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ADDIS ABABA UNIVERSITY COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

OVARIAN FOLLICULAR DYNAMICS AND CONCEPTION RATE IN BORAN COWS PREVIOUSLY SUBJECTED TO REPEATED OVUM PICK UP

BY

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Ovarian follicular dynamics and conception rate in Boran cows previously subjected to repeated ovum pick up

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DEDICATION

This work is dedicated to my grandfather Ato Tesfaye Aga and my aunt W/ro Asnaku Tesfaye who passes away during my research work.

STATEMENT OF AUTHOR

First, I declare that this thesis is my work and that all sources of material used have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced degree (MVSc) at Addis Ababa University, College of Veterinary Medicine, and is deposited at the University/College library to be made available to borrowers under the rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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ABBREVIATIONS

ADH	Angiotensin-converting enzyme
AI	Artificial Insemination
AIT	Artificial Insemination Technician
BMP-15	Bone morphogenic protein-15
CL	Corpus Luteum
COCs	Cumulus Oocyte Complexes
CSA	Central Statistical Agency
DF	Dominant Follicle
DGF-9	Growth Differentiation factor-9
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization
FSH	Follicle Stimulating Hormone
GDP	Growth Development Program
GnRH	Gonadotropin-Releasing Hormone
IGF-I	Insulin-like Growth Factor I
IOI	Inter Ovulatory Interval
IVEP	In Vitro Embryo Production
KL	Kit-ligand
LH	Luteinizing Hormone
OPU	Ovum Pick up
P4	Progesterone
PGF2α	Prostaglandin F2α
PMSG	Pregnant Mare Serum Gonadotropin

ABSTRACT

The study was conducted to investigate the effect of repeated ovum pick-up on ovarian follicular dynamics, serum estrogen and progesterone pattern, and the conception rate of Boran cows. Eight Boran cows were estrous synchronized by 500µg PGF2α at 11 days intervals. After the second PGF2α injection ovaries were scanned by transrectal ultrasonography every 12 hrs for the first two days and then every 6 hrs until ovulation. From the day of ovulation onwards, the ovaries were examined twice daily (at 12 hrs interval) to characterize the growth and regression pattern of ovarian follicles during two consecutive estrous cycles. A blood sample was collected every other day to evaluate estrogen and progesterone concentrations. The mean (±SEM) IOI was 21.1±1.19 days for cows with two waves and 22.66±0.57 days for cows with three waves. The length of the IOI was not significant (P>0.05) by follicular waves. The mean (±SEM) number of days from the emergence of cohort follicles to ovulation of dominant follicle of the ovulatory wave was significantly (P<0.05) greater (10.80±1.03 days) for two wave cows than three-wave cows (7.50±0.70 days). The mean (±SEM) diameter of the preovulatory follicle was significantly (P<0.05) larger for cows with two waves (14.29±1.36mm) than for cows with three waves (12.30±1.01mm). The mean (±SEM) growth rate of the dominant follicle that finally ovulated was significantly (P<0.05) higher (1.16±1.2mm/day) in the three-wave cycle than in two-wave cycles (0.85±0.1mm/day). The mean (±SEM) serum concentrations of estrogen and progesterone were 28.73±6.56 pg/ml and 0.88±0.40 ng/ml, respectively during the proestrous and estrous. While during the late diestrous cycle, serum estrogen and progesterone were 11.01±1.61pg/ml and 2.67±0.12 ng/ml, respectively. 87.5 % of cows were found conceived on ultrasound 32 days after insemination. In conclusion, based on ovarian follicular dynamics, serum estrogen and progesterone pattern, and conception rate repeated ovum pick does not significantly affect the fertility of Boran cows. Boran cows were characterized by a higher incidence of two-wave cycles.

Keywords: *Boran cows, estrous cycle, follicular wave, hormones, ovum pick-up*

1. INTRODUCTION

The Boran cattle breed is one of the most commonly used cattle breeds in Africa and Ethiopia. The Ethiopian Boran is descended from the first Zebu to be introduced into Africa from West Asia. The breed is well suited to semi-arid tropical conditions and the arid pastoral Borana plateau of southern Ethiopia, with a high degree of heat tolerance, resistance to many tropical diseases, and the ability to survive long periods of feed and water scarcity (Hannotte *et al*., 2000). The Ethiopian Boran spread to Ethiopia's eastern rangelands, as well as northern Kenya and southwestern Somalia, as a result of pastoralist movements and migrations. These migrations gave rise to the Orma Boran, Ethiopian Boran, and Kenya Boran, with only the Orma and Ethiopian Boran remaining on the Borana plateau (Hannotte *et al*., 2002; DAGRIS, 2008).

Boran has a large body size, large and long legs, tall height, broad back and wide pin-bones, well-developed dewlap and udder, and well-developed hindquarters. The male's body weight ranges from 318 to 680 kg, while the females' body weight ranges from 225 to 454 kg (Mekonnen *et al*., 2010; OARI, 2010). The survival of this breed is now threatened by several factors, the most serious of which are bush encroachment, frequent droughts, inadequate herd management, and market access issues (Coppock, 1994).

Ovarian follicular growth in cows occurs in waves. A wave of follicular growth occurs when a group of follicles develops at the same time, one of which becomes dominant and grows to the maximum diameter, suppressing the growth of the smaller follicles. Emergence, selection, and dominance are all part of each wave of development, which is followed by either atresia or ovulation of the dominant follicle (Pierson, 1987).

Ultrasonography has improved scientific understanding of the physiological processes in the estrous cycle, including ovarian follicle growth, regression, and ovulation. These results indicate that follicles in an increasing cohort are at various stages of differentiation, implying that there is a developmental hierarchy within a growing cohort (Ginther *et al*., 1996). Ovum pick-up is a well-established method of collecting oocytes that involves puncturing follicles on the ovaries of a donor heifer or cow and collecting the fluid and oocytes inside. High-quality oocytes can be fertilized and early matured in a laboratory before being transferred to recipient cattle. Although ultrasound-guided transvaginal follicular aspiration is assumed to be a non-invasive technique for ovum pick-up (OPU) for recovering oocytes from antral follicles in live animals (Dellenbach *et al.*, 1984), there are contradicting reports on the fertility of cattle after repeated ovum pick up. Therefore, it was hypothesized that repeated ovum pick-up in Boran cows has a negative impact on ovarian cyclic activity and fertility; based on this hypothesis the current study proposed with the following objectives:

Therefore, the objectives of this study were:-

- ❖ To assess impacts of repeated follicular punctures on ovarian follicular dynamics and estrous related hormones
- ❖ To evaluate the potential negative impacts of repeated OPU procedures on the fertility of donor cows through assessing the conception rate

2. LITERATURE REVIEW

2.1. Boran cattle

Boran, a popular cattle breed, is widely used and distributed throughout various African countries. The Boran evolved in eastern Africa, with the Borana plateau in southern Ethiopia serving as the primary Boran hotspot (DAGRIS, 2006). That was the point at which all of the different breeds passed through on their way to their various destinations in Africa. The Boran's genetic make-up was discovered to be 24 percent European *Bos Taurus*, 64 percent *Bos indicus*, and 12 percent African *Bos Taurus*, according to DNA research (Wayne, 2007).

The phenotypic characteristics of Boran cattle can be divided into two subtypes based on their origin: the large-framed Qorti and the smaller Ayuna (Haile Mariam *et al*., 1998).A true type of Ethiopian Boran cattle, the Qorti is known for its physiological adaptation to heat stress, drought tolerance, walkability, good mothering abilities, docility, longevity and tall height. Relatively they has also long legs, broad backs, long necks, short horns, short tails, and a pendulous preputial sheath on males and the umbilical fold on females (Haile Mariam, 1994). Under range conditions, the second form, Ayuna, is known for its high fertility, good growth, and milk production potential (Homann *et al*., 2003; Kerstin and John, 2004).

The Ethiopian Boran, with its distinctive hump and pendulous dewlap, is a member of the zebu cattle (*Bos indicus*) family. According to archaeological records, zebu cattle are the most recent types of cattle to be introduced into Africa (Marshall, 2000; Hanotte *et al.*, 2002). Zebu cattle are known to regulate body temperature better than humpless cattle, resulting in lower body water requirements. Long migrations are made possible by their hardened hooves and lighter bones. These adaptable characteristics have aided their importation and spread across the Red Sea by Indian and Arabian merchants to the drier agro-ecological regions of the Horn of Africa (Loftus and Cunningham, 2000).

The Large East African Zebu is represented by 13 breeds that are restricted to relatively dry areas of Sudan, Eritrea, Ethiopia, Somalia, Kenya, Tanzania, and Uganda. Isolations imposed by tribal borders, whether physical or cultural, as well as those imposed by ecological limitations, are partly responsible for genetic differentiation, resulting in the existence of various breeds and strains (Rege and Tawah, 1999). The Ethiopian Boran breed is descended from the first introduction of zebu into Africa from West Asia. The breed first appeared in the semi-arid and arid pastoral Borana plateau of southern Ethiopia. Pastoral movements and migrations spread the Ethiopian Boran to the eastern rangelands of Ethiopia, as well as northern Kenya and southwestern Somalia. These migrations resulted in the evolution of the Orma Boran, Somali Boran, and Kenya Boran. Boran is now thought to be divided into two groups: unimproved and improved Boran (DAGRIS, 2006).

The Boran breed of cattle is distinct to northern Kenya and southern Ethiopia. In 1992, the FAO named the Boran as one of five tropical cattle breeds that should be prioritized for further development and conservation (Philipson, 1992). This is because the area where Boran cattle originate in Ethiopia is dominated by a semi-arid climate characterized by pastoral or agro-pastoral systems, with surface water serving as the primary limiting resource (Hailemariam, 1994). The Borana pastoralists achieved a high level of production efficiency while maintaining an optimum balance of people, livestock, grass, and water by implementing relatively sophisticated rangeland management and a coherent, well-structured social organization. The Boran, a breed developed by Borana pastoralists in Ethiopia and improved by ranchers in Kenya, is now reported from 11 countries (nine in East, Central, and Southern Africa, as well as Australia and Mexico) (Homann *et al.,* 2006; FAO, 2007).

Boran cattle are relatively large and have a good overall body conformation. Their coat is mostly white, light gray, fawn, or light brown, with gray, black, or dark brown shading on the head, neck, shoulders, and hindquarters. The horns are thick at the base, short, upright, and pointing forward. In the male, there is a well-developed hump that is pyramidal in shape and overhangs to one side. The dewlap is well developed. The preputial sheath is pendulous in the male, whereas the udder is well developed in the female. The average wither height is 118 to 124 cm (Alberro and Haile-Mariam, 1982).

The Ethiopian Boran breed is one of the most common cattle breeds in Ethiopia. The breed is well adapted to semi-arid tropical conditions, has a high level of heat tolerance, is resistant to many tropical diseases, and can survive long periods of feed and water scarcity (Ojango *et al.*, 2006). When compared to other indigenous cattle breeds in Ethiopia, the breed is fast-growing, fertile, and a good milk producer. The performance of Boran's growth, reproduction, and milk production has been improved in various parts of the world, including Kenya, South Africa, Australia, and the United States (Haile *et al*., 2009).

Additionally, Boran's performance has also improved significantly as a result of improvements in management and selection. The improved Boran, for example, is heavier at birth, weighing an average of 30 kg, and the weaning weight at Abernossa Ranch in Ethiopia was estimated to be 158 kg (Banjaw and Haile-Mariam, 1994; DAGRIS 2006). Female reproductive traits are commonly used to evaluate reproductive performance. Regularly considered traits include age at first calving, calving interval, and calving rate. Comparing the reproductive performance of Ethiopian Boran cattle to that of other indigenous Ethiopian breeds revealed that Boran cattle calve at a younger age and have shorter calving intervals. The improved types, on the other hand, outperformed the Ethiopian Boran (Ouda *et al*., 2001; DAGRIS, 2006).

In general, Ethiopian Boran is superior to other indigenous Ethiopian cattle breeds in terms of growth, reproduction, milk production, and carcass traits; second, improved Boran in Kenya, South Africa, Australia, and the United States is a better animal than unimproved Ethiopian Boran; and third, with a properly designed selection scheme and management intervention, there is an enormous opportunity for improvement. Ethiopia focuses on the genetic improvement of cattle through cross-breeding. Some of the efforts in Ethiopian Boran cattle breeding and improvement can be found at the Adami-Tulu and Abernossa cattle improvement and multiplication centers (Haile *et al.*, 2009).

2.2. Ovarian follicular dynamics in cattle

2.2.1. Follicular development

Folliculogenesis is the process by which a pool of non-growing, primordial follicles in the ovary develops to a preovulatory size after the oocyte and its surrounding granulosa cells grow and differentiate (Knight and Glister, 2001). Ovarian follicles are fluid-filled structures surrounded by granulosa cells on the inside and thecal cells on the outside. The oocyte is held in place within the antrum by the cumulus oophorous, a specialized pedicle of granulosa cells (Pierson and Ginther, 1988). There are two phases of follicular development: a gonadotropin-independent (FSH and LH) phase in which the follicle grows from primordial to large antral follicle, and an FSH- and LH-dependent phase. The follicle will ovulate when there are adequate levels of frequency and amplitude of LH pulses during the preovulatory stage (Webb *et al.*, 2004).

The total time required by an activated bovine primordial follicle to reach preovulatory size has been estimated to be 80 to 100 days in bovine, regulated by signals between the oocyte and surrounding epithelial cells, other somatic cells in the ovarian cortex, and signals emanating from systemic circulation (Britt, 2008). Primordial follicles may undergo progressive development soon after formation, or they may remain dormant for years before resuming development (Cushman *et al*., 2002; Yang and Fortune, 2008). The transformation of a primordial follicle into a preantral follicle entails cellular growth, proliferation, and differentiation (Braw-Tal, 2002).

Even though the mechanisms that control the differentiation of pregranulosa to granulosa cells during primordial follicle activation and subsequent granulosa proliferation are unknown, molecules such as kit ligand (KL) and its receptor (c-Kit), growth differentiation factor 9 (DGF-9), and bone morphogenetic protein 15 (BMP-15) have been implicated (Fortune, 2003; Oktem and Oktay, 2008; Moniruzzaman and Miyano, 2010). In the follicular developmental hierarchy, the primordial follicle contains an oocyte with a visible nucleus surrounded by a single flattened layer of pregranulosa cells, whereas the primary follicle contains a larger oocyte with a visible nucleus surrounded by cuboidal granulosa cells (Cushman *et al*., 2002).

The larger secondary follicle has an enlarged oocyte surrounded by 3 to 4 layers of cuboidal granulosa cells and a thicker cytoplasmic membrane due to the appearance of a zona pellucida layer secreted by the oocyte. Once progressive follicle growth begins a variety of local and systemic regulatory factors such as insulin, IGF-I, steroids (estradiol, testosterone), gonadotropins, and angiotensin-converting enzyme (ADH) come into play (Britt, 2008). The final development of large antral follicles occurs in a wavelike pattern, with a cohort of follicles of similar size continuing to grow in response to an increase in FSH (Ginther *et al*., 1989; Rathbone *et al.*, 2001).

Each wave of follicular growth in cattle lasts 7 to 9 days, during which there is a transient peak of FSH, a selection phase that results in a reduction in the number of growing follicles, and a decrease in the concentration of circulating FSH. This coincides with the onset of atresia in the majority of follicles (subordinate follicles), but follicles with diameters equal to or greater than 8 mm may remain dominant and ovulate if the animal hormonal milieu is adequate. If the ovulation of the dominant follicle coincides with the lysis of the CL and the subsequent fall of P4, the inhibitory effect of P4 on GnRH is eliminated, and an LH preovulatory peak is reached, resulting in follicle ovulation (Mihm *et al.*, 2000; Fortune *et al.*, 2001).

Due to the regression of the corpus luteum, there is a hormonal environment of basal progesterone during the follicular phase of the estrous cycle (CL). The increased E2 concentrations caused by the rapid proliferation of the pre-ovulatory dominant follicle (DF), combined with the decrease in circulating progesterone concentrations, cause a surge in GnRH and allows for the display of behavioral estrus, during which heifers/cows are sexually receptive and will stand to be mounted. Because of negative feedback, the progesterone-dominant luteal phase of the estrous cycle only allows for the secretion of larger amplitude but less frequent LH pulses (one pulse every 3 to 4 hours), which are insufficient (Rahe *et al*., 1980). Thus, the DFs produced during the luteal phase of the estrous cycle undergo atresia, E2, and inhibin production decreases, removing this negative feedback block to the hypothalamus/pituitary, FSH secretion can increase, and a new follicle wave emerges (Crowe and Mullen, 2013).

2.2.2. Follicular wave pattern during an estrous cycle

A follicular wave begins with the emergence of a group or cohort of small antral follicles just before the day of ovulation. During the next few days, one of the follicles in this cohort grows and becomes dominant, suppressing subordinate follicles within the wave from which it originated as well as the emergence of follicles in an ensuing follicular wave. As the dominant follicle grows, the growth of the remaining follicles in the cohort stops or slows, and these subordinate follicles eventually develop atresia. The second wave of growth appears around Day 10 after ovulation, and for three-wave cycles, an additional wave appears around Day 16 after ovulation. The ovulatory follicle emerges from the final wave in both two and three-wave cycles (Ginther *et al*., 1996).

The ovarian follicular dynamics of cows and heifers during estrous cycles are characterized by waves of follicular growth and regression (Knopf *et al*., 1989; Taylor and Rajamahendran, 1991). Each cycle consists of two or three waves of follicular growth, followed by a period of growth, and ending with the growth of a large dominant follicle while the others become atretic (Webb *et al*., 1999). Follicular dynamics refers to the process of continuous growth and regression of antral follicles that leads to the development of the preovulatory follicle. In a single estrous cycle, there are one, two, three, or four waves of follicular growth, and the preovulatory follicle is derived from the last wave (Ginther *et al*., 1989; Forde *et al*., 2010; Crowe and Mullen, 2013).

The main characteristics of follicular growth and atresia are affected by wave order (Savio *et al*., 1988; Ginther *et al*., 1989) and can also vary between animals due to a variety of factors such as breed, reproductive stage, season, heat stress, energy balance, and body score condition (Rhodes *et al.,* 1995; Figueiredo *et al.*, 1997; Burke *et al.*, 1998; Wilson *et al.*, 1998; and Crowe and Mullen, 2013). From the cohort, one follicle is chosen for continued growth and becomes dominant. Final maturation and ovulation occur if luteolysis occurs during the growth phase of the dominant follicle. Atresia results when luteolysis does not occur during the growth and maintenance phase of the follicle (Bao & Garverick, 1998). Each wave is preceded by a small increase in serum FSH levels, followed by the recruitment of a pool of follicles within 2-5 mm, which progresses ovarian development (Ginther *et al*., 1997).

Recruitment is the process by which a group of follicles begins to mature in an environment with enough pituitary gonadotropic stimulation to allow progress toward ovulation. During each wave of follicular growth, a cohort (usually 1 to 6) of follicles 4 to 5 mm in diameter emerges and begins to grow (recruitment). Each wave of follicular growth is preceded by a transient increase in FSH that begins about 2.5 days before the initiation of the new wave of follicular growth and begins to decline around the time the cohort of follicles in the wave appears (Adams *et al*., 1992; Hamilton *et al*., 1995).

Selection is the process by which a follicle (or follicles) avoids atresia, develops further, and becomes competent to achieve timely ovulation. FSH and LH regulate the process of follicle selection on a systemic level, while factors that modulate the actions of gonadotropins regulate it on a local level (Ginther *et al*., 2002). The transient FSH rise was responsible for the initiation of follicular growth wave peaks when the largest bovine follicles reach a diameter of 4–5 mm and then decrease (Kulick *et al*. 1999). Maximum FSH levels in the first growth wave of the cycle are typically expressed 28 hours after the onset of estrous (Mihm and Austin, 2002). The production and secretion of estradiol and inhibin by growing follicles suppresses FSH release, even though these follicles are still dependent on FSH for growth (Gibbons *et al*., 1999).

Dominance is the process by which a single recruited follicle achieves and maintains dominance over the other recruited follicles that undergo atresia. In cattle, the largest follicle of a follicular wave emerges at 3 or 4 mm and 6 or 7 hours before the second-largest follicle (Ginther *et al*., 1997; Kulick *et al*., 1999). The beginning of the difference in growth rates between the two largest follicles is known as follicle deviation, and it occurs in cattle when the largest follicle reaches a mean diameter of 8.5 mm and differentiation occurs between the future DF and the remaining subordinate follicles (Beg and Ginther, 2006).

At deviation, the largest follicle continues to grow and becomes the dominant follicle, while the smaller follicles begin to regress and become subordinate follicles (Ginther *et al*., 2000). The difference in diameter between the two largest follicles at the start of deviation seems to allow enough time (8 hrs) for the largest follicle to establish dominance before the next largest follicle reaches a similar diameter. This establishment of dominance at the start of deviation involves inhibition of the smaller follicles, given that all growing follicles of the wave are capable of dominance until such inhibition is complete (Gibbons *et al*., 1997; Ginther *et al*., 1999).

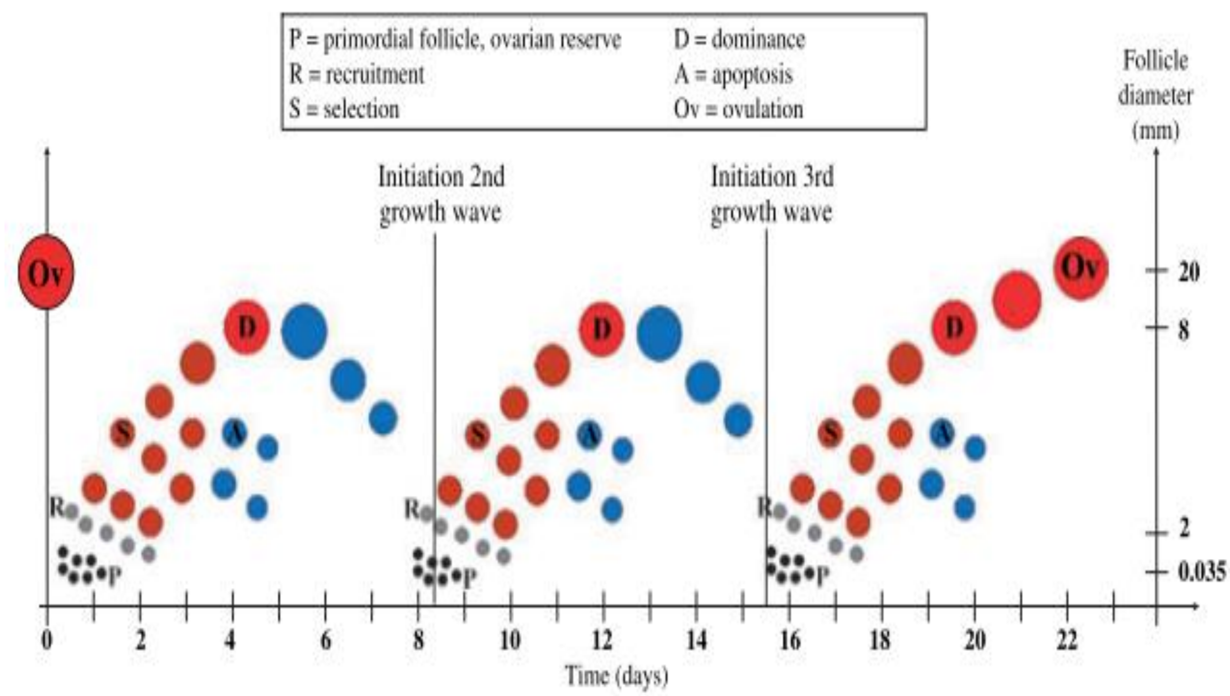

Figure 1:- Recruitment, selection, and dominance phase in a three-wave growth pattern during a bovine estrous cycle

Source: - (Aerts and Bols, 2010)

2.3. Reproductive technologies in cattle

Reproductive biotechnologies are designed to be used regularly to shorten generational intervals and propagate genetic material among breeding animal populations. To achieve this goal, several reproductive technologies have been developed over generations, such as artificial insemination, superovulation, in vitro fertilization, and embryo transfer, to overcome reproductive problems, increase offspring from selected females, and reduce generation intervals in farm animals. This advanced reproduction technology will undoubtedly play an important role in future perspectives and visions for efficient reproductive performance in livestock (Vikrama Chakravarthi & Sri Balaji, 2010).

2.3.1. Artificial Insemination

Artificial insemination (AI) is a process in which sperm is collected from a male, processed, stored, and artificially introduced into the female reproductive tract to conceive. It is a tool for increasing livestock reproductive performance and genetic quality. This technique is commonly used in the dairy and beef cattle industries to more rapidly improving desired characteristics through intensive genetic selection (Webb, 2003). The first successful artificial insemination was performed in Italy in 1780, and it was used for horse breeding over a century later, in 1890 (Tegegn *et al*., 1989). Artificial insemination was introduced in Ethiopia in 1938 in Asmara, then a part of Ethiopia, but was halted due to the Second World War and restarted in 1952 (Yemane *et al.,* 1993).

Artificial insemination reduces the incidence of sexually transmitted diseases in cattle while also increasing the use of genetically superior sires to improve herd performance. The greatest advantage of AI is that it allows for the most effective use of superior sires. Natural service would most likely limit the use of one bull to less than 100 matings per year (Webb, 2003). In 1968, AI enabled one dairy sire to provide sperm for over 60,000 services. The use of AI prevents sires from being exposed to infectious genital diseases, reducing the risk of such diseases spreading (Zewdie, 2007). The use of AI reduces the time required to establish reliable proof on young bulls. Other benefits include the early detection of infertile bulls, the use of old or crippled bulls, and the elimination of the dangers associated with handling unruly bulls. Artificial insemination is the process of artificially implanting fresh, stored, or cryopreserved sperm intra-vaginally, transcervical, or intrauterine (Sinishaw, 2005).

There are a few disadvantages of AI that can be overcome with proper management, such as poor conception rates due to poor heat detection and inefficiency of AI technicians, the spread of reproductive diseases, and poor fertility rates if AI centers are not equipped with appropriate inputs and are not well managed (GebreMedhin, 2005). Other disadvantages include high production costs (collection and processing), storage and transportation of sperm, as well as budget and administrative issues, and Artificial insemination technician inefficiency (Pope, 2000; GebreMedhin, 2005).

Individual performance as regularly recorded starting from the time of birth, which should include birth weight, subsequent weight increments, and later on progeny testing, as well as general health status, should be included in the selection criteria of bulls for AI service (Herman *et al*., 1994). Appropriate and specialized facilities, equipment, and procedures were used during semen collection to avoid injury to the bulls and their handlers, maximize the physiological responsiveness of the bulls in producing semen, and increase the quantity and quality of semen that could be collected. The area for sperm collection has been chosen to be clean, relatively quiet, and free of distractions and other stressful procedures. It has been reported that proper sexual stimulation of bulls increases spermatozoa motility by 50%. (Garner, 1991).

Insemination is delayed as a result of insufficient and/or inaccurate estrus detection. Because the fertile life of eggs in most species is relatively short, and sperm may require capacitation before they are capable of fertilizing ova, insemination should come before ovulation. Because ovulation is difficult to predict regularly, inseminations are typically timed to coincide with the onset of estrus. The psychic manifestation of heat characterizes estrous in cows. The cow may bawl frequently, be restless, attempting to mount other animals, and stand to be mounted/standing heat (Herman *et al.,* 1994).

Maximum fertility in cows has been achieved by inseminating from mid estrus to the end of estrus (Gomes, 1977). Capacitation of the spermatozoa is required for fertilization to take place. Capacitated sperm cells are hyper-motile and have undergone the acrosome reaction. Spermatozoa have a finite lifespan. If insemination occurs too early, the sperm cells will die before the ovum can be fertilized. When insemination is postponed, the ovum loses its ability to be fertilized. Because estrus can last from 10 to 25 hours, there is a wide range of possible insemination times. A successful heat detection program, followed by proper insemination timing, will pay dividends in terms of increased reproductive efficiency (Daris, 1998).

Table 1:- Showing proper timing of insemination

Cows showing estrus	**Should be inseminated**	**To be late for good results**
In morning	Same day	Next day
In afternoon	Morning of next day or Early afternoon	After 3 pm the next day

Source: **-** (Webb, 2003).

The main factors that influence AI efficiency are heat detection abilities, herd fertility, sperm quality, and inseminator efficiency. Similarly, successful insemination requires the acquisition of quality semen from a bull, the detection of estrus in the female, and the ability to properly place the semen in the female's reproductive tract (Damron, 2000). While the success of AI has been large because the technique is simple, inexpensive, and has a high success rate, the introduction of freezing protocols with glycerol as a cryoprotectant for sperm has greatly expanded the possibilities of AI. The use of frozen-thawed sperm accelerated the spread of valuable genes and allowed for the establishment of testing and breeding programs for promising bulls (Verberckmoes *et al*., 2004).

2.3.2. Estrous synchronization in cattle

The estrous cycle can be manipulated in cattle with active ovaries by using prostaglandin to induce early corpus luteum regression and progestagens to act as an artificial corpus luteum (Daris, 1998; Bekana *et al.*, 2005). Estrus synchronization is a technique by which most of the females in a population or a herd can be brought into estrous at a predetermined time (Hadgu and Fisseha, 2020). It can be conducted by the use of either, PGF2α or GnRH or the combination of the two where the former is injected 7 days before the latter to induce a new follicular wave (Sugawara, 2004).

The complexity and labor requirements of synchronization protocols can vary significantly. The simplest and least expensive protocols combine prostaglandin (PGF2α) strategic timing with intensive heat detection. Prostaglandin plays several roles in the normal estrus cycle, some of which are still unknown. The most important and notable role of PGF2α is its lysis of the corpus luteum. After the CL is destroyed, the cow will enter estrus (standing heat) and can be inseminated. The timing of PGF2α administration is critical because the CL requires time to develop PGF2α receptors. If PGF2α is given too soon after the previous heat, the injection will be ineffective (Levy *et al*, 2000).

The synchronization of estrous allows for fixing the breeding time within a short predetermined period and thereby scheduling the time of parturition at the most favourable season, when the newborns can be raised in a suitable environment with adequate food to improve their chances of survival. Fertility in farm animals may be expected to rise as a result of this technique, which allows for timely breeding of the animals. Estrus synchronization increases economic returns by improving animal production efficiency. Instead of females being bred over 21 days, synchronization can reduce the breeding period to less than five days, depending on the treatment regimen (Prusley *et al*., 2004).

Cows enter estrus at all times of the day and only stay in heat for 12-18 hours, making it difficult to detect estrus, especially in hot weather. Keeping cows in groups of three to five and performing two to three visual heat checks per day will improve the chances of detecting cycling animals. The use of synchronization and heat-detection aids can greatly reduce the time spent observing heat, but they will not benefit non-cycling cows or anestrous cows a condition in which the cow does not cycle due to a lack of natural hormonal stimuli (Pennington, 2013). The rate of conception to the first service was significantly higher in Insemination at detected estrous than in ovarian synchronization (Tenhagen *et al*., 2004).

A synchronization program's benefits include the ability to breed a high percentage of females in a given group of heifers or cows in a short period, using either artificial insemination or natural service (bulls) (Noseir, 2003). We achieve the following through effective implementation: Concentration of the breeding season, calves are concentrated during the calving season; a more uniform calf crop typically improves returns (increases calf crop value) and facilitates the use of artificial insemination by concentrating estrus detection requirements (Stephen, 2000). The basic approach to controlling the timing of the onset of estrus is to control the length of the estrous cycle (Ozill *et al.*, 2011). Estrus and ovulation can be synchronized by using either PGF2α or GnRH, or a combination of the two, with the former injected 7 days before the latter to induce a new follicular wave (Sugawara, 2004).

Prostaglandin (PGF2α)-based protocols

Prostaglandin (PGF2α) is a naturally occurring hormone that signals the corpus luteum to regress if a pregnancy does not occur, allowing the cow to return to standing estrus. During a normal estrous cycle of a non-pregnant animal, PGF2α is released from the uterus 16 to 18 days after the animal has been in heat. This PGF2α release destroys the corpus luteum. The Corpus luteum is a structure in the ovary that produces the hormone progesterone and prevents the animal from returning to estrus. The release of PGF2α from the uterus is the mechanism that causes the animal to return to estrus every 21 days. There are several prostaglandin products on the market that can be used to synchronize estrus in heifers and lactating dairy cows. Originally, these products were used to treat individual cows that had not exhibited heat by the time of desired first service. A commercially available PGF2α (Lutalyse, Estrumate, and Prostamate) allows the herd owner to simultaneously remove the Corpus luteum from all cycling animals at a predetermined time that is convenient for heat detection (Patterson *et al*., 2003; Dejarnette, 2004).

The corpus luteum is not responsive to PGF2α during the first 5 days of luteal development or natural corpus luteum regression (after day 17 of the estrous cycle). Consequently, PGF2α is only effective in regressing CL on days 5 to 17 of the estrous cycle. When PGF2α is injected during the responsive period (days 5 to 17), the corpus luteum regresses and the animal exhibits standing estrus 48 to 120 hours later (Salverson & Perry, 2005). If an animal does not have a corpus luteum (for example, a cow in postpartum anestrus or a heifer that has not reached puberty), she will not respond to a PGF2α injection. To respond to PGF2α injection, an animal must be cycling and between days 5 and 17 of the estrous cycle. Despite these limitations, prostaglandins are the most straightforward method for synchronizing estrus in cattle. These products sync estrus and fertility in cyclic females, such as virgin heifers, but are unable to induce estrous cycles in non-cycling cows (Bader, 2003).

Two-shot based

The most common method of synchronization with PGF2α is to inject all animals and breed those that come into heat within the next 5 to 7 days. Animals that are not detected in estrous after the first injection are re-injected 14 days later and bred over the next 5 to 7 days. Animals found in standing heat should be inseminated 8-12 hours later (DeJarnette, 2004). Regardless of the stage of the estrous cycle in which the first injection was given, all cycling cows should respond to the second injection. The program could be modified by breeding all females who show signs of estrus after the first PGF2α injection. A second injection is then administered only to females who have not been bred. Although this option saves money and time, it results in two synchronized groups instead of one, and a longer breeding cycle (Sahatpure and Patil, 2008).

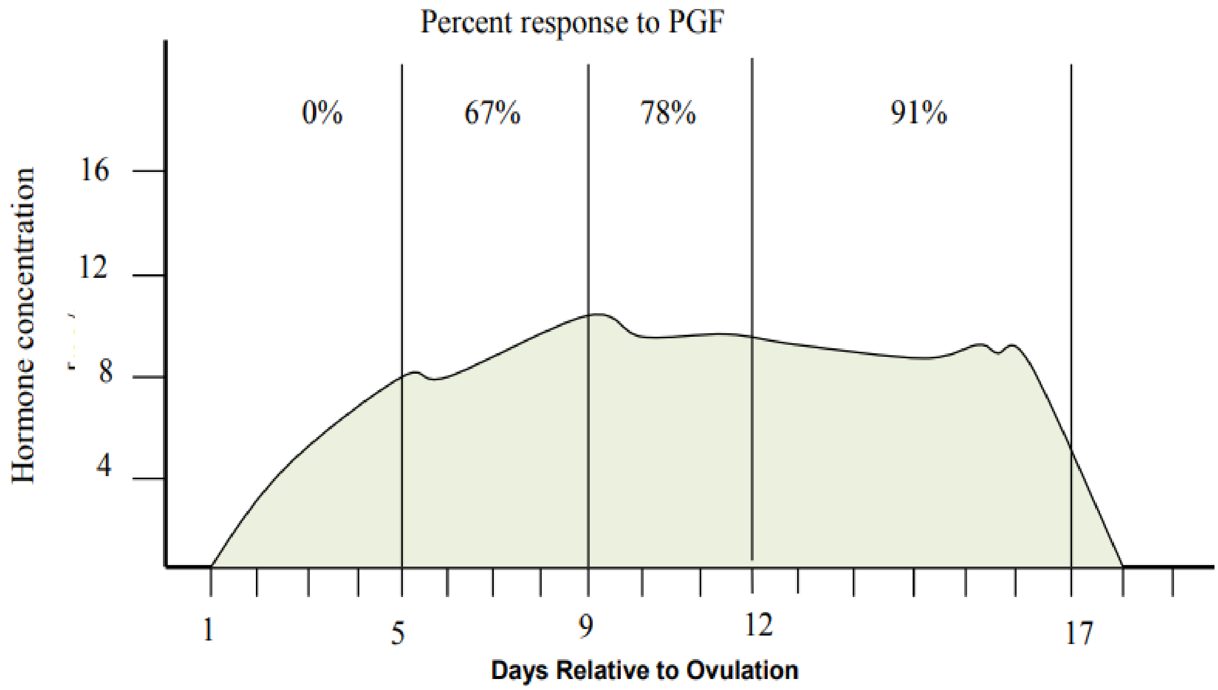

Figure 2:- Stage of the Estrous Cycle and CL Regression with PGF2α
Source: (Day and Geary, 2007)

GnRH based

The follicular phase of the estrous cycle is regulated by gonadotropin-releasing hormone (GnRH). Gonadotropin-releasing hormone (GnRH) is a naturally occurring hormone which triggers the release of luteinizing hormone (LH), resulting in ovulation of the dominant follicle, even in the presence of progesterone. Following the induced ovulation of the dominant follicle, a CL forms and new Follicles grow in wave-like patterns, with each estrous cycle consisting of two or three follicular waves. Following the regression of the CL by PGF2α, the new follicular wave that was initiated by the induced ovulation by GnRH will develop normally, and around day 7, a new dominant follicle will be present and ready to ovulate. Each of these waves has a dominant follicle capable of ovulating (releasing an egg) and being fertile. However, when progesterone is present, it prevents a dominant follicle from ovulating. The animals that do not have a dominant follicle at the time of the GnRH injection will not ovulate. Depending on the stage of the estrous cycle, these animals may exhibit standing estrous before the PGF2α injection (Wood *et al*., 2001).

2.4. Ovum-pick up in cattle

Canadian researchers used endoscopy through the right paralumbar fossa to collect oocytes in vivo in cattle for the first time. When a suction device was used instead of a syringe system, the rate of oocyte recovery increased. The best results were obtained with a 19-gauge (G) needle with a 45-degree bevel and a vacuum pressure of 250 mmHg (Lambert *et al*., 1983). An ultrasonically guided aspiration of bovine follicular oocytes was first proposed by a Danish team (Callesen *et al*., 1987), and in 1988, a Dutch team established in vivo oocyte collection by transvaginal ultrasound-guided follicle aspiration (Ovum Pick-Up: OPU) in cattle, and it has become possible to collect COCs many times from the same donor animal. OPU can be performed for an extended period without harming the animal's health, well-being, or ability to become pregnant afterward (Pieterse *et al*., 1988).

Oocyte retrieval via ultrasound-guided follicle puncture, or ovum pickup (OPU), is inextricably linked to in vitro embryo production procedures because it allows the most elite males and females to combine for accelerated genetic gain. Another important parameter for OPU efficiency is the sensitivity of the ultrasound equipment. A 5.0 MHz sector scanner probe was used in the first OPU study in the Netherlands, which allowed visualization of follicles larger than 3 mm. The number of visible follicles and collected oocytes increased significantly when a 6.5 MHz curved array probe was used (Kruip *et al.*, 1994). Another aspect of OPU efficiency is its consistency when compared to twice-weekly OPU application by using continuous or discontinuous (i.e. limited to day’s 0-12 of the estrous cycle) schemes. The mean number of punctured follicles and collected oocytes as well as the oocyte quality and the cleavage rate did not differ between the two OPU schemes. The discontinuous OPU scheme permits a normal ovulation and corpus luteum (CL) formation that shows characteristics similar to those of the pre-OPU period (Petyim *et al*., 2003).

The inability to collect competent oocytes from the same individual was one of the potential drawbacks. Only oocytes contained within follicles involved in follicular wave dynamics have the proper developmental competence required for IVEP and a thickening of the tunica albuginea accompanied by a hardening of the ovaries after very long use of the same animal. Anomalies in hormonal patterns have also been reported as a result of the formation of progesterone-secreting Corpus luteum (CL)-like structures after large follicle puncture (Boni *et al.,* 1997).

2.5. Ultrasonography of Bovine ovary

The use of transrectal real-time ultrasonography in the study of bovine reproduction is a technological breakthrough that has revolutionized reproductive biology knowledge. The nature of complex reproductive processes in cattle, such as ovarian follicular dynamics, corpus luteum function, and fetal development, has been clarified by new research information generated by ultrasonic imaging. Early applications of ultrasound technology in the dairy industry included transvaginal follicular aspiration and oocyte recovery, as well as use as a supplement to embryo transfer procedures (Pieterse *et al.*, 1991; Meintjes *et al*., 1993).

A cross-sectional view of the uterus is displayed as a "rosette" and is easily distinguished from other peripheral tissues, whereas the longitudinal section is less recognizable, but a trained technician can distinguish between the elongated view of the uterus and other tissues that may appear similar. Physiological changes during the estrous cycle cause physical changes (such as tone) in the uterus, which alters the echogenic properties of the uterus (Pierson and Ginther, 1987). Ultrasound is a rapid method for pregnancy diagnosis that improves reproductive efficiency and pregnancy rate in cattle by decreasing the interval between AI services and increasing the rate of AI services (Kastelic *et al.,* 1999). Although ultrasound evidence of pregnancy from days 18 to 22 of gestation yields excellent results, a technician must ensure that there is no confusion between fluid accumulations in the chorioallantois during early pregnancy (Kastelic *et al.,* 1989).

Today, the use of ultrasound has influenced the monitoring of follicular dynamics associated with wave emergence, follicular atresia, and follicular dominance. These findings imply that follicles in a growing cohort are at different stages of differentiation, implying that a developmental capacity hierarchy exists within a growing cohort (Ginther *et al*., 1996). CL ultrasonographic properties such as cross-sectional diameter, luteal area, and echogenicity have been linked to luteal structure and function (Singh *et al*., 1997; Battocchio *et al*., 1999). While ultrasound is more effective than rectal palpation for assessing ovarian follicles, both techniques make it difficult to differentiate between developing and older regressing corpora lutea (Pieterse *et al*., 1990).

Bovine reproductive organs are typically scanned per rectum with a linear-array transducer designed specifically for transrectal use. However, specialized applications such as ovum pickup and follicle ablation require a transvaginal approach with a sector transducer. Small and preovulatory follicles from clinically infertile animals, cows in their first trimester of pregnancy, and cows stimulated biweekly with pregnant mare serum gonadotropin (PMSG) or FSH to increase follicle numbers can all be aspirated using ultrasound-guided technology (Singh *et al*., 1997; Singh *et al*., 1998).

Most veterinary ultrasound scanners are compatible with probes of various frequencies, and linear-array transducers in the 5.0 and 7.5 MHz frequency ranges are most commonly used in cattle for reproductive ultrasound examinations. The frequency of the transducer effects and is inversely related to the depth of tissue penetration of sound waves and image resolution. Thus, a 5.0-MHz transducer results in a greater depth of tissue penetration but less image detail, whereas a 7.5-MHz transducer results in less depth of tissue penetration but more image detail (Pierson and Ginther, 1988).

When the diagnostic limitation of ultrasonic imaging is exceeded and an incorrect therapy or reproductive intervention is recommended, the diagnostic limitation of ultrasonic imaging becomes important. A through understanding of ovarian physiology and the mechanisms by which hormonal programs succeed or fail is required for the accurate interpretation of ultrasonic imaging data (Fricke, 2016).

3. MATERIALS AND METHODS

3.1. Study area

This study was carried out at, College of Veterinary Medicine and Agriculture. Bishoftu town is found in east Shewa Zone, Oromia Regional State, located about 45 km South-east of the capital city, Addis Ababa. The area is located at 9°N latitude and 40°E longitude at an altitude of 1850 meters above sea level. According to the national meteorology agency, annual rainfall of 866 mm of which 84% is in the long rainy season (June to September) with annual minimum and maximum temperatures of 11and 29°C, respectively (Bishoftu City Administration Agricultural Desk, 2014).

3.2. Experimental animals

The study animals were eight Boran cows that were previously subjected to twice-weekly ultrasound-guided ovum pick-up for 24 weeks. The mean (±SEM) body condition score of the cows was 4.37±0.74 (on a scale of 3-5). The time gap between the last ovum pick-up and the start of this experiment was two months. Cows were grazing for about 8 hrs and supplemented with grass hay and concentrate mix of wheat bran and noug (*Guizotia abyssinica*) seed cake. All animals had free access to clean water.

3.3. Follicular dynamics

To estrous synchronize, cows received 500µg PGF2α (Synchromate®, cloprostenol sodium, Warburg, Germany) at 11 days intervals. After the second PGF2α injection cows were observed three times daily for signs of estrous. Similarly, the ovaries were scanned transrectal by ultrasound (Mindray DP.50vet, China) with a 7.5 MHz linear array rectal transducer every 12 hrs for the first 48 hrs of PGF2α injection and then every 6 hrs until ovulation occurred or to the maximum of 120 hrs of PGF2α injection if no ovulation. The day of ovulation is considered as day zero of the experiment. From day zero onwards, the ovaries were examined twice daily (at 12 hrs interval) by transrectal ultrasound technique until the next ovulation. Each animal was scanned for two consecutive cycles and follicular diameter was measured by the internal electronic calipers.

Follicular dynamics and the time of ovulation were determined as described by Ginther *et al*., 1993) and (Duchens *et al*., 1995). The number of follicular waves, length of Inter-ovulatory interval, diameter of the preovulatory follicle and second subordinate follicle, growth, and atresia rate were recorded. The inter-ovulatory interval (IOI) is defined as the number of days between two consecutive ovulations in the same cow. Ovulation is confirmed based on the sudden disappearance of the largest diameter follicle and the subsequent appearance of a corpus luteum (CL) at the site of ovulation. The first day of detection of the disappearance of the dominant follicle is defined as the day of wave emergence on each wave (day zero).

About 10 ml of blood were collected using plain vacutainer tubes from all cows via jugular vein puncture every other day to determine the estrogen and progesterone profile by stages of the estrous cycle. The serum was separated and stored at -20 °C until analysis. Serum estrogen and progesterone concentrations were determined by an Elecsys Estradiol III and Elecsys Progesterone III assay system respectively by using Modular analytics E170 or Cobas e 411analyzer (Cobas E®, Roche Diagnostics GmbH, Mannheim, Germany). Estradiol and Progesterone has a detection limit of 5pg/ml-3000pg/ml and 0.05ng/ml-60ng/ml, respectively.

3.4. Pregnancy diagnosis

Conception was checked on D32. On ultrasound, a fluid-filled uterine horn and a conceptus were considered positive indicators of conception (Fricke *et al*., 1998).

3.5. Ethical clearance

Ethical approval for this research was obtained from Addis Ababa University, College of veterinary medicine and agriculture animal research ethical review committee (Ref. No:

VM/ERC/15/05/13/2021), Bishoftu campus (indicated in Annex 6). All the Sampling process from the animals was undertaken as per the standards approved by the animal welfare and research ethics committee at the college, which is under the international guidelines for animal care and welfare.

3.6. Data management and Statistical analysis

During each estrous cycle, data were arranged according to follicular characteristics. A descriptive analysis was used to determine the proportions of animals that showed different patterns of follicular activity. The diameters of the follicles occupying the largest, second-largest, and total count of follicles greater than 4mm were plotted daily during the IOI, regardless of individual identity. Profiles of the mean diameter of the largest two follicles throughout the IOI, as determined by the non-identity method, were normalized to the mean IOI for cows exhibiting two or three waves of follicle development during the cycle to 21 days and 23 days IOI for two and three waves, respectively, according to (Mapletoft *et al*., 2009).

For each wave a descriptive analysis mean (±SEM) were performed for the following characteristics: Inter-ovulatory Interval (IOI) [day], Size of preovulatory follicle [mm], Size of second large follicle [mm], Follicular growth rate [mm/day] and Atresia rate [mm/day]. Data were grouped according to the number of follicular waves present in each estrous cycle. Among animals with the same number of follicular waves per cycle, parameters were evaluated by ANOVA. The relationship between the number of waves and Inter-ovulatory interval was estimated by Pearson's correlation method. Graphical analyses were performed using Microsoft offices excel. Results are shown as mean (±SEM) and the level of significance was held at $P<0.05$.

4. RESULTS

4.1. Follicular dynamics

The details of follicular characteristics and follicular wave numbers were indicated in Table 2 and the pattern of the cycle wave in figure 3. Seventy-five percent of cows (n=6) had exhibited estrous cycles with two follicular waves and only 25% (n=2) had three follicular waves. The mean (±SEM) maximum diameter of the preovulatory follicle was significantly higher (P<0.05) for cows with two waves than for cows with three waves. There was a positive correlation (r = 0.38, P<0.05) between the number of follicular waves in a cycle and IOI which is that as the inter-ovulatory interval is prolonged there is a tendency for cows to have three follicular waves.

Table 2:- Characteristics of follicular dynamics in two and three waves.

Parameter	2-wave cows		3-wave cows		
mean (±SEM)	First	Second	First	Second	Third
Wave length [day]	14.3 ± 1.88^a	10.8 ± 1.03^b	12.66 ± 1.52^a	9.33 ± 1.15^b	7.50 ± 0.70^c
Day of Maximum diameter [day]	10.4 ± 1.95^a	21.1 ± 1.19^b	10.5 ± 0.70^a	17.33 ± 0.57^b	22.66 ± 0.57^c
Size of preovulatory follicle [mm]	12.75 ± 1.78^a	14.29 ± 1.36^b	11.4 ± 1.01^a	10.23 ± 0.86^a	12.30 ± 1.01^b
Size of second large follicle [mm]	7.8 ± 0.80^a	8.63 ± 0.63^a	6.8 ± 0.37^a	6.43 ± 0.85^a	7.56 ± 1.72^a
Growth rate [mm/day]	0.83 ± 0.20^a	0.85 ± 0.10^a	0.74 ± 0.58^a	0.8 ± 0.42^a	1.16 ± 1.02^b
Atresia rate [mm/day]	1.37± 0.85	------------	0.81 ± 0.29^a	0.89 ± 0.24^a	-------------

$^{a\ b\ c}$ mean value with different letters within the row differ significantly (P<0.05).

The mean (±SEM) IOI for the cows with two follicular waves was 21.1±1.19 days and that of three waves was 22.66±0.57 days. The difference in IOI was not significant ($P>0.05$) by follicular waves. The mean (±SEM) number of days from the emergence of cohort follicles to ovulation of dominant follicle of the ovulatory wave was significantly ($P<0.05$) greater (10.80±1.03 days) for two wave cows than three-wave cows (7.50±0.70 days). The mean (±SEM) diameter of the preovulatory follicle was significantly ($P<0.05$) larger for cows with two waves (14.29±1.36mm) than for cows with three waves (12.30±1.01mm). The mean (±SEM) growth rate of the dominant follicle that finally ovulated was significantly ($P<0.05$) higher (1.16±1.2mm/day) in the three-wave cycle than in two-wave cycles (0.85±0.1mm/day) (details of growth rate, and atresia rates are indicated in Table2). The overall mean (±SEM) total number of ovarian follicles greater than 4mm was 19.36±10.47 (range=8-25) for Boran cows. However, more follicles were found on the right than left ovaries.

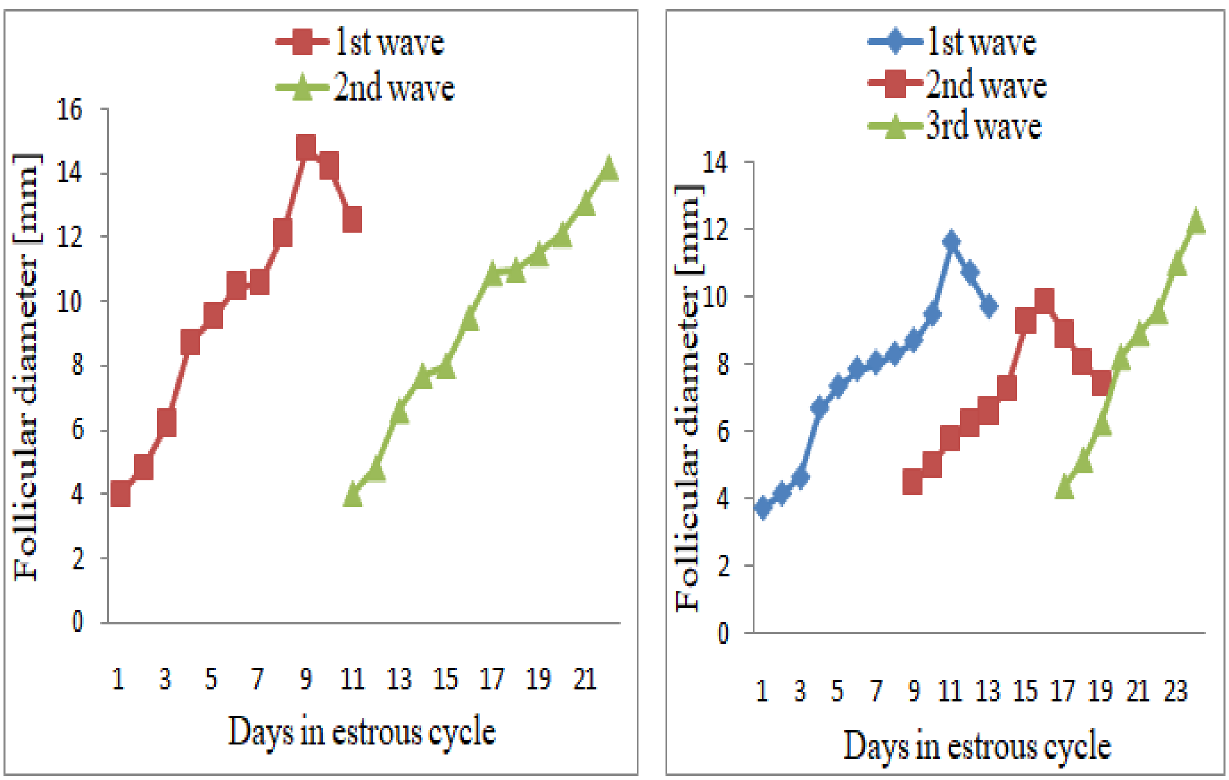

Figure 3:- Follicular wave patter in two and three waves

4.2. Serum Estrogen and Progesterone concentration

Table 3 shows the serum estrogen and progesterone concentrations by day of the estrous cycle, while Figure 4 shows the patterns of serum estrogen and progesterone changes by estrous stage. Estrogen concentrations in the blood began to rise during the pro-estrous period and peaked during estrous. In proestrous and estrous periods, serum estrogen concentrations were significantly higher ($P<0.05$) than in metestrous and diestrous periods. During the proestrous and estrous periods, the mean (SEM) serum estrogen and progesterone concentrations were 28.73±6.56 pg/ml and 0.88±0.40 ng/ml, respectively. The corpus luteum, on the other hand, measured 17.53±4.36 mm (range=11.8-23.2) in diameter.

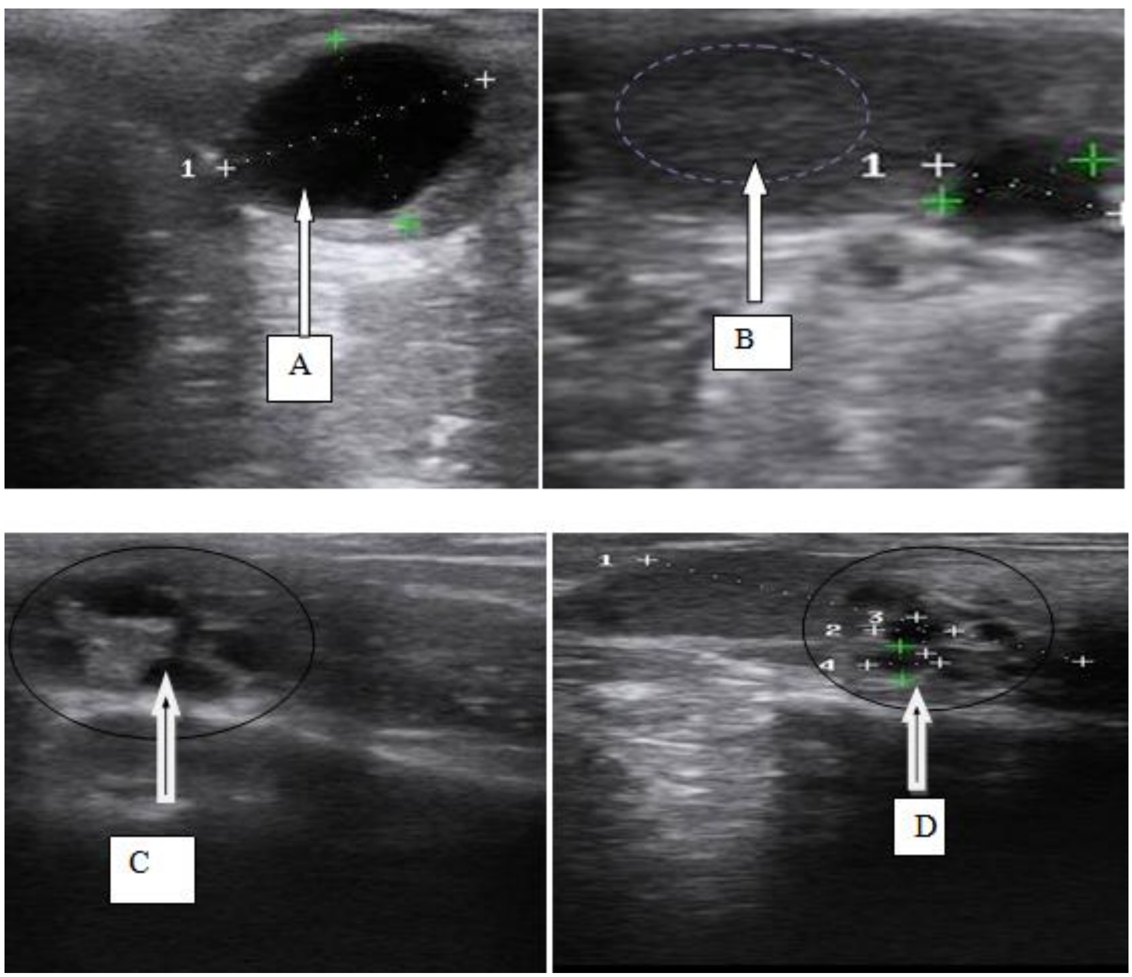

Figure 4:- Ultrasonographic image of CL and ovarian follicles in Boran cows: a= DF; b= Mature CL; c= medium and large sized follicles; d= Small follicles

Table 3:- The mean (±SEM) serum concentration of estrogen and progesterone in different periods of the estrous cycle in Boran cows

Stage of estrous cycle	Met estrous (1-5 days)	Early diestrous (7-11 days)	Late diestrous (13-17 days)	Proestrous and Estrous (19-23 days)
Estrogen (pg/ml)	22.68±6.92[a]	13.26±2.66[b]	11.01±1.61[a]	28.73±6.56[b]
Progesterone (ng/ml)	1.05±0.25[a]	2.42±0.70[b]	2.67±0.12[a]	0.88±0.40[b]

[a b] values with different superscript within the row are significant (P<0.05).

The serum estrogen level started to fall in the metestrous period and remains at its lowest concentrations throughout the diestrous period. Serum concentrations of progesterone were started to increase during the met estrous period and reached a peak during the late diestrous stage (Figure 4). The progesterone concentration in the late diestrous was significantly (P<0.05) higher than during the Proestrous and estrous phases of the estrous cycle. The mean (±SEM) serum estrogen and progesterone concentration were 11.01±1.61pg/ml and 2.67±0.12 ng/ml respectively during the late diestrous cycle (Table 3).

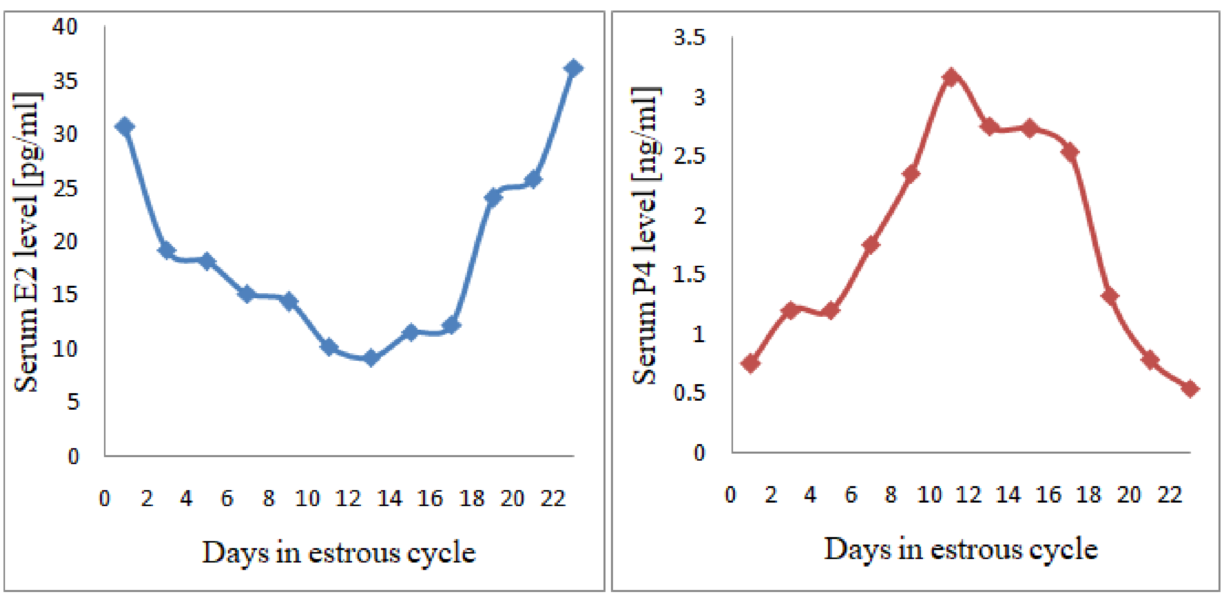

Figure 5:- Serum Estrogen and progesterone concentration in cycling Boran cows

4.3. Conception Rate

All Boran cows (n=8) were inseminated on standing estrous of which 87.5% conceived and only one cow open at day 32 of AI.

Figure 6:- ultrasonographic image of 32 days old embryo in Boran cows

5. DISCUSSION

Cows were followed for two consecutive estrous cycles (a total of 16 estrous cycles). From these estrous cycles twelve (87.5%) cows showed two follicular waves and only the remaining 12.5% showed three follicular waves and this was agreed with the work of Degefa *et al*. (2016), Alvarez *et al*. (2000), and Anivaldo *et al*. (2012) who reported 100% in Boran cows, 55.6% in Braham cows and 67.6% in Nelore heifers that exhibited two waves of follicular growth per estrous cycle, respectively. However, the present finding was different from the Kenyan Boran which showed a predominance of three follicular waves per cycle (Muraya, 2013). This difference may be due to the long-lasting adaptation of the breed in the tropical region and unexpected energy imbalance. Similarly, different works from elsewhere show different wave numbers among the *Bos indicus* breed.

The mean (±SEM) IOI for the cows with two waves was 21.1±1.19 days and that of three waves was 22.66±0.57 days. Sartorelli *et al*. (2005) reported IOI 23.1±0.7 days for three wave and 20.7±0.3 days for two waves in Nelore (*Bos indicus*) cows and this was very close to the present finding. This finding also agrees with Muraya (2013) and Anivaldo *et al*. (2012) who reported IOI 23.6±1.05 days in Kenyan Boran and 21.61±0.35 days in Nelore heifers for three wave cycles, respectively. However, Muraya (2013) and Anivaldo *et al*. (2012) reported shorter IOI 18.6±1.9 days in Kenyan Boran and 20.53±0.26 days Nelore heifers for two wave cycles, respectively. The variation in the length of the IOI observed may be due to the climatic condition in which the animals reared.

The diameter of the preovulatory follicle was 14.29±1.36 mm and 12.30±1.01mm in Boran cows with two and three waves, respectively. This finding was comparable with the previous study by Jemal *et al*. (2020) who reported the diameter of preovulatory follicles 14.2±1.63 mm and 14.6±2.06 mm in Boran heifers with two and three waves, respectively. Similarly, Muraya (2013) also reported 14.0±0.85mm and 13.52±0.50 mm diameter of the preovulatory follicle in two and three waves Kenyan Boran, respectively. The larger size of the preovulatory follicles may be related to higher estrogen concentration in the blood.

The total number of ovarian follicles greater than 4 mm was 19.36±10.47 (range=8-25) for Boran cows in the present study was consistent with the findings of Degefa *et al*. (2016), who reported 18.34±0.145 (range=11-28) total number of follicles >4 mm in diameter for Ethiopian Boran cows. However, Jemal *et al.* (2020) reported a smaller follicular population of 9.4±3.0 in Boran heifers. The mean (±SEM) estrogen concentration 18.92±8.61 pg/ml in Boran cows in the present study was higher than Alvarez *et al.* (2000) and Figueiredo *et al*. (1997) who reported lower estrogen concentration 8.9±1.6 pg/ml in Brahman cows and 12.71±0.98 pg/ml in Nelore cows, respectively. The difference in estrogen concentration may be due to breed difference, climate in which animals reared and the physiological state of cows.

The mean (±SEM) progesterone concentration 1.75±0.90 ng/ml in Boran cows of the present finding was lower than the report of Degefa *et al*. (2016) and Figueiredo *et al*. (1997) who recorded a higher mean progesterone concentration 2.01±0.11 ng/ml in pure Boran cows and 4.92±0.22 ng/ml in Nelore heifers, respectively. The mean (±SEM) diameter of corpus luteum 17.53±4.36 mm in Boran cows of the present study was lower than Degefa *et al*. (2016), Alvarez *et al*. (2000) and Figueiredo *et al*. (1996) who reported a higher mean diameter of corpus luteum 19.33±0.12 mm in pure Boran cows, 16.4±0.3 mm in Braham cows and 15.56±0.44 mm in Nelore cows, respectively. However, Figueiredo *et al*. (1996) reported comparable diameter of corpus luteum 17.87±0.35mm in Nelore heifers. The difference in diameter of CL may be due to breed.

In this study 87.5% Boran cows were pregnant to insemination. Brown *et al*. (1996) reported lower (53%) conception rates to insemination in cows undergone repeated ovum pick-up performed in prepubertal heifers twice a week for 3 months, thrice a week for 6 months, and twice for 14 months. However, Figueiredo *et al*. (2020) also indicated that after varying numbers of OPU sessions, beginning as early as 10.6 months of age, the fertility of virgin heifer donors was negatively impacted by OPU and pregnancy rate to the first insemination was approximately decreased by 15%.

6. CONCLUSION AND RECOMMENDATIONS

Follicular dynamics during the estrous cycle occur in a wave-like pattern and that most cows exhibit two to three waves. The present study revealed that Boran cows predominantly were characterized by two-wave follicular dynamics. The difference in inter-ovulatory was not significant by the number of follicular waves in the estrous cycle. The mean size of the preovulatory follicle of the ovulatory wave was larger for two waves than for three waves. The mean growth rate (mm/day) of the dominant follicle was larger for three waves than for two waves. The increase or decrease pattern of serum estrogen and progesterone was similar for cows with two and three follicular waves. Based on ovarian follicular dynamics, serum estrogen and progesterone pattern, and conception rate, we concluded that repeated ovum pick does not significantly affect the fertility of Boran cows.

Based on the above conclusion, the following recommendations are forwarded;

- In Boran cows due to higher number of ovarian follicles in each estrous cycle it can be used for ovum pick-up. So it should be wisely used for the multiplication of these breed through in vitro embryo production technologies.
- There was no significant change in follicular wave activity of conception rate after repeated ovum pick-up in Boran cows so it should be successfully carried out regardless of the reproductive status of the donor.

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8. ANNEXS

Annex 1. Photos during Boran cows shows estrous sign

Annex 2. Hormone analyzer machine

Annex 3. During Artificial insemination

Annex 4. Ethical approval sheet

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/15/05/13/2021

Name of Applicant: **Gezahegn Berhan (DVM, MVSc fellow)**

Address: Department of Clinical Studies, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Ovarian follicular dynamics and conception rate in Boran cows previously subjected to repeated ovum pick up*

Date of application:	**December, 2020**
Nature of the project:	**Mildly invasive**
Target animal species:	**cattle**
Number of animals involved:	**8**
Study area:	**Bishoftu, Ethiopia**

Minutes No. and date of review: **VM/ERC/05/13/021, 21/03/2021**

The above indicated research project is acceptable from ethical perspective, relevance, originality and technical competence points of view. Hence the project is ethically sound to be executed provided that:

1. All procedures and conditions stipulated in the proposal are respected, minor comments are corrected and any deviation or changes be reported to the committee
2. The project activities be open for occasional supervision by the committee when deemed necessary

Getachew Terefe (DVM, PhD)
Chairman

Signature

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Please quote Our Ref. No. When replying

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