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Article

Follicular and Luteal Dynamics during Natural and Induced Estrous Cycle in Arunachali Yak (*Bos grunniens*)

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Simple Summary

The yak farming is very important for people living in the high-altitude Himalayan region. Reproductive efficiency in yak is low, which limits productivity and income for people. One reason for this problem is poor understanding of how the reproductive cycle works in yaks. This study aimed to compare natural heat cycles with hormone-controlled heat cycles in *Arunachali Yaks*. A total number of thirty-six healthy female yaks were observed, with one group showing natural heat and the other treated with hormones to control the heat cycle. The ovaries were checked daily using an imaging method throughout one complete cycle. We found that yaks in natural heat usually showed two patterns of egg development, while hormone-treated yaks mostly showed three patterns and had a longer heat cycle whose oestrous cycle length was also significantly longer and the corpus luteum reached a significantly greater maximum diameter. These findings help improve understanding of yak reproduction and can support better breeding planning, higher pregnancy rates, and improved livelihoods for people who depend on yak farming in mountainous regions.

Abstract

To study follicular growth pattern and luteal dynamics during natural and induced estrous cycle in *Arunachali Yak* maintained at the Nyukmadung farm of ICAR-NRC on Yak of Arunachal Pradesh. A total of 36 animals of apparently healthy cyclic female yaks of first to fourth parity and free from any post parturient complications, maintained under intensive system of rearing were selected for the study and equally divided into two groups. Female yak with natural oestrus was placed in Group-I and the female yak subjected to Double Ovsynch protocol were placed in Group-II. Transrectal-ultrasonography was performed daily for one oestrous cycle to monitor the follicular growth pattern and Luteal dynamics. Three wave patterns were observed in natural oestrus and four wave patterns were observed in induced oestrous female yak. The common follicular wave pattern in *Arunachali Yak* was two wave pattern (66.67%) in natural oestrus and three wave pattern (77.78%) in induced oestrus. The overall mean oestrous cycle length in natural oestrous (Group I) and induced oestrous (Group II) female yak was found to be 20.00±0.86 and 22.50±0.57 days respectively. Two follicular wave patterns in natural oestrus and three follicular wave patterns in induced oestrus was the most common in *Arunachali Yak*. The length of first follicular wave was found to be maximum in both natural and induced oestrous. The length of follicular wave decreases successively as the number of follicular waves increases in *Arunachali Yak*. The size of Corpus Luteum was found significant difference in between the group.

Keywords: *Arunachali yak*; estrous cycle; follicular dynamics; luteal dynamics; ultrasonography

Introduction

The yak is a domestic herbivore found at high altitudes (2,500- 5,500 meters) above sea level in the Himalayan region [37]. It belongs to the Bovidae family and is scientifically named *Bos grunniens*. In words, the yak population accounts for 14.2 million (FAO), and In India, yaks are mainly distributed in the states of Jammu-Kashmir, Himachal Pradesh, Uttarakhand, Sikkim, Arunachal Pradesh, and West Bengal [5]. The world's total yak population accounts for 14 million in which China shares about 90 percent of the total yak population [35]. According to the Livestock Census Report, 2019 the estimated population of yak is 58,000 in India. Yak is the principal livestock for the people living in high-altitude regions of the Himalayas and Tibetan regions. Therefore, Yak can become a key factor for the upliftment of their socio-economic conditions [26]. Yak fulfils their nutritional requirements from grazing, therefore pasture availability across the year plays a major role in reproductive performance [34]. Products obtained from yak are mainly meat, milk, and hide, these are the main sources of income for highlanders [26]. Yaks are seasonal breeders/polyoestrous animals whose mating season in their native high-altitude environment typically occurs between July and November [26]. In yak, oestrous cycle (19-21 days) comprises 4 phases viz. proestrus (1-5 days), estrous (12-36 hours), metestrus, and diestrus. Ovulation takes place at the end of estrous after 12-24 hours [25]. Yak has an average inter-calving period of 2 years [6]. Therefore, by enhancing the reproductive ability of this species, the livelihood of highlanders as well as the economy of the country are likely to be improved.

Yak suffers from many reproductive problems like repeat breeding, post-partum anestrous, poor estrous expression, and delayed maturity [26]. Prolonged calving to the estrous period is also a major reproductive problem in yak [37]. Yak expresses behavioral signs of estrus only for very few hours during the estrous cycle. The success rate of AI in *Arunachali Yak* is limited due to poor estrous symptoms, unpredictable spontaneous ovulation, and difficulty in estrus detection [6, 27]. In the natural oestrous cycle, it was reported that yak showed a low (40- 60%) annual fertility rate [38]. Artificial insemination in yak can be used for genetic improvement and the improvement of production traits in a short period [6]. Therefore, there is a need for estrous synchronization protocol in yak so that fixed-time AI can be performed in a group of animals through which an increase in the fertility of yak can be achieved [38]. In the past few decades, several protocols were developed for estrous synchronization in the yak.

The use of ultrasonography in the understanding of follicular growth patterns became possible in the late 1980s in cattle [2]. A very extensive study of follicular growth patterns has been conducted in cattle by using ultrasonography but knowledge of ovarian ultrasonography is limited in yak.

Follicular dynamics is a continuous process of follicular growth and regression [1], where the reserve primordial follicle develops into primary follicles followed by secondary and tertiary or mature follicles [3]. Rajakoski (1960) proposed a wave theory in cattle ovaries, the theory was based on speculation that the recruitment of follicles continues throughout the oestrous cycle and follicles selected for ovulation coincidentally [23]. Each follicular wave is divided into 4 parts i.e., recruitment, selection, deviation, and dominance [20, 9]. In cattle, it is found that 1st and 2nd and 3rd follicular wave arises during the luteal phase and this phase leads to the development of the dominant follicle [33]. In cattle, the number of dominant follicles is more than in yak and buffalo, the size of the dominant follicle is >7mm in diameter, and it continues to grow, while the remaining smaller follicles undergo atresia [34]. There are 2-3 follicular wave patterns seen in cattle and these follicular patterns originate successfully on 0, 9, and 16 days from an estrous period in the Three Follicular Wave Model [11].

Oestrous synchronization can be better achieved after assessment of follicular growth pattern and endocrine change during various stages of the oestrous cycle in the yak. Moreover, various advanced reproductive techniques such as superovulation, embryo transfer, and in vivo fertilization can be implemented only after gaining information on the follicular dynamics and endocrine profile of yak.

Material and methods

Experimental animals

The study was conducted in *Arunachali Yak* maintained at the animal farm of ICAR-NRC on Yak, Dirang in West Kameng district of Arunachal Pradesh, India (Figure 1.a). A total of 36 animals (n=36) of apparently healthy cyclic female yak of second to fourth parity and free from any post-parturient complications were selected for the study. The animals were maintained under intensive system of rearing with standard feeding and manage mental conditions.

Experimental design

Thirty-six numbers of female yak were equally divided into two groups viz. Group-I and Group II. Group-I animals were kept as the control without any treatment, while Group-II animals were subjected to modified Double Ovsynch protocol specially for yak (Figure 2) i.e. on the day of the synchronization (Day 0), the female yak received 500 mcg (2ml) of cloprostenol (Pragma, Intas Pharmaceutical Ltd., India) which is a prostaglandin (PGF2α) analogue by intramuscular route.

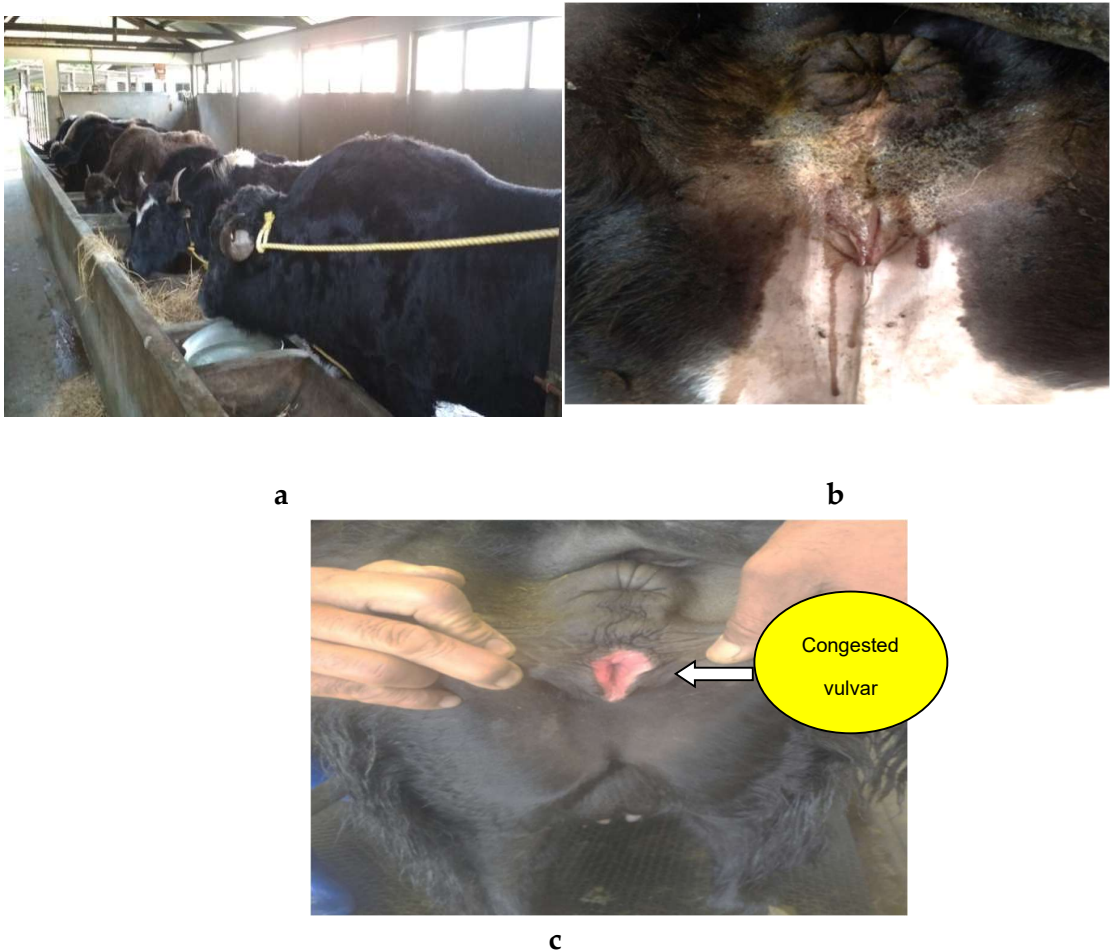


Figure 1. a: Yak shed at ICAR-NRC on Yak farm; b:Cervicovaginal mucosal discharge; c Congestion of vulvar mucus membrane.

After 48 hours (Day 2) of the first cloprostenol treatment, they were given first dose of buserelin acetate which is a GnRH analogue (Gynarich, Intas Pharmaceutical Ltd., India) @ 10 mcg intramuscularly. After 7 days of the first dose of buserelin acetate administration (Day 9), a second dose of cloprostenol was injected with the same dose and route as the previous one. Two days after the second dose of cloprostenol, i.e. on day 11, a second dose of Buserelin acetate was administered

maintaining the dose and route same as the first one. The estorus in experimental animals was confirmed by clinical signs and symptoms (Figure 1.b, 1.c) All the oestrus animals in Group I and Group-II were monitored for follicular and luteal dynamics for one estrous cycle using real-timetransrectal ultrasonography (Figure 3).

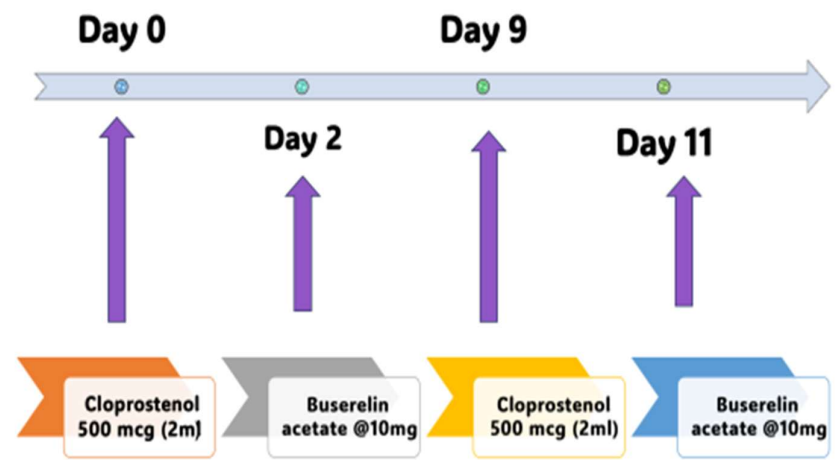


Figure 2. Double Ovsynch protocol for group II Arunachali Yak.



Figure 3. Transrectal ultrasonography in yak.

Ultrasonographic observations of ovarian structures

Follicular dynamics

The Length of oestrous cycle was calculated by taking the difference between two consecutive heats considering the wave pattern [28]. The day of follicular wave emergence was considered when few numbers of follicles ≥ 1.5 mm in diameter were observed by per-rectal ultrasonography on the ovary [28]. Duration of wave (wave length) in each oestrous cycle was calculated from day of emergence of the wave up to the atretic phase of the dominant follicle [30]. Number of recruited

follicles was calculated as the total number of follicles (≥ 1.5 mm in diameter) which were recruited in one follicular wave.

The life span of dominant follicle (DF) was defined from the stage of deviation Life span of anovulatory dominant follicle was calculated by subtracting the day of follicular deviation from the day of atresia. The life span of ovulatory dominant follicle was calculated by subtracting the day of follicular deviation from the day of last appearance before ovulation.

The DF was considered as the largest follicle with a diameter of >7.5 mm and being at least 2 mm larger than other follicles. The diameter of the dominant ovulatory follicle was calculated by taking average size of dominant ovulatory follicle of two consecutive oestrus and the maximum diameter of the dominant anovulatory follicle was calculated from the observations of one oestrous cycle only [36]. The day of oestrous cycle on which the highest diameter of dominant follicle was observed and was recorded as day of maximum diameter of dominant follicle.

Luteal dynamics

The life span of CL was calculated by taking the difference between the day of CL regression and the day of appearance of the granular echogenic structure at the place of DF after ovulation [4].

Maximum diameter of corpus luteum was estimated by taking the measurements of the mean of two perpendicular diameters (length and width) acquired with the ultrasonic scanner's built-in digital calipers [15]. Growing phase of corpus luteum was estimated as the number of days required to grow by CL after ovulation to achieve its maximum diameter [10]. Regression phase of corpus luteum was calculated as the number of days taken for complete regression of CL after the achievement of its maximum diameter [10].

Statistical analysis

The statistical analysis was carried out with standard statistical methods advocated by Snedecor and Cochran [32]. Descriptive statistics was used to calculate the mean, standard deviation and standard error of means. Comparisons between the groups were done by applying t-test: unpaired two samples assuming unequal variance.

Results

Follicular dynamics

The overall mean estrous cycle length in natural and induced Arunachali female Yak was found to be 20.00 ± 0.86 and 22.5 ± 0.57 days, respectively (Table 1). The overall estrous cycle length was significantly ($P < 0.05$) longer in the induced group as compared to the natural group.

Table 1. Oestrous cycle length (mean $\pm$ SEM) and frequency of occurrence (%) of oestrous in natural and induced Yak cows.

Parameter	Two wave	Three wave	Three wave	Four wave
Length of oestrous cycle in different wave pattern	19.60 ± 0.51	22.50 ± 1.50	22.33 ± 0.49	24.00 ± 0.0
Frequency of occurrence of different wave pattern of oestrous cycle in female Arunachali Yak	66.67	33.33	77.78	22.22

The frequency of occurrence of two follicular wave patterns was found more (66.00%) as compared to the three follicular wave pattern (44.00%) in naturally occurring estrous group (Figure 4.a, 4.b).

In induced estrous group, frequency of three follicular wave patterns (77.78%) was higher as compared to four (22.22%) follicular wave patterns. Two follicular wave patterns were not observed in induced estrous animals.

The mean day at emergence of first (1.67 ± 0.21 vs 1.75 ± 0.85 days), second (9.50 ± 0.67 vs 8.50 ± 0.89 days) and third (16.00 ± 0.67 vs 14.34 ± 0.65 days) follicular waves in Group I natural and Group II induced female yak did not vary significantly between the groups (Table 2). In induced estrous group, the mean day of emergence of fourth follicular wave was found to be on day 19.00 ± 0.00 of estrous cycle (Table 2).

Table 2. Detail study of follicular growth pattern, dominant follicle, subordinate follicle and characteristics of corpus luteum in natural and induced oestrus female yak.

	Natural	Induced	P- value
Length of oestrous cycle	20.00 ^a ± 0.86	22.50 ^b ±0.57	0.02
Day at emergence of follicular wave			
1 st follicular wave	1.67±0.21	1.75± 0.85	0.40
2 nd follicular wave	9.50±0.67	8.50± 0.89	0.19
3 rd follicular wave	16.00±1.00	14.34±0.65	0.15
4 th follicular wave		19.00± 0.00	
Length of follicular wave			
1 st follicular wave	11.16± 1.30	13.50± 0.50	0.70
2 nd follicular wave	9.80± 0.90	9.12± 0.54	0.26
3 rd follicular wave	7.00± 0.00	7.87± 0.51	0.25
4 th follicular wave		6.00 ±0.00	
Number of recruited follicles			
1 st follicular wave	4.50± 0.42	4.12± 0.30	0.07
2 nd follicular wave	3.80± 0.30	4.38±0.38	0.26
3 rd follicular wave	4.50± 0.50	4.50± 0.60	0.25
4 th follicular wave		3.00 ±0.00	
Life span of dominant follicle (DF) from the stage of deviation			
1 st follicular wave	7.53 ^a ±0.48	10.88 ^b ± 88 ^b ±0.61	<0.05
2 nd follicular wave	5.21± 0.34	5.75 ±0.65	0.24
3 rd follicular wave	4.50 ±0.50	4.75 ±0.45	0.37
4 th follicular wave		4.00 ±1.00	
Maximum diameter of the dominant follicle			
1 st follicular wave	12.98±0.43	13.20± 0.33	0.07
2 nd follicular wave	11.45± 0.58	10.93± 0.36	0.26
3 rd follicular wave	13.25± 1.05	12.20± 0.79	0.25
4 th follicular wave		12.60 ±0.50	
Maximum diameter of ovulatory follicles	12.46± 0.56	12.97 ±0.50	0.25
Maximum diameter of anovulatory dominant follicles	12.28± 0.55	11.50± 0.39	0.15
Day at maximum diameter of follicle			
1 st follicular wave	9.00± 0.77	9.38 ±0.50	0.33
2 nd follicular wave	17.83± 0.79	16.00± 0.80	0.07
3 rd follicular wave	22.00± 2.00	20.00± 0.79	0.27
4 th follicular wave		24.00± 0.00	
Growth rate of dominant follicle			
1 st follicular wave	0.76± 0.07	0.92± 0.12	0.13
2 nd follicular wave	0.77± 0.13	0.78 ±0.13	0.46
3 rd follicular wave	1.35 ±0.20	0.91 ±0.12	0.10
4 th follicular wave		1.17± 0.23	
Life span of different subordinate follicle from the stage of deviation			
1 st follicular wave	3.83 ±0.48	3.88±0.35	0.47
2 nd follicular wave	3.67± 0.33	3.81±0.32	0.34
3 rd follicular wave	2.50± 0.50	3.75± 0.56	0.09

4 th follicular wave	3.50±0.50		
The maximum diameter of the subordinate follicle			
1 st follicular wave	7.20± 0.58	8.00± 0.43	0.15
2 nd follicular wave	6.73± 0.22	6.88 ±0.40	0.38
3 rd follicular wave	6.60 ^a ± 0.20	8.14 ^b ±0.66	0.03
4 th follicular wave	0.00	7.50 ±0.00	
Day at maximum diameter of subordinate follicle			
1 st follicular wave	6.00± 0.68	6.50± 0.50	0.28
2 nd follicular wave	13.83± 0.70	13.00± 0.13	0.26
3 rd follicular wave	19.00± 2.00	18.63± 0.75	0.15
4 th follicular wave		23.00± 0.00	
Growth rate of subordinate follicle			
1 st follicular wave	0.40 ^a ± 0.03	0.83 ^b ± 0.10	<0.05
2 nd follicular wave	0.68 ±0.09	0.54± 0.14	0.21
3 rd follicular wave	1.30 ±0.20	0.96± 0.13	0.13
4 th follicular wave		1.19± 0.36	
Luteal dynamics			
Life span of corpus luteum (days)	18.25±0.82	19.25±0.82	0.20
Maximum diameter of corpus luteum (mm)	18.23 ^a ±0.98	21.16 ^b ±0.29	<0.05
Day at maximum diameter of corpus luteum	10.13±0.55	11.00±0.62	0.15
Growing phase of corpus luteum (days)	11.63±0.84	12.63±0.84	0.21
Regression phase of corpus luteum (days)	6.63±0.38	7.63 ^b ±0.38	<0.05

The pattern of follicular wave length showed a gradual decrease from first to third or fourth waves in both the groups and a shorter life span of the last follicular wave. The length of the first follicular wave was longer in both Group I (11.16 ±1.30 days) and Group II (13.50 ± 0.50 days) female yak and length gradually decrease in the second (9.80 ±0.90 vs 9.12 ±0.54 days) and third waves (7.00 ±0.00 vs 7.87 ±0.51) in Groups I and Groups II, respectively (Table 2). The fourth follicular wave observed only in Group II yak was found to be shorter (6.00 ± 0.00 days) than the previous three waves (Table 2). No significant variation was recorded in the length of the follicular waves between groups.

The number of recruited follicles ranged from 3-5 numbers in the entire follicular wave pattern in both the groups.

There was a decreasing pattern of life span of dominant follicle from first follicular wave to third or fourth follicular wave in both the groups. Life span of dominant follicle from stage of deviation to atresia in first follicular wave differed significantly (P<0.05) between natural (7.53 ±0.48 days) and induced (10.88 ±0.61 days) estrous female yak group. In second and third follicular waves, no significant difference was observed (Table 2).

The life span of dominant ovulatory follicles (third and fourth follicular wave) was numerically lower in Group I (4.50 ±0.50 days) and Group II (4.00 ±1.00 days) female yak as compared to dominant anovulatory follicles. The maximum diameter of dominant follicles was (12.46 ± 0.56 mm) and (12.97 ± 0.50mm) in Group I and Group II female yak, respectively, with no significant difference between groups (Table 2).

The maximum size of the anovulatory dominant follicles was 12.28±0.55 mm and 11.50±0.39 mm in Group I and Group II female yak, respectively, with no significant difference (P<0.05). No significant variation was observed in day at maximum diameter of dominant follicle and growth rate of dominant follicle between the groups (Table 2).

Subordinate follicle

There was no significant difference (P>0.05) in the maximum diameter of subordinate follicle between the first and second follicular wave in natural (Group I) and induced (Group II) female yak. In contrast, significant difference (P<0.05) in maximum diameter of subordinate follicle was observed

in third follicular wave between Group I (6.60 ± 0.02 mm) and Group II (8.14 ± 0.66 mm) female yak (Table 2).

In growth rate of subordinate follicle, significant ($P<0.05$) difference was observed in the first follicular wave between Group I (0.40 ± 0.03 mm/day) and Group II (0.83 ± 0.10 mm/day). No significant variation was observed in the growth rate of subordinate follicle in second and third follicular wave between the groups (Table 2).

Luteal dynamics

The life span of corpus luteum in Group I and Group II female yak was 18.25 ± 0.82 and 19.25 ± 0.82 days, respectively, with no significant variation between the groups. The maximum diameter of corpus luteum was significantly ($P<0.05$) larger in Group II (21.16 ± 0.29 mm) female yak as compared to Group I (18.23 ± 0.98 mm) (Figure 4.c). The day at maximum diameter of corpus luteum in Group I and Group II female yak was 10.13 ± 0.55 and 11.00 ± 0.62 days, respectively, with no significant variation between the groups. The growing phase of corpus luteum was 11.63 ± 0.84 and 12.63 ± 0.84 days in Group I and Group II, respectively, with no significant variation.

The regression phase of corpus luteum was significantly ($P<0.05$) longer in Group II (7.63 ± 0.38 days) as compared to Group I (6.63 ± 0.38 days) (Figure 4.d).

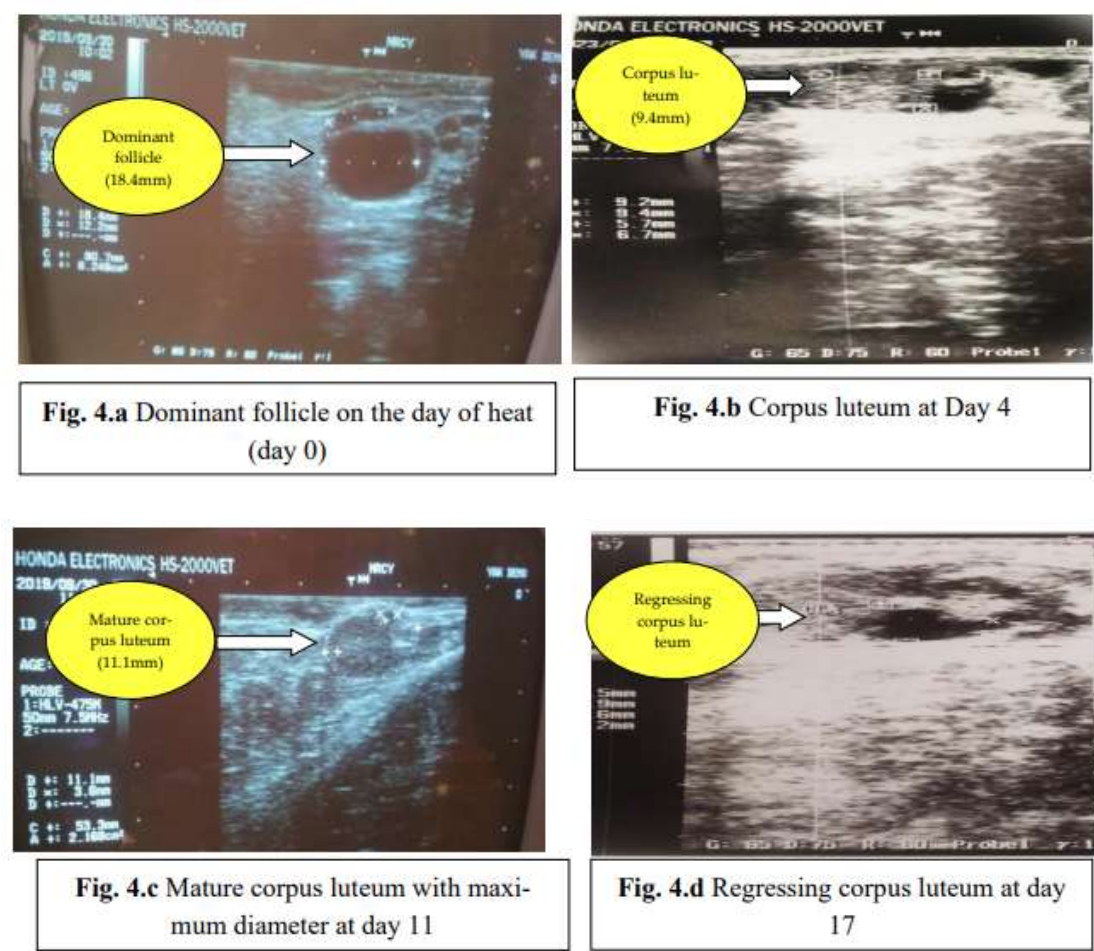


Figure 4.

Discussion

Similar observation of higher frequency of two follicular wave was also reported in cattle [14,15,17] and in *Bos taurus* and Nellore cows [30]. Contrary to the present findings, [32] reported

lower frequency of occurrence of two follicular waves pattern as compared to three and four wave patterns in oestrous cycle of cattle heifer.

Mithun cow recorded higher frequencies of three follicular wave patterns in induced oestrus Mithun along with longer oestrous cycle length in induced oestrus animals with four follicular wave patterns, which corroborate the present findings [23].

The longer oestrous cycle length in four follicular wave patterns might be attributed to the longer mean inter-ovulatory interval due to delayed onset of regression of CL as luteal regression occurs after the emergence of the ovulatory wave [23,25].

Similar number of recruited follicles was recorded by [32] in cattle heifers. However, higher number of recruited follicles were reported in Holstein Friesian heifer and cow [9], in induced Mithun cow [23], and in Thai native cattle [7]. The recruitment of follicle depends on the number of primordial follicles and hormonal stimulation. The lesser recruitment of follicles in yak might be due to species differences [18].

Similar findings on life span of dominant follicle were reported by [25] in cattle heifer. However, longer life span of dominant follicle was observed by [7,13,20,22,32] in cattle.

The significantly larger corpus luteum in induced oestrus group might be due to the effect of GnRH analogue administration, which stimulates terminal growth of dominant follicle leading to increased oestradiol production, oestrus, ovulation and formation of large sized corpus luteum. Similar findings were reported in Surti buffalo [13] and Mithun [23].

The longer regression phase of corpus luteum in induced group might be due to the presence of four follicular waves leading to longer oestrous cycle [19], as luteal regression occurs after emergence of the ovulatory wave [25]. Similar and contrasting findings have been reported by [7,22,23].

The variations in follicular growth pattern and luteal dynamics among different studies might be due to species, breed, management practices, geographical location, age and parity of animals.

Conclusion

In *Arunachali Yak* follicular growth pattern were found in wave like pattern. Two follicular wave patterns in natural oestrus and three follicular wave patterns in induced oestrus was the most common follicular wave pattern in *Arunachali Yak*. The length of first follicular wave was found to be maximum in both natural and induced oestrous. The length of follicular wave decreases successively as the number of follicular waves increases in *Arunachali Yak*. The size of CL was found significant in between the group.

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Ethical approval: All animal procedures were approved by Committee for Control and Supervision of Experiments on Animals (CCSEA) of Govt. of India (V-11011(13)/13/2022- CPCSEA-DADF) and were conducted in accordance with the institutional guidelines for the care and use of laboratory animals.

Conflicts of Interest: The authors declare no competing interests.

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