran and their crosses Jemal *et al*.(2020) and in Kenyan Boran Muraya (2013a) in which the majority of them had shown two and three follicular waves. The length of the luteal phase is considered as the main factor determining the number of follicular waves Figueiredo *et al*.(1997).

In this current observation in few animals the dominant follicle remained for longer times (more than three days) without ovulation. This phenomenon also reported in local breeds Jemal *et al*.(2020). The failure of the dominant follicles to ovulate could be due to lack of LH serge. LH pulse frequency is the key determinant of the dominant follicle growth Duffy *et al*.(2000) . Follicular growth and maturation are dependent on a high frequency of LH pulses Roche and Boland (1991), which is suppressed during diestrus due to the negative feedback effect of the progesterone. The mean length of inter ovulatory interval had shown no difference between the breeds. This result is in agreement with previous work (Degefa *et al*., 2016; Jemal *et al*., 2020) in Ethiopian Boran and their crossbred cows' estrous cycle length.

Jersey cows had higher diameter of ovulatory follicle than Fogera heifers. Muraya, (2013a) reported that the maximum diameter of ovulatory follicle in Kenyan Boran cows' was smaller than those reported in European cows by Ginther *et al.* (1989). But comparable in size with other *Bos indicus* cows Figueiredo *et al*.(1997). There was no difference in dominant or subordinate follicles growth or atresia rates between the breeds and between animals with different follicular waves this is inagreement with the report of Jemal *et al.* (2020). The dominant follicles in both breeds developed in the right ovaries in higher proportion than the left ovaries. The higher incidence of ovulatory DFs in the right ovary is because of 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 left ovary (Muraya, 2013b). All the characteristics observed for follicular dynamics in two and three follicular wave for both breeds compare well with others documented for other breeds of zebu cattles ( Savio *et al.*, 1988; Taylor and Rajamahendran, 1991; Viana *et al*., 2000; Muraya, 2013b; Jemal *et al*., 2020)

There was significant difference in total number of follicular population between the breeds. Fogera cows have lower follicular population compered to Jersey cows,but It is known that *Bos indicus* cattle have 3-4 times more follicle population than *Bos taurus* cattle Pontes *et al*.(2011). Fogera heifers were bought from the farmers. During pregnancy they may deprived of nutrition, because in our country the farmer does not take enough care of his animals. Feed shortages During pregnancy affects the follicular population of progeny. That is why fogera heifers had lower follicle population than jersey cows. In the present study mean number of follicular population for both breed is lower than that of Mossa *et al*.(2012) for Holstein-Friesian dairy cows,however the present finding is higher than that of Jemal *et al*.(2020) for Ethiopian Boran breed and its HF crossbred heifers.Follicle count is a good predictor of subsequent superovulation response and embryo production ability of female cattle Center (2015). Cattle with relatively average to high numbers of follicles during follicular waves respond best to superovulation and the number of follicle which at each follicular wave predicts ovarian reserve (primordial follicle) and this indicates that the animal can continuously produce eggs Ireland *et al*.(2007).Depending on this fact Fogera and Jersey can be responsive to supper ovulation and good embryo producer since their follicle population is average.

# 6. CONCLUSIONS AND RECOMMENDATIONS

Behavioral signs of heat were weaker in fogera heifers as compared to Jersey cows. Prolonged estrus signs (more than three days) were exhibited in both Fogera and Jersey cows. The length of the estrous cycle of Fogera heifers is similar to that of other zebu breeds with predominantly three follicular waves within the estrous cycle, with a fourth wave also present in this cattle. Jersey cow have shown two and three follicular wave during consecutive three estrus cycles. Relatively higher number of ovarian follicles observed in Jersey than Fogera breed.

- 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 to maximize on super ovulatory response.

- The prolonged estrus signs (more than three days) without ovulation were exhibited in these breeds as there is confirmed by this study, we just recommend, it is preferable to inseminate semen (AI) two days after they show these signs.

- Our research is limited to this point and the remaining ones such as: supper ovulation test, ovum-pickup and invitro maturation,invitro fertilization and embryo transfer, and hormonal profile (progesterone ,estrogen,FSH,LH) has to be done.

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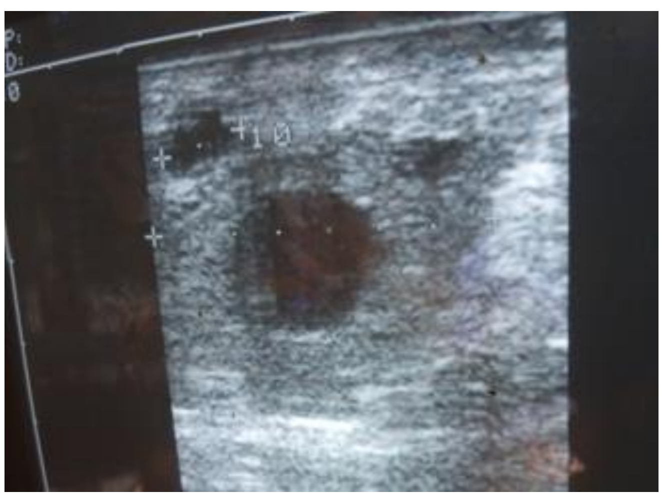
# ANNEXES

## **Annex A** PHOTO SHOWING SERIAL SCANNING OF THE COW'S UTERUS, AND OVARIAN STRUCTURES BY ULTRASOUND

Annexes A-1: Ultrasound searching of cow's ovary

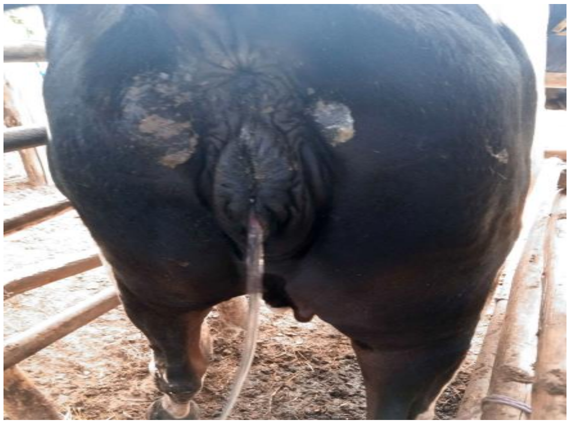


Annexes A-2: Ovarian structure with dominant follicle and subordinate follicles

Annexes A-3: Measuring the size of follicles, ovarian size, and counting follicle population

Annexes A-4: Installation of ultrasound to start scanning

**Annex B**. PHOTO SHOWING COW'S ESTRUS BEHAVIOR



Annexes B-1: Fogera cattle breed showing vulva discharge

Annexes B-2: Jersey cattle breed showing vulva discharge

Annexes B-3: Fogera cattle breed showing mounting and stands to be mounted

Annexes B-4: Jersey cattle breed mounting and fogera cattle breed stands to be mounted

Annexes B-5: Jersey cattle breed chin resting over fogera cattle breed

Annexes B-6: Jersey cattle breed mounts and other jersey stands to be mounted

## **Annex C.** PHOTO OF SHOWING EXPERIMENTAL ANIMALS

Annexes C-1: Photo showing experimental animals (jersey breed)

Annexes C-2: Photo of showing experimental animals (fogera breed)

Annexes D-1: Table of follicular dynamics data sheet

| Follicular Dynamics Data Sheet for Fogera and Jersey | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Animal ID | Largest | | | | | | | | | | | |
| No | Follicles | mm | mm | mm | mm | mm | mm | mm | mm | mm | mm | mm |
| F10 | f1 | | | | | | | | | | | |
| RO | f2 | | | | | | | | | | | |
| | f | | | | | | | | | | | |
| | f4 | | | | | | | | | | | |
| | f5 | | | | | | | | | | | |
| | TNF | | | | | | | | | | | |
| | OVS | | | | | | | | | | | |
| | CLS | | | | | | | | | | | |
| LO | f1 | | | | | | | | | | | |
| | f2 | | | | | | | | | | | |
| | f | | | | | | | | | | | |
| | f4 | | | | | | | | | | | |
| | f5 | | | | | | | | | | | |
| | TNF | | | | | | | | | | | |
| | OVS | | | | | | | | | | | |
| | CLS | | | | | | | | | | | |
| | f1 | | | | | | | | | | | |
| RO | f2 | | | | | | | | | | | |
| | f | | | | | | | | | | | |
| | f4 | | | | | | | | | | | |
| | f5 | | | | | | | | | | | |
| | TNF | | | | | | | | | | | |
| | OVS | | | | | | | | | | | |
| | CLS | | | | | | | | | | | |
| LO | f1 | | | | | | | | | | | |
| | f2 | | | | | | | | | | | |
| | f | | | | | | | | | | | |
| | f4 | | | | | | | | | | | |
| | f5 | | | | | | | | | | | |
| | TNF | | | | | | | | | | | |
| | OVS | | | | | | | | | | | |
| | CLS | | | | | | | | | | | |
| | f1 | | | | | | | | | | | |
| RO | f2 | | | | | | | | | | | |
| | f | | | | | | | | | | | |
| | f4 | | | | | | | | | | | |
| | f5 | | | | | | | | | | | |
| | TNF | | | | | | | | | | | |
| | OVS | | | | | | | | | | | |
| | CLS | | | | | | | | | | | |
| LO | f1 | | | | | | | | | | | |
| | f2 | | | | | | | | | | | |
| | f | | | | | | | | | | | |
| | f4 | | | | | | | | | | | |
| | f5 | | | | | | | | | | | |
| | TNF | | | | | | | | | | | |
| | OVS | | | | | | | | | | | |
| | CLS | | | | | | | | | | | |

Annex E. 1: Photo of showing estrus behavior data sheet

| Animal ID number | Estrus cycle | | |
|---|---|---|---|
| | First estrus cycle | Second estrus cycle | Third estrus cycle |
| F008 | | | |
| | mounting | mounting | mounting |
| | Vaginal discharge | Vaginal discharge | Vaginal discharge |
| | Stands to be mounted | Stands to be mounted | Stands to be mounted |
| | Swelling of vulva | Swelling of vulva | Swelling of vulva |
| F017 | Vaginal discharge | Vaginal discharge | Vaginal discharge |
| | mounting | mounting | mounting |
| | Swelling of vulva | Swelling of vulva | Swelling of vulva |
| F010 | Vaginal discharge | Vaginal discharge | Vaginal discharge |
| | mounting | mounting | mounting |
| | Swelling of vulva | Swelling of vulva | Swelling of vulva |
| | restlessness | restlessness | restlessness |
| F026 | Vaginal discharge | Vaginal discharge | Vaginal discharge |
| | Stands to be mounted | Stands to be mounted | Stands to be mounted |
| | Swelling of vulva | Swelling of vulva | Swelling of vulva |
| | mounting | mounting | mounting |
| F014 | Vaginal discharge | Vaginal discharge | Vaginal discharge |
| | Chin resting on the rump | Chin resting on the rump | Chin resting on the rump |
| | sniffing | sniffing | sniffing |
| | restlessness | restlessness | restlessness |
| | mounting | mounting | mounting |
| | Swelling of vulva | Swelling of vulva | Swelling of vulva |
| F016 | Vaginal discharge | Vaginal discharge | Vaginal discharge |
| | mounting | mounting | mounting |
| | Stands to be mounted | Stands to be mounted | Stands to be mounted |
| | Swelling of vulva | Swelling of vulva | Swelling of vulva |
| F020 | Vaginal discharge | Vaginal discharge | Vaginal discharge |
| | Chin resting on the rump | Chin resting on the rump | Chin resting on the rump |
| | sniffing | sniffing | sniffing |
| | restlessness | restlessness | restlessness |
| | mounting | mounting | mounting |
| | Swelling of vulva | Swelling of vulva | Swelling of vulva |
| F036 | Vaginal discharge | Vaginal discharge | Vaginal discharge |
| | mounting | mounting | mounting |

| | | | |
|---|---|---|---|
| | Swelling of vulva | Swelling of vulva | Swelling of vulva |
| F012 | mounting | mounting | mounting |
| | Swelling of vulva | Swelling of vulva | Swelling of vulva |
| | Vaginal discharge | Vaginal discharge | Vaginal discharge |
| J1359 | mounting | mounting | mounting |
| | Vaginal discharge | Vaginal discharge | Vaginal discharge |
| | Swelling of vulva | Swelling of vulva | Swelling of vulva |
| | Stands to be mounted | Stands to be mounted | Stands to be mounted |
| J1415 | Vaginal discharge | Vaginal discharge | Vaginal discharge |
| | restlessness | restlessness | restlessness |
| | mounting | mounting | mounting |
| | Chin resting on the rump | Chin resting on the rump | Chin resting on the rump |
| | Swelling of vulva | Swelling of vulva | Swelling of vulva |
| J1437 | Vaginal discharge | Vaginal discharge | Vaginal discharge |
| | Stands to be mounted | Stands to be mounted | Stands to be mounted |
| | Chin resting on the rump | Chin resting on the rump | Chin resting on the rump |
| | Swelling of vulva | Swelling of vulva | Swelling of vulva |
| | mounting | mounting | mounting |
| J1211 | Vaginal discharge | Vaginal discharge | Vaginal discharge |
| | Stands to be mounted | Stands to be mounted | Stands to be mounted |
| | sniffing | sniffing | sniffing |
| | Chin resting on the rump | Chin resting on the rump | Chin resting on the rump |
| | Swelling of vulva | Swelling of vulva | Swelling of vulva |
| | mounting | mounting | mounting |
| J1323 | Vaginal discharge | Vaginal discharge | Vaginal discharge |
| | Stands to be mounted | Stands to be mounted | Stands to be mounted |
| | Swelling of vulva | Swelling of vulva | Swelling of vulva |
| | mounting | mounting | mounting |
| | restlessness | restlessness | restlessness |
| J1516 | Vaginal discharge | Vaginal discharge | Vaginal discharge |
| | Chin resting on the rump | Chin resting on the rump | Chin resting on the rump |
| | sniffing | sniffing | sniffing |
| | mounting | mounting | mounting |
| | Swelling of vulva | Swelling of vulva | Swelling of vulva |
| | Stands to be mounted | Stands to be mounted | Stands to be mounted |