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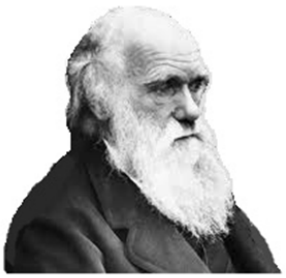

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Ovarian Follicular Dynamics in Fogera and Jersey Cattle Breeds in Ethiopia

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ABSTRACT

Understanding the reproductive physiology of female animals is used to optimize reproductive management of dairy cattle. This study was conducted at Adea Berga Dairy Research sub Center of the Ethiopian Institute of Agricultural Research. A total of 15 experimental animals were used to characterize the follicular dynamics of Fogera (n=9) and Jersey (n=6) dairy cattle breeds. Characterization of ovarian follicular dynamics was done using a trans-rectal real-time B-mode ultrasound system for three consecutive estrus cycles; and behavioral estrus signs were visually observed and recorded. Fogera cows manifested two (n=2, 29.62%), three (n=5, 55.55%), and four (n=1, 14%) follicular waves whereas Jersey cows showed two (n=2, 38.9%) and three (n=4, 61.11%) follicular waves. Cycle length or inter ovulatory interval was 20.35 ± 2.8 and 20.67 ± 2.6 days for Fogera and Jersey cows, respectively. The mean number of follicular population per animal was 13.9 ± 5.9 for Fogera and 17.9 ± 7.0 for Jersey cows. There was a significant ($p<0.001$) difference in follicular number 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 15.22 ± 1.3 mm. There was no statistical difference between the breed in terms of inter-ovulatory interval (IOI), however, there was significant difference ($p<0.001$) between the breeds in number of follicular population and ovulatory follicle size. In conclusion, behavioral signs of estrus were weaker in fogera breeds as compared to their Jersey counterpart, and relatively higher number of ovarian follicles recorded in Jersey than Fogera cattle breed.

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

1. INTRODUCTION

Ethiopia has the largest cattle population in Africa with an estimated herd of about 60.4 million heads of animals [1]. Livestock production is the most important subsector in the country's economy. The majority of these animals are indigenous breeds or populations that live in a variety of geographic and climatic situations throughout the country [2]. The country is regarded as one of the original locations of Africa's indigenous cattle. Fogera cattle is one of Ethiopia's recognized indigenous cattle breeds which kept for multipurpose functions at crop livestock mixed agriculture production system. The daily milk yield and lactation length of Fogera cattle is high as compared to other indigenous breeds under exceptionally challenging environment [3]. Jersey dairy cattle breed originated from the Island of Jersey[5] and is one of the exotic breeds found in Ethiopia. Generally exotic cattle breeds population in the country accounts only about 0.1% [4].

Follicular dynamics in cattle is the growth of ovarian follicle in a wave like 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 [6]. 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) on approximately ovulation day or day zero of the estrus cycle. 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 ovulatory or atretic.

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 [7]. 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 [8].

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 [9]. Although Ethiopia has a great deal of livestock population, the profits the country is earning from dairy and beef industry are low [10].

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. The mechanisms that control follicular dynamics 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 physiology, ovarian follicular population and estrus behavior for Fogera and Jersey dairy cattle breeds.

2. MATERIALS AND METHODS

2. 1. Description of study area

The experiment was conducted between the months of January to march 2022 at Adea Berga Dairy Research substation of the Ethiopian Institute of Agricultural Research which is located in West Shewa Zone of Oromia Regional State, Ethiopia. Adea Berga is located in Ethiopia's central highlands at 90 16' N latitude and 380 23' E longitude, 70 km West of Addis Ababa. 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 °C and 1225 mm, respectively. The perennial grasses and sedges are the main vegetation of the area. 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.

2. 2. Experimental 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 every morning before the start of the experiment. 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 ultrasound scanning operations.

2. 3. Experimental design

The experiment was conducted on 15 selected multiparous non-lactating Jersey (n=6) and Fogera (n=9) cows. Experimental 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 cows were about 5 years whereas the Jersey cows were in the range of 5 to 8 years old.

A single intramuscular injection of 2ml PGF2α (Estrumate, Holland) hormone was used to synchronize and bring all animals to the same physiological status before the start of the experiment. Behavioral estrus signs were visually observed during early morning data collections sessions and by the herdsmen at the evening. Characterization of ovarian follicular dynamics was done using a trans-rectal real-time B-mode ultrasound system for three consecutive estrus cycles. Follicular population, day of follicle emergence, growth and atresia rate of the dominant follicles, follicular diameter, and ovary size during estrous cycles were evaluated.

Dominant follicles growth rate was calculated by subtracting the diameter on the day of detection from the maximum diameter and dividing this by the interval in days [13]. The number of days between two consecutive ovulations in the same female was taken as the inter-ovulatory interval (IOI).

2. 4. Data Management and Analysis

All recorded information during the experiment including the daily scans was filled in Microsoft offices excel. Data were grouped according to breed of animals and follicular characteristics in each estrous cycle. Descriptive statistics was used to calculate the means and standard deviation of means where the results were presented in tabular, and graph.

Comparisons between animals and the wave groups (two-wave, three-wave) were done using analysis of variances (ANOVA). The data was analyzed using SPSS (20). Results are shown as mean ± standard deviation (SD) and level of significance was held at $P<0.05$.

3. RESULTS

3. 1. Ovarian follicular dynamics

3. 1. 1. Estrus behavior characteristics

All experimental animals except a 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, standing 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 signs, vaginal discharge and swelling of vulva were the most consistent (100%) estrous signs for both breed. Proportion of animals exhibited behavioral signs of mounting, attempting to mount, restlessness, chin resting on the rump, sniffing, standing to be mounted, swelling of vulva and vaginal discharge were presented in (Table 1).

Table 1. Proportion and number of animals showed different signs of estrus behavior

Behavioral estrus signs	Types of breed			
	Fogera		Jersey	
	N	%	N	%
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 less intense behavioral estrus signs during heat period. However, Jersey breed outperformed the Fogera breed in exhibiting more intense behavioral estrus signs during the estrus cycles. Prolonged estrus signs (more than three days) were exhibited in both Fogera and Jersey cows.

3. 1. 2. Follicular waves

Estrous cycles were evaluated for 63 days on Fogera and Jersey cows. Fogara cows (n=9) manifested two (29.62%) (Fig. 1A), three (55.55%) (Fig. 2A) and four (14%) (Fig. 3) follicular waves whereas, Jersey cows (n=6) showed two (38.9%) (Fig. 1B) and three (61.11%) follicular waves (Fig. 2B) during the estrus cycles.

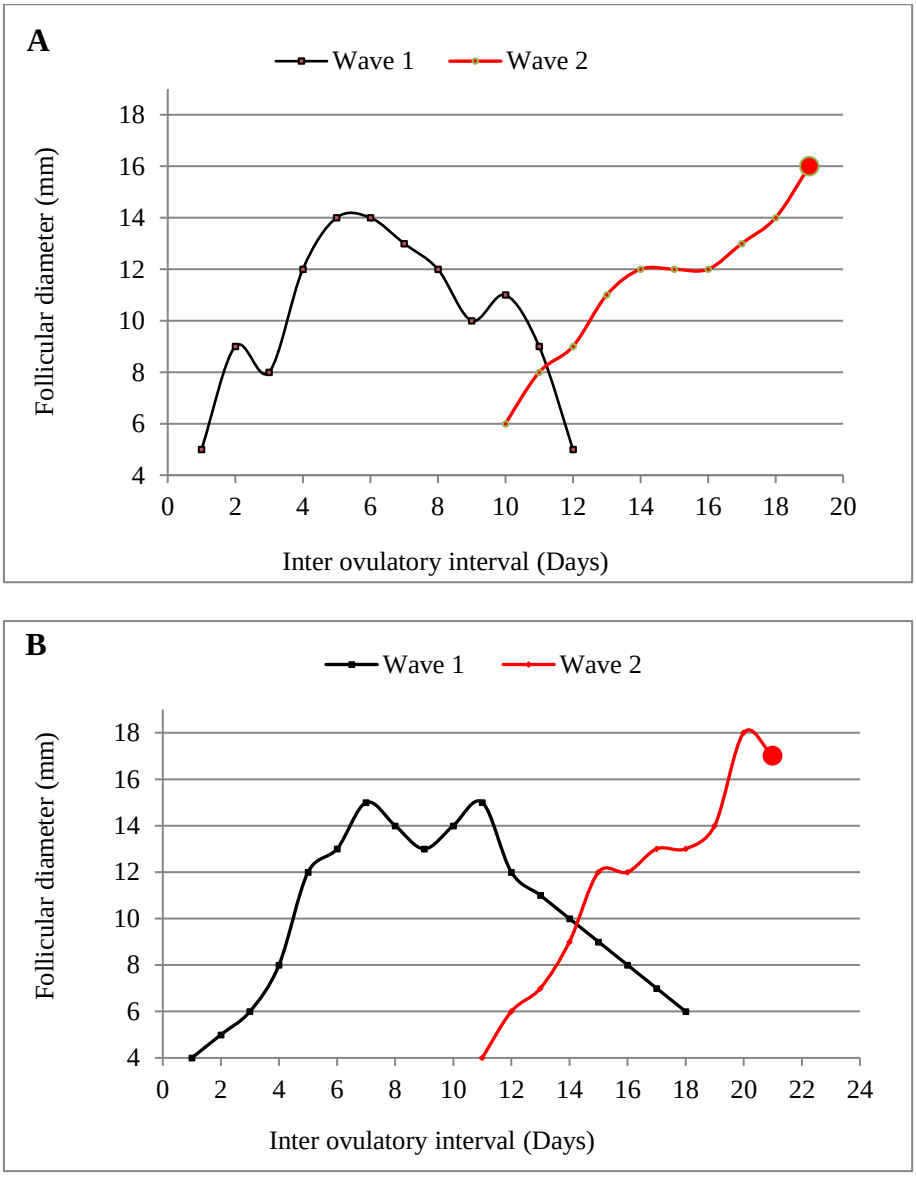

Figure 1. Dynamics of follicular development in a Fogera (A) and Jersey (B) cows during two-wave estrus cycle

The majority of Fogera cows (n=8, 88.89%) exhibited estrus cycles with two and three follicular wave. 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 cycle. The size of ovulatory follicle remained constant and slightly reduced in size (1 mm) until they eventually ovulated.

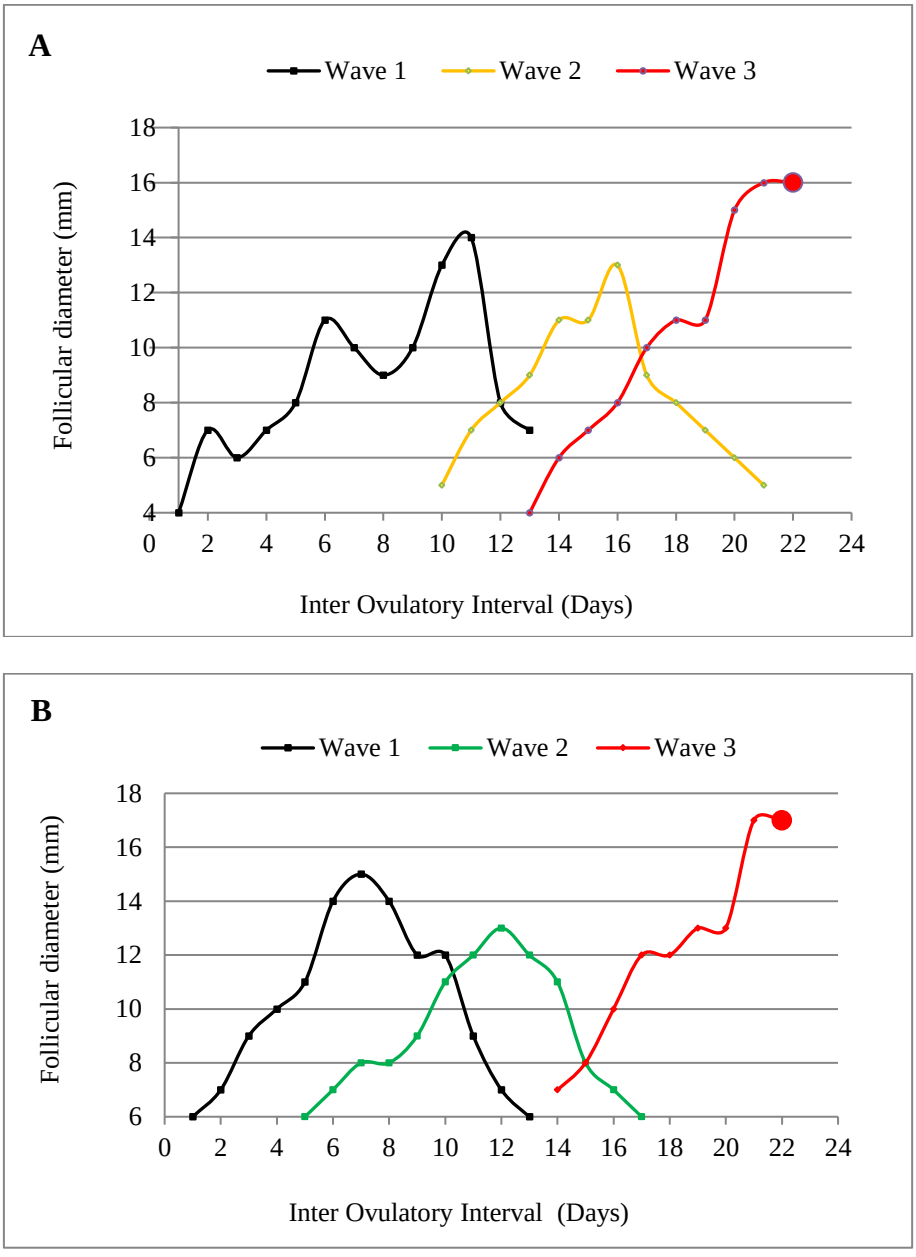

Figure 2. Dynamics of follicular development in a Fogara (A) and Jersey (B) cows during three-wave estrus cycle

The estrous cycle length or inter ovulatory interval of Fogera and Jersey cows was (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 difference ($p>0.05$) in dominant follicles growth or atresia rates

between the breeds and between animals with different follicular waves. Table 2 shows the characteristics of follicular dynamics in the both breeds with two follicular waves during the estrous cycles.

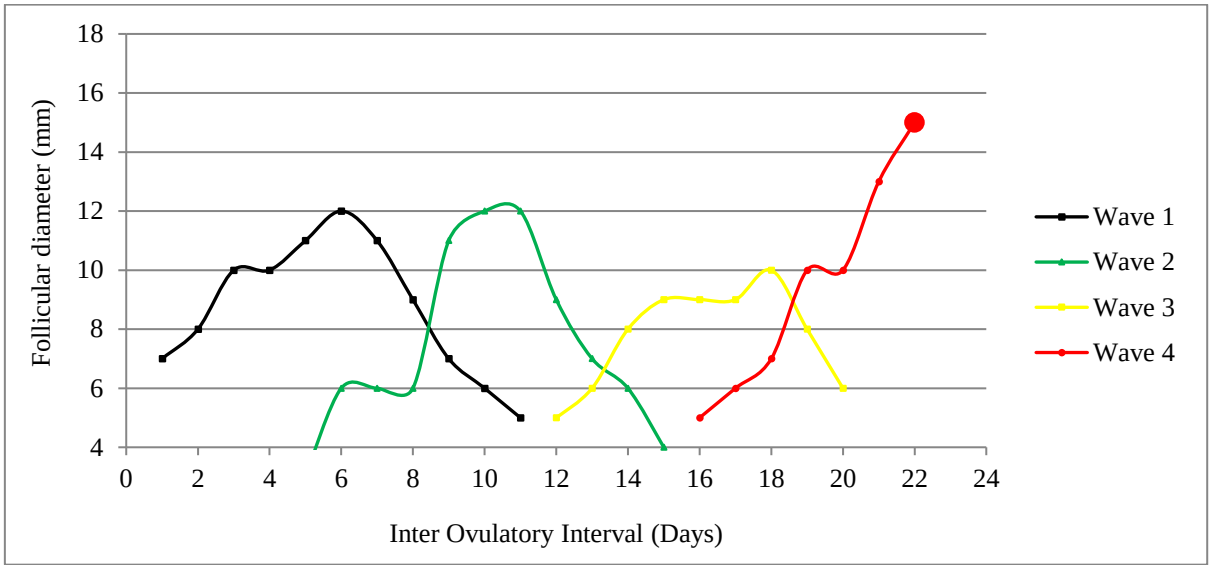

Figure 3. Dynamics of follicular development in a Fogera cows during four-wave estrus cycle

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 2). The dominant follicle reached its maximum diameter on day 7.88 ± 2.9 for Fogera and 7.43 ± 2.7 for Jersey cows (Table 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 2).

Table 2. Characteristics of follicular waves, growth and atresia rate of dominant follicle in Fogera 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 ± 0.5[a]	15.13 ± 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 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.

The main characteristics of the follicular dynamics in the cows with three follicular waves are shown on Table 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 3).

Table 3. Characteristics of follicular waves, growth and atresia rate of dominant follicles in Fogara and Jersey cows with three follicular waves during the estrus cycle (mean ± SD)*

Characteristics	Follicular Waves		
	First	Second	Third
Fogera			
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 ($p < 0.05$).*

The dominant follicle growth pattern of the first follicular wave for Fogera 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 3.3). The second wave showed up on day 7.5 ± 1.9 for Fogera 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 and Jersey cows, respectively (Table 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 3). There was difference (p<0.001) between the size of both right and left ovaries of Fogera and Jersey cows. Table 4 shows the size (mm) of ovaries of both Fogera and Jersey breeds.

Table 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 5). The mean number of follicular population per animal was 13.9 ± 5.9 for Fogera 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.

Table 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]		

4. DISCUSSION

The relationship between cardinal signs of estrus is not common to all the cows and varies with breed [14], [15]. 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 [16]. 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 [17]. [18] Had reported that estrus behavior expression in tropical breed is less than that of temperate. This is in agreement with the present study where weaker estrus signs were observed in Fogera cows compared to Jersey cows. The present finding is also in concordance with the previous findings of [19] for boran and boran X holstein friesian crossbred cattle and [20] for Brahman and Charolais cows. Vaginal discharge, sniffing, mounting, standing to be mounted, swelling of the vulva, chin resting and restlessness are among estrus signs shown by the Fogera breed. The same finding was reported for the same breed by [21].

This is the first time this study (follicular dynamics) has been done on this breed (Fogera). 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 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[22].

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 [23], Zebu cows [24], Gir breeds (Bos indicus) [22] and in Ethiopian Boran and their Crosses [11]. In the present finding Fogera cows showed predominance of three follicular waves per cycle (55.55%). This is in agreement with the previous report of [12] for Kenyan Boran in which the majority of them had shown three follicular waves

In a two-wave cycle for Fogera cattle the first wave emerged on day 0.25 ± 0.46 of the cycle and reached its maximum diameter at 7.88 ± 2.90 day, whereas the second wave emerged 10.0 ± 3.21 and attained maximum diameter of 15.13 ± 0.99. This was previously seen in Kenyan Boran [12] and in agreement with the present finding. The day of maximum diameter of follicle of Fogera cow with three waves was 6.55 ± 1.67, 12.91 ± 3.02, 21.27 ± 1.74 for first, second and third wave respectively. This is in agreement with report of [22] for Brazilian Zebu breed cattle.

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 [11]. 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 fate of the dominant follicle [25] . Follicular growth and maturation are dependent on a high frequency of LH pulses [26], which is suppressed during diestrus due to the negative feedback effect of the progesterone.

The length of inter ovulatory interval had shown no statistically difference between the breeds. This result is in agreement with previous work [11], [27] in Ethiopian Boran and their

crossbred cows' estrous cycle length. The mean length of inter ovulatory interval for Fogera cow was (20.35 ± 2.8). It is comparable with the previous finding of [27] for Ethiopian Boran (19.44 ± 0.23)

Jersey cows had higher diameter of ovulatory follicle than Fogera cows. [12] reported that the maximum diameter of ovulatory follicle in Kenyan Boran cows' was smaller than those reported in European cows by [6]. But comparable in size with other *Bos indicus* cows [28].

There was significant difference in total number of follicular population between the breeds. Fogera cows have lower follicular population compered to Jersey cows. The mean number of follicular population per animal was 13.9 ± 5.9 for Fogera and 17.9 ± 7.0 for Jersey cows. This is lower than that of [9] for Holstein-Friesian dairy cows. Whereas the present finding was higher than that of [11] 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 [29]. 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 [30].

5. CONCLUSIONS

Behavioral signs of heat were weaker in fogera 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 cows was 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 cows have shown two and three follicular wave during consecutive three estrus cycles. Relatively higher number of ovarian follicles observed in Jersey than Fogera breed. Due to the fact that both breeds have good follicle population during the estrus cycle, they can be considered as candidate breed for supper ovulatory treatment and OPU activities.

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