Original Article

Ovarian Follicular Patterns and Hormone Profile in Thai Native Cattle (*Bos indicus*)

Denpong Sakhong[1,2] Thevin Vongpralub[2*] Suporn Katawatin[2] Saksiri Sirisathien[3]

Abstract

The objective of this study was to characterize the patterns of ovarian follicular waves, and FSH and P4 concentration during estrous cycle in Thai native cattle (*Bos indicus*). Transrectal ultrasound examination of ovaries was performed daily using a 7.5 MHz transducer and blood samples were collected after the ultrasound examination during May to August, 2010. There were 1-, 2- or 3-follicular waves per cycle in heifers, and 1-, 2-, 3- or 4-follicular waves per cycle in cows. Moreover, in cows, only one cycle exhibited 4-follicular wave pattern. The majority of TNC cows (82.76%) exhibited a 3-follicular wave pattern, whereas the majority of heifers exhibited 2-(38.10%) and 3-(47.62%) follicular wave patterns, respectively. The numbers of follicles per wave were 6.67±0.69, 7.71±0.45, and 6.29±0.45 in heifers and 4.50±0.85, 4.00±0.85, 4.82±0.29, and 5.00±1.20 in cows, respectively. The length of estrous cycle was 9.33±0.79, 15.13±0.48, and 18.57±0.51 days in heifers and 10.50±0.96, 15.50±1.46, 21.63±0.51, and 25.00±1.36 days in cows. The maximum diameters of dominant ovulatory follicles in heifers were 12.13±0.62, 11.31±0.38, and 11.28±0.34 and in cows 12.65±0.76, 12.45±0.76, 12.82±0.22, and 12.80±1.08 mm. The growth rates were 1.17±0.14, 1.12±0.08, and 1.16±0.07 and 1.16±0.17, 1.34±0.17, 1.16±0.05 and 1.29±0.24 mm/day in heifers that had 1-, 2-, or 3- follicular waves and cows that had 1-, 2-, 3-, or 4-follicular waves, respectively. The greater numbers of small follicles emerged more in heifers than in cows ($p<0.05$), while the duration of the dominant ovulatory follicle was longer in cows than in heifers ($p<0.05$). The plasma FSH concentrations slightly increased on days 0-1, 6-7, 13-14, and 18 of the estrous cycle, then the wave emerged on the next day. Plasma P4 concentrations slightly increased on days 5-6 and remained high 3-5 days before ovulation.

In conclusion, the ovarian follicular dynamic in Thai native cattle developed into 1-, 2-, 3- or 4-follicular waves. In cows 3-follicle waves were mostly found whereas in heifers 2- and 3- follicular waves were mostly found. The duration of estrous cycle correlated to the number of follicular wave. The waves of follicular development were consistent with the increase in amount of FSH. Moreover, when the level of P4 decreased, the largest follicle usually underwent ovulation. The results revealed differences in ovarian follicular dynamics, and in plasma concentrations of FSH and P4, which may account for the differences in the characteristics of ovarian follicular development between heifers and cows of Thai native cattle.

Keywords: follicular wave, FSH, progesterone, Thai native cattle

[1]*Veterinary Research and Development Center (Upper Northeastern Region), Department of Livestock Development, Khonkaen, 40260 Thailand*

[2]*Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002 Thailand*

[3]*Department of Surgery and Theriogenology, Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen, 40002 Thailand*

***Corresponding author: E-mail:** *vthevi@kku.ac.th*

บทคัดย่อ

รูปแบบการพัฒนาของฟอลลิเคิลและการเปลี่ยนแปลงของฮอร์โมนในโคพื้นเมืองไทย

เด่นพงษ์ สาฆ้อง [1,2] เทวินทร์ วงษ์พระลับ [2] สุภร กตเวทิน [2] ศักดิ์ศิริ ศิริเสถียร [3]*

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาลักษณะรูปแบบของคลื่นการพัฒนาของฟอลลิเคิลบนรังไข่และระดับความเข้มข้นของฮอร์โมน FSH และ P4 ในกระแสเลือด ระหว่างวงรอบการเป็นสัดของโคสาวและแม่โคพันธุ์พื้นเมืองไทย ติดตามการพัฒนาของฟอลลิเคิลบนรังไข่โคทุกๆ วัน โดยใช้เครื่องอัลตร้าซาวน์ หัวตรวจชนิดสอดเข้าทวารหนัก คลื่นความถี่ขนาด 7.5 เมกะเฮิรท์ และเจาะเก็บเลือดทุกครั้ง หลังการอัลตร้าซาวน์ ในระหว่างเดือน พฤษภาคม ถึง สิงหาคม 2553 พบว่าในโคสาวพบการพัฒนาของคลื่นฟอลลิเคิล เป็น 1, 2 หรือ 3 คลื่นการพัฒนา สำหรับแม่โคพบการพัฒนาของคลื่นฟอลลิเคิล เป็น 1, 2, 3 หรือ 4 คลื่นการพัฒนา ซึ่งพบการพัฒนาแบบ 4 คลื่น ในแม่โคเพียง 1 วงรอบการเป็นสัด โดยในแม่โคพบการพัฒนาของคลื่นฟอลลิเคิลเป็นแบบ 3-คลื่น (ร้อยละ 82.76) มากที่สุด ในขณะที่ในโคสาวพบการพัฒนาของคลื่นฟอลลิเคิลเป็นแบบ 2-คลื่น (ร้อยละ 38.10) และ 3-คลื่น (ร้อยละ 47.62) มากที่สุด จำนวนฟอลลิเคิลเฉลี่ยในโคสาวเท่ากับ 6.67±0.69, 7.71±0.45, 6.29±0.45 ฟอลลิเคิล และในแม่โคเท่ากับ 4.50±0.85, 4.00±0.85, 4.82±0.29, 5.00±1.20 ฟอลลิเคิลต่อคลื่นการพัฒนาที่ 1, 2, 3 และ 4 ตามลำดับ นอกจากนี้พบว่าโคสาวและแม่โคที่พบการพัฒนาของฟอลลิเคิลเป็น 1, 2, 3, หรือ 4 คลื่นการพัฒนา มีความยาววงรอบการเป็นสัดเฉลี่ย 9.33±0.79, 15.13±0.48, 18.57±0.51 และ 10.50±0.96, 15.50±1.46, 21.63±0.51, 25.00±1.36 วัน ฟอลลิเคิลเด่นที่เกิดการตกไข่มีขนาดโตที่สุดเฉลี่ย 12.13±0.62, 11.31±0.38, 11.28±0.34 และ 12.65±0.76, 12.45±0.76, 12.82±0.22, 12.80±1.08 มม. อัตราการเจริญเติบโตเฉลี่ย 1.17±0.14, 1.12±0.08, 1.16±0.07 และ 1.16±0.17, 1.34±0.17, 1.16±0.05, 1.29±0.24 มม./วัน ตามลำดับ โคสาวมีจำนวนฟอลลิเคิลขนาดเล็กที่พัฒนาในแต่ละคลื่นมากกว่าแม่โค ($p<0.05$) แต่อายุการคงอยู่ของฟอลลิเคิลเด่นที่นำไปสู่การตกไข่ในแม่โคยาวนานกว่าในโคสาว ($p<0.05$) ระดับความเข้มข้นของฮอร์โมน FSH ในกระแสเลือดจะเพิ่มขึ้นในวันที่ 0-1, 6-7, 13-14, และ 18 ของวงรอบการเป็นสัด และจะพบการพัฒนาของคลื่นฟอลลิเคิลในวันถัดมา ในขณะที่ระดับเข้มข้นของฮอร์โมน P4 จะเพิ่มสูงขึ้นในวันที่ 5-6 และลดลงระดับลงก่อนการตกไข่ 3-5 วัน

จากผลการวิจัยครั้งนี้สรุปได้ว่า การพัฒนาของฟอลลิเคิลบนรังไข่ของโคพื้นเมืองไทย มีการพัฒนาเป็น 1, 2, 3 หรือ 4 คลื่นการพัฒนาต่อวงรอบการเป็นสัด โดยโคสาวมีการพัฒนาของคลื่นฟอลลิเคิลเป็นแบบ 2-คลื่น และ 3-คลื่น มากที่สุด ในขณะที่แม่โคมีการพัฒนาของคลื่นฟอลลิเคิลเป็นแบบ 3- คลื่น มากที่สุด ความยาวของวงรอบการเป็นสัดมีความสัมพันธ์กับจำนวนคลื่นการพัฒนาของฟอลลิเคิล โดยคลื่นการพัฒนาของฟอลลิเคิลจะสอดคล้องกับการเพิ่มขึ้นของปริมาณ FSH ในกระแสเลือด และเมื่อระดับความเข้มข้นของ P4 ลดลง ฟอลลิเคิลขนาดใหญ่จะสามารถเกิดการตกไข่ได้ จากผลการเปลี่ยนแปลงของฟอลลิเคิลบนรังไข่และระดับความเข้มข้นของฮอร์โมน FSH และ P4 ในกระแสเลือดโค สามารถบ่งบอกถึงความแตกต่างของลักษณะการพัฒนาของฟอลลิเคิลระหว่างโคสาวและแม่โคพันธุ์พื้นเมืองไทยได้

คำสำคัญ: คลื่นฟอลลิเคิล เอฟ เอส เอช โปรเจสเตอโรน โคพื้นเมืองไทย

[1]*ศูนย์วิจัยและพัฒนาการสัตวแพทย์ภาคตะวันออกเฉียงเหนือ (ตอนบน) กรมปศุสัตว์ จ. ขอนแก่น 40260*

[2]*ภาควิชาสัตวศาสตร์ คณะเกษตรศาสตร์ มหาวิทยาลัยขอนแก่น 40002*

[3]*ภาควิชาศัลยศาสตร์และเธนุเวชวิทยา คณะสัตวแพทยศาสตร์ มหาวิทยาลัยขอนแก่น 40002*

***ผู้รับผิดชอบบทความ E-mail:** vthevi@kku.ac.th*

Introduction

Thai Native Cattle (TNC) is originated in South Asia and later spread across South-East Asia (Jaturasitha et al., 2009). They adapt well to high temperature, tolerate harsh sunshine, are resistant to ticks and disease (Dobson and Kamonpatana, 1986) and can utilize poor quality diets of the humid tropical regions (Kawashima et al., 2003). They are major beef cattle for breeding in Thailand. However, the information on the TNC's reproductive physiology is rare, especially in ovarian follicular wave and endocrine factors that affect follicular pattern. As a result, a better understanding, particularly of the ability to control the growth of the dominant follicle, together with understanding of the competing mechanisms controlling follicular recruitment and growth, should enable more precise control of ovarian function and superovulation in TNC.

Ultrasonic monitoring of individually identified ovarian follicles has been used to confirm the wave-like pattern of follicular development in *B. taurus* dairy cows (Savio et al., 1988; Sirois and Fortune, 1988; Ginther et al., 1989[a,b]), *B. taurus* beef cows (Evans et al., 1994; Sunderland et al., 1994; Bo et al., 1995), and *B. indicus* (Rhodes et al., 1995; Figueiredo et al., 1997; Castilho et al., 2007; Gimenes et al., 2008). The ovarian follicular dynamics during the estrous cycle in *B. indicus* females are similar to that observed in *B. taurus* females, but comparable data in TNC have not been reported. The differences in the physiology of estrous cycle may have implications for the success of treatments to manipulate follicular development in TNC.

Folliculogenesis in cattle is characterized by waves of follicular development, each wave corresponding to the initial development of a population of small follicles. Emergence of a new follicular wave is preceded by a surge in plasma concentration of follicle stimulating hormone (FSH; Adams et al., 1992, 2008), after which a single follicle is selected for continual growth called the dominant follicle, whereas the remainder, called subordinate follicles, becomes atretic and regressive (Adams et al., 2008). The largest follicle present at the onset of decreasing in Progesterone (P4) level usually undergoes ovulation, two (Ginther et al., 1989[c]; Knopf et al., 1989), three (Savio et al., 1988) or four (Rhodes et al., 1995) follicular waves are found during an estrous cycle. Greater than 95% of bovine estrous cycles are composed of either two or three follicular waves (Adams, 1999). Rhodes et al. (1995) reported on Brahman heifers that 26.5%, 66.7%, and 6.8% of estrous cycles had either two, three, and four waves of ovarian follicular developer during an estrous cycle, respectively. The majority of Nelore cows have two (83.3%) and heifers have three (64.7%) waves of ovarian follicular development (Figueiredo et al., 1997). Others have reported that up to 27% of estrous cycles in *B. indicus* cows consist of four waves of follicular development compared with only 7% in *B. indicus* heifers (Bo et al., 2003).

The FSH plays a significant role in regulating the follicular wave emergence and deviation in cattle (Adams et al., 1992). After each FSH surge, a group of small follicles appear in the ultrasonographic examination. However, circulating concentrations of inhibin increase concurrently with emergence of waves, which suppress the synthesis and secretion of FSH (Kulick et al., 2001). Plasma FSH concentration decreases within 2-3 days after the emergence of wave and this decrease in the FSH concentration has been implicated in the mechanism of follicular deviation (Ginther et al., 1999; Kulick et al., 2001) and selection of a dominant follicle (Adams et al., 1993). Estradiol (E2) content in the follicular fluid of dominant follicle has a major inhibitory action on FSH, growing follicles produce other factors such as IGFs, Inhibin and Follistatin (Singh et al., 1999) that also regulate FSH release and availability. The release of Luteinizing Hormone (LH) is enhanced by E2 and low concentrations of P4 (Kaneko et al., 1991). Increased LH pulse frequency has been shown to be associated with maintenance of a dominant follicle (Savio et al., 1993). If this dominant follicle develops during the follicular phase with increasing LH frequency pulse, it ovulates. Changes in the pulsatility of FSH and P4 are the potential endocrine signaling mechanism that has not been reported in associations with follicular wave in TNC.

Therefore, the objectives of this study were to identify patterns of growth and developmental characteristic of ovarian follicles (≥3.0 mm) using real-time ultrasonography and to characterize the temporal relationship between follicular waves and the circulating concentration of FSH and Progesterone during estrous cycle in TNC.

Materials and Methods

Animals and treatments: TNC with a 3.0-3.5 BCS (1-5 score) were divided by reproductive status into two groups (heifers; n=7; averaged 2.57±0.07 years of age and 182.29±4.46 kg body weight (BW) and cows; n=7; averaged 7.43±0.65 years of age and 242.57±6.61 kg BW). During the experimental period, all animals were kept on pen. They were fed *ad libitum* of hay and mineral block, had free access of water, and received the concentrate (14% of crude protein) at 1.5% of the BW.

The estrous cycle of each animal was synchronized with two injections of 25 mg of PGF2α (Lutalyse®; Pfizer Inc, New York, USA) 11 days apart. On day 7 after heat signs occurred, all animals were inserted with CIDR® (EAZI-BREED™, InterAg, Hamilton, New Zealand) for a 7 day period. On day 6 after CIDR® insertion, there were injected with 2.5 mg of Estradiol-17β (E-17β; InterAg, Hamilton, New Zealand), and given 25 mg of PGF2α (Lutalyse®; Pfizer Inc, New York, USA). Estrous detection was performed daily for 3 days after CIDR® removal by personal observation at 06.00-07.00 am and 05.30-06.30 pm and examined daily by ultrasonography to detect ovulation (Day 0). Animals in which ovulation was detected were assigned into completely randomize design to characterize the patterns of ovarian follicular waves during 3 to 4 consecutive cycles.

Ultrasonographic examination: Transrectal ultrasonography was used to characterize ovarian follicular dynamics during estrous cycles. A Honda HS-2000 equipped with a 7.5 MHz linear-array transducer (Honda Inc Tokyo, Japan) was used to monitor individual follicles larger than 3.0 mm in diameter. Follicles were measured with a ruler calibrated against the in-built provided with the ultrasound unit. An ovarian map was drawn in each examination, the relative positions and sizes of ovarian follicles and corpora lutea were recorded. Ultrasonographic examination began on the day at which CIDR® was removed and performed daily for at least 3 consecutive estrous cycles. During estrous period, the examination was preformed every 6 hour until the ovulation was detected. All of observations were carried out by the same person and data from the first estrous cycle were not included.

Blood collection and hormonal assays: Blood samples were collected daily from the jugular vein in a 12 ml heparinized tubes from each animal and immediately stored on ice. After centrifugation at 2000xg for 20 min at 4^{0}C, the plasma was separated and stored at -20^{0}C until analysis. Daily plasma samples from 6 animals through one estrous cycle were used for detection of the concentrations of FSH and P4. The hormone assays were preformed in duplicate with the use of a double-antibody Radioimmunoassay system commercially supplied by ICN Pharmaceuticals, Inc (California, USA) were measured at the Radiology Laboratory (Faculty of Medicine, Khon Kaen University, Thailand). The intra-assay coefficients of variation were 2.6% at 13.1 mIU/ml and 10.8% at 1.58 ng/ml for FSH and P4, respectively, while the inter-assay coefficients of variation were 8.0% at 13.7 mIU/ml and 11.1% at 0.72 ng/ml for FSH and P4, respectively.

Follicular wave characteristics: Individual follicles were identified and followed when they reached a diameter of 5.0 mm. The individual follicular growth was scored over the days. The day of wave emergence was defined as the day at which follicles developed over 3.0 mm. The day of emergence of the ovulatory follicle was defined retrospectively, as the day on which the ovulatory follicle was first detected at the size of larger than 8.0 mm in diameter. The life-span of the ovulatory follicle was defined as the time interval between the day of emergence of the ovulatory follicle and the time of ovulation.

The growth rate and maximum diameter were analyzed. The time interval from the first measurable diameter to the last day of the maximum diameter recorded was defined as the growth period. Growth rate was calculated by subtracting the minimum diameter from the maximum diameter and dividing by the growth period. The dominant follicle was defined as the largest follicle with a diameter of ≥8.0 mm. Ovulation was defined as the disappearance of a dominant follicle, followed by the development of a corpus hemorrhagicum at the position previously occupied by the ovulatory dominant follicle.

Statistical Analysis: The follicular wave pattern during estrous was tested by general lineal model procedures of Statistical Analysis System (SAS Inst Inc, Cary, NC, USA). Diameters and growth rates of the dominant follicle were analyzed according to the time of ovulation after normalizing the data. Differences and means were calculated using Least square means and *t*-test.

Results

Follicular wave in TNC was characterized by 1-, 2-, 3- or 4-follicular wave pattern. The majority of TNC cows (82.76%) exhibited a 3-wave pattern, whereas the majority of heifers (38.10%; 47.62%) exhibited a 2- and 3-wave pattern. However, just only 1 cycle in cow exhibited 4-wave pattern; as shown in table 1.

Table 1 Characteristics of ovarian follicular development in TNC.

Items	Number of follicular waves during estrous cycle.[1/]							Means
	1-wave		2-wave		3-wave		4-wave	
	heifers	cows	heifers	cows	heifers	cows	cow	
Number of estrous cycle (%)	3/21(14.29)	2/29(6.90)	8/21(38.10)	2/29(6.90)	10/21(47.62)	24/29(82.76)	1/29(3.45)	
Emergence of first wave (day)	2.50±0.42	2.50±0.42	1.50±0.21	1.50±0.42	1.43±0.22	1.41±0.14	2.00±0.59	1.54±0.10
Emergence of second wave (day)			7.38±0.38	7.00±.77	6.71±0.41	7.53±0.26	8.00±1.08	7.31±0.18
Emergence of third wave (day)					12.57±0.56^{a}	14.29±0.36^{b}	14.00±1.49^{ab}	13.92±0.34
Emergence of fourth wave (day)							18.00±0.00	
Number of follicles emergence								
in first wave (follicles)	6.67±0.69^{ab}	4.50±0.85^{bc}	7.71±0.45^{a}	4.00±0.85^{d}	6.29±0.45^{abc}	4.82±0.29^{bc}	5.00±1.20^{bc}	5.69±0.19
in second wave (follicles)			6.86±0.50^{a}	3.50±0.96^{b}	6.00±0.50^{a}	4.59±0.32^{ab}	7.00±1.33^{a}	5.35±0.23
in third wave (follicles)					5.38±0.46	4.58±0.30	5.00±1.30	4.82±0.25
in fourth wave (follicles)							8.00±0.00	
Length of estrous cycle (day)	9.33±0.79^{e}	10.50±0.96^{e}	15.13±0.48^{d}	15.50±1.46^{d}	18.57±0.51^{c}	21.63±0.51^{b}	25.00±1.36^{a}	18.00±0.22
Length of days between emergence of waves:(day)								
1st wave to 2nd or 1st wave to ovulation.	7.00±1.11	8.00±1.11	7.63±0.55	8.50±1.11	6.57±0.59	8.25±0.39	7.00±1.57	7.71±0.26
2nd wave to 3rd or 2nd wave to ovulation.			7.75±0.54	8.50±1.09	7.43±0.58	8.81±0.38	6.00±1.55	8.18±0.27
3rd wave to 4th or 3rd wave to ovulation.					6.14±0.44^{ab}	8.19±0.29^{a}	5.00±1.18^{b}	7.46±0.24
4th wave to ovulation.							7.00±0.00	
Ovulatory follicle.								
Maximum diameter (mm).	12.13±0.62^{ab}	12.65±0.76^{ab}	11.31±0.38^{a}	12.45±0.76^{ab}	11.28±0.34^{a}	12.80±0.22^{b}	12.80±1.08^{ab}	12.20±0.15
Growth rate (mm/day).	1.17±0.14	1.16±0.17	1.12±0.08	1.34±0.17	1.16±0.07	1.16±0.05	1.29±0.24	1.16±0.04

[1/] Least square means with different superscripts within a row different significantly (p<0.05)

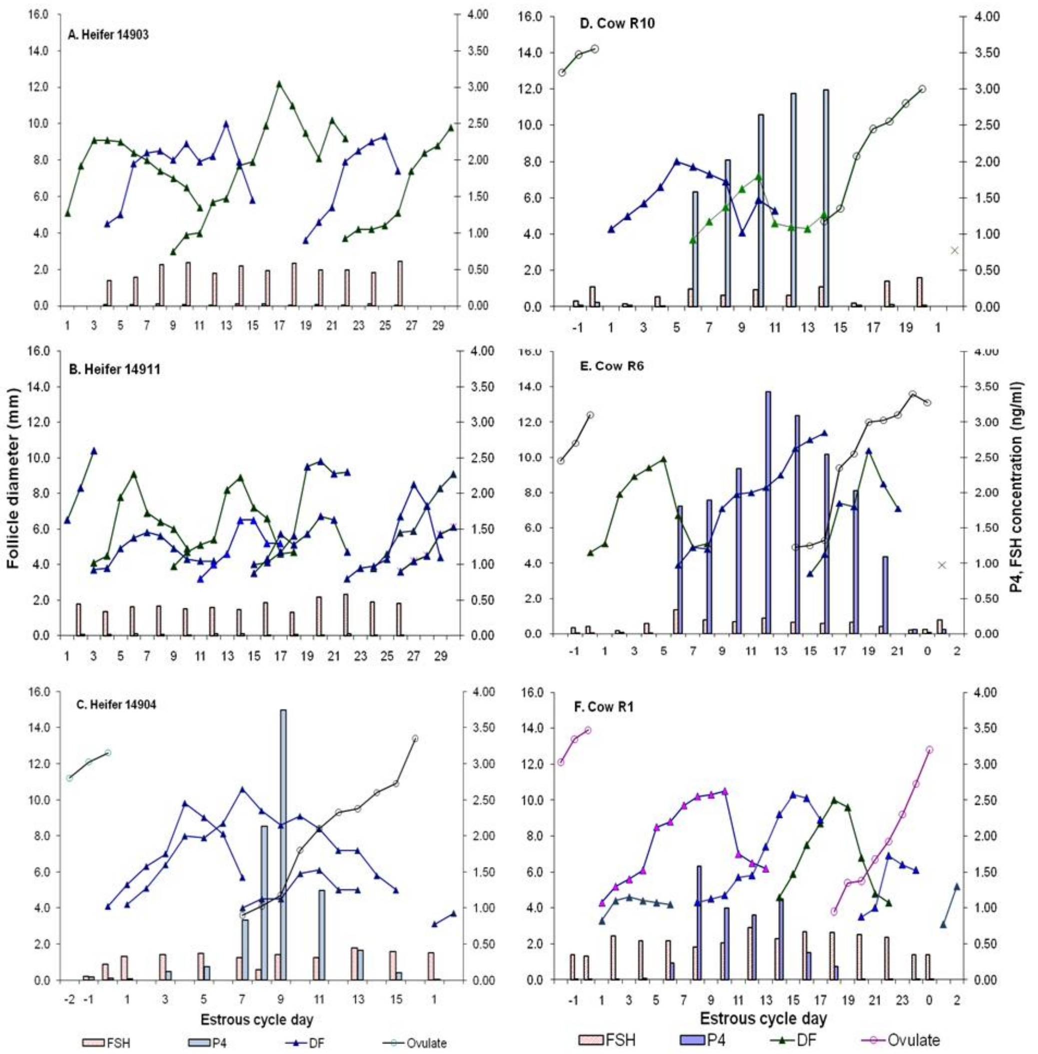

Figure 1 Patterns of follicular development and endocrine changes in TNC as individual.

Panels C, D, E and F; show patterns of development and regression of individual follicle, progesterone and FSH concentration in heifers and cows during complete estrous cycles. The estrous cycles with 2-follicular waves were the cycles of heifer number 14904 that are shown in panel C, cycles with 3-follicular waves were the cycle of cows number R10 and R6 that are shown in panel D and E respectively. The estrous cycle with 4 follicular waves were the cycles of cow number R1 that are shown in panel F. Day 0 indicates the day of ovulation. The estrous cycle of heifer number 14903 and 14911, showed in panel A and B respectively, haven't observed ovulation and there were excluded for data analysis.

FSH: Serum Follicle stimulating hormone concentration, P4: Serum progesterone concentration, DF: dominant follicle diameter, Ovulate: ovulated follicle diameter

The first and second waves in both heifers and cows of all patterns were not different. For the third wave of 3-wave pattern, it appeared earlier in heifers than in cow (12.57±0.56, 14.29±0.36 d; $p<0.05$). The number of follicles emerged in the first and second waves of heifers were higher than those of cows ($p<0.05$), while the number of the third wave of each group were not different. Lengths of estrous cycle of the 2-wave pattern of both heifers and cows were longer than those of the 1-wave pattern ($p<0.05$), but shorter than those of the 3-wave pattern. The longest estrous cycle appeared in the 4-wave pattern ($p<0.05$). The estrous cycle length was 9.80±0.23, 15.20±0.23, 20.70±0.23, and 25.00±0.23 days on average for 1-, 2-, 3-, and 4- follicular wave patterns, respectively. The interval between emergences of wave in the first and the second wave of each pattern was not different except for the third wave of the 4-follicular wave pattern, which was shorter than the others ($p<0.05$).

There were no differences among the patterns in the linear growth rate of dominant ovulatory follicle. However the first and second wave in both heifers and cows of all patterns were not different. The maximum diameters and estrous cycle length in the 3-wave pattern for heifers was smaller and shorter than in cows ($p<0.05$). These dominant follicles reached ovulation on day 9.33±0.79, 15.13±0.48, 18.57±0.51 in 1-wave, 2-wave and 3-wave heifers, and on day 10.50±0.96, 15.50±1.46, 21.63±0.51, 25.00±1.46 in 1-wave, 2-wave, 3-wave and 4-wave cows, respectively.

The sequential location (right versus left ovary) of the ovulatory follicles and corpus luteum did not follow any specific pattern during 3 consecutive cycles. The overall ovulatory follicle was from the right ovary in 62.00% (31/50) cycles and from the left ovary in 38.00% (19/50). The heifers had ovulation from the right ovary in 71.43% (15/21) of cycles and on the left ovary in 28.57% (6/21), cows had ovulation on the right ovary in 55.17% (16/29) cycles and on the left ovary in 44.83% (13/29).

The patterns of follicular dynamic, FSH and progesterone profile during estrous cycle from 3 heifers and 3 cows as individual were shown in Fig 1. Panel C shows the pattern of the 2-follicular waves development of heifers number 14904. The first wave emerged on day 1, reached the longest diameter on day 7 and gradually declined along with an emergence of a new wave. The preovulatory follicle was approximately 3.4 mm in diameter on day 7, grew slowly to day 9 and then rapidly during day 9 to 16, reached 13.4 mm in the diameter and got ovulation on day 16 of estrous cycle. Plasma FSH concentrations slightly increased on day 0 and fluctuated throughout the estrous cycle. Plasma progesterone concentrations increased slowly from day 3 to 5, reached the peak of approximate 3.74 ng/ml on day 9 and then declined to baseline by day 15.

Two cows had 3-wave pattern (Panel D and E) with waves emerging on day 1, 6 and 14. The dominant ovulated follicle started from a follicle approximately 4.8 mm in diameter on day 14, grew rapidly and reached its maximum size of 12.0 to 13.6 mm, then ovulated on day 20 to 23 of estrous cycle. Plasma FSH concentrations were similar to the 2-wave pattern in heifers. The levels of plasma P4 increased progressively from day 6 after ovulation. Peak P4 values approximately 2.99 to 3.43 ng/ml have been recorded about 12 to 14 days after estrous and then decreased to minimal values being observed just 3 to 5 days before ovulation.

Follicular wave of cow number R1 (Panel F) exhibited 4-wave pattern. Their estrous cycle had duration of 25 days. Each wave persisted from 7 to 12 days with the maximum diameter of dominant ovulated follicle 12.8 mm, and the life-span was 8 days. Plasma progesterone levels increased from day 6 to 18, reached the peak of approximately 1.58 ng/ml on day 8 and then declined to baseline by day 20. Circulating concentrations of FSH were 0.33-0.72 ng/ml through estrous cycle, and peaked at 0.72 ng/ml on day 12 after ovulation.

Two heifers (Panel A and B) exhibited the follicular wave pattern, but neither estrous nor ovulation was detected. The concentration of plasma progesterone was at its nadir (0.01-0.08 ng/ml) along with higher FSH levels (0.34-0.62 ng/ml) being observed during the estrus cycle. The greater numbers of small follicles emergence corresponded well to the higher concentrations of FSH. Two of three heifers showed this pattern shown in panel A and B.

Discussion

According to this work, TNC have 1, 2, 3 and 4 waves of follicular growth during the estrous cycle. The wave of follicular growth begins with the synchronous growth of a cohort of follicles. Each wave was characterized by the development 1 large follicle and variable number of smaller follicles. In a single wave pattern, the dominant follicle was observed to steady growth and became ovulatory. The result of this study was found in 3 estrous cycles of heifers (14.29%) and in 2 estrous cycles of cows (6.90%). These phenomena seem to be related to the deficiency of P4 released, along with the enhancement of E2 content in the follicular fluid of dominant follicle. The positive relationship between the size of dominant follicle and E2 secretion may contribute to the onset of luteolysis, as a result of ovulation. Short estrous cycles with one follicular wave has been reported around the time of puberty (Evans et al., 1994) and postpartum after first ovulation (Savio et al., 1990). The two-wave pattern has a second-wave dominant follicle that goes on to ovulate. The first-wave dominant follicle becomes atretic at mid-cycle, and the second-wave begins afterward. In the 3-wave pattern, the second-wave dominant follicle becomes atretic and the third follicular wave gives rise to the ovulatory follicle. The four-wave pattern has a fourth-wave dominant follicle that goes on to ovulate. The majority of cows (82.76%) exhibited a 3-wave pattern, whereas the majority of heifers (38.10%; 47.62%) exhibited a 2- and 3-wave pattern. These patterns of follicular growth are similar to those reported on European breeds, in which 2 and 3 follicular waves predominate (Kanitz, 2003; Sartori et al., 2004; Adams et al., 2008). For Zebu cattle (*B. indicus*), Rhodes et al. (1995) reported that 26.5%, 66.7%, and 6.8% of estrous cycles had either 2, 3, or 4 waves of ovarian follicular developer during the estrous cycle, respectively. Other studies in Nelore heifers (Sartorelli et al., 2005) Gir cows (Gambini et al., 1998), and Brahman cows (Zeitoun et al., 1996) reported a predominance of 3-follicular waves during estrous cycle. Moreover, Figueiredo et al. (1997) reported on 2- to 3-follicular waves in Nelore breed, with predominance of cows having 2 (83.3%) and heifers 3 (64.7%) waves of ovarian follicular development, which is different from this study, which found that the majority of cows (82.76%) had 3-, whereas heifers (38.10%; 47.62%) had 2- and 3- follicular waves during the estrous cycle.

During the onset of each follicular wave, approximately 3.50-8.00 small antral follicles were detected in TNC. However, the number of follicles emerged per wave in heifers were greater than in cows, but it seem to be less than others reported. *B. taurus* cattle were recorded approximately 24 follicles (Ginther et al., 1996). However, in *B. indicus* cattle, a greater numbers of small follicles have been recorded, 50 follicles in Nelore heifers (Buratini et al., 2000), 39 follicles in Brahaman and in 33 follicles in Senepol (Alvarez et al., 2000), 33.4 follicles in Nelore and 25.4 follicle in Gir breed (Carvalho et al., 2008), respectively.

The lengths of estrous cycle in 3-wave patterns of heifers are shorter than cows, correlating to the emergence of the third wave, length of days between emergence of the third waves to ovulation, and the maximum diameter of the ovulatory follicle. The circulating concentration of progesterone was less in heifers than in cows. High concentrations of progesterone may block further preovulatory LH surges and ovulation, cause delayed ovulation and greater size of ovulatory follicle more in heifers than in cows. The estrous cycle length was 15.20±0.23 and 20.70±0.23 days on average for 2- and 3-wave pattern, which is shorter than that reported in Nelore breed (Figueiredo et al., 1997), Brahman breed (Rhodes et al., 1995) and European breeds (Ginther et al., 1989[a]; Bo et al., 2003). The means length of the estrous cycle positively correlated to the number of waves per estrous cycle.

The means of maximum diameter of dominant ovulatory follicles and their growth rate were 12.20±0.15 mm and 1.16±0.04 mm/day, respectively, which are similar to Nelore breed (B. indicus) (Figueiredo et al., 1997; Sartoerlli et al., 2005; Carvalho et al., 2008) but smaller than in *B. taurus* (Ginther et al., 1989b; Kastelic et al., 1990; Bo et al., 2003). Therefore, lower serum E2 levels in *B. indicus* than in *B. taurus* may lead to smaller preovulatory LH-surge and a different timing to ovulation.

Two heifers exhibited the follicular waves developed, but neither estrous nor ovulation was detected. Likewise Yavas and Walton (2000) reported on the postpartum cow that the dominant follicles of these waves failed to ovulate, due to a failure to undergo terminal maturation. As a result, anovulatory dominant follicles are smaller than the ovulatory follicles in cyclic cows. Failure of dominant follicles to undergo terminal maturation may be due to absence of appropriate LH pulses, a prerequisite for follicular terminal maturation prior to ovulation. Absence of LH pulses is due to continued sensitivity of the hypothalamic GnRH pulse-generator to the negative feedback effect of ovarian estradiol-17β, which results in absence of GnRH pulses. Undetectable circulating progesterone concentrations indicate absence of an ovulation and of a functional CL (Rawlings et al., 1980).

One heifer and 3 cows exhibited 2-, 3-, or 4-follicular wave patterns during estrous cycles. The dominant follicle was observed to steady growth and became ovulatory. Plasma FSH concentrations slightly increased on days 0-1, 6-7, 13-14, and 18 of the estrous cycle, the wave emerged on the next day. Plasma progesterone concentrations slightly increased on days 5-6 and remain high to the day 3-5 before ovulations. Similarly, Adams et al. (1992) and Suntherland et al. (1994) reported on dairy cattle that the emergence of successive follicular waves during the estrous cycle was associated with increases in circulating concentrations of FSH, which preceded of emergence of the wave within 1 day (Adams et al., 1992). In addition, luteal regression occurred after emergence of the ovulatory wave, and the next wave did not emerge until near the day of ovulation at the onset of the subsequent interovulatory interval (Ginther et al., 1989[c]).

A different status of the endocrinological events leads to differences in ovarian follicular dynamics between heifers and cows of TNC. In this study we used heifers averaged 2.57±0.07 years of age and 182.29±4.46 kg BW, which just passed puberty, therefore the endocrinological status might be different. According to Adams et al. (2008), the reproductive aging in cattle is characterized by an elevation in plasma FSH concentration, a decrease in the number of follicles recruited into each wave, a lower superovulation response, and a lower oocytes fertilization rate. The remaining oocytes in the ovary of cows are less than of heifers. Likewise, reported by Orihuela (2000), the age and physiological status affect the estrous cycle and fertility.

In conclusion, this study demonstrated that Thai Native Cattle was characterized by 1-, 2-, 3- or 4-follicular waves during estrous cycle. The viable dominant follicle present at the time of luteolysis became the ovulatory follicle. Most estrous cycles, consisting of 2 or 3 ovarian follicular waves, revealed the predominance of 3-follicular waves in cows and 2- or 3-follicular waves in heifers. The duration of estrous cycle correlated to number of ovarian follicular wave. The waves of follicular development were consistent with the increase in amount of FSH. When the level of P4 decreased, the largest follicle usually underwent ovulation.

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