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# Follicle Dynamics of Aceh Cattle During Estrous Cycle

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**Abstract:** Follicular dynamics studies can be used as main reference in reproduction technology implementation in Aceh cattle. The aim of this research is to observe the dynamic of follicle growth on aceh cattle. Ten Aceh cattle were used with the criteria of 5-8 years old, weighing 150-250 kgs, having at least 2 regular cycles and having good body score index value. Follicular dynamic observation during estrus cycle was done by using ultrasonography. The follicles were classified into three categories: small follicles with diameter of 0.3-0.5 cm (Class I), medium follicle with diameter of 0.6-0.9 cm (Class II) and big follicle with diameter of ≥ 1 cm (Class III). Qualitative and quantitative data of follicular dynamic were presented descriptively. Three follicle waves appeared during estrus cycle with the average score for class I, II and III were 10.54±2.74; 5.68±2.18; and 2.42±1.10, respectively. The average estrous cycle length for Aceh cattle was 17.8±0.4 days. The onset of ovulation after synchronization was 3.8±0.8 days. In conclusion, Aceh cattle have a total of three follicular growth waves.

**Key words:** Follicle Wave · Aceh Cattle · Ultrasonography

## INTRODUCTION

Aceh cattle is one of four local beef cattle origin from Indonesia (Aceh, Pesisir, Madura and Bali cattle). Sumban-Ongole and Javan-Ongole cattle (Ongole crossbred, or PO, Ongole crossbred) are also considered as Indonesian local cattle breed [1]. The previous study on the phenotypically genetic characteristic identification and DNA analysis of aceh cattle revealed that it is closer genetically to *Bosindicus* [2]. In addition, its phylogenetic tree showed that it is within one cluster of Ongole crossbreed and forms phylogeny branch with Pesisir and Madura cattle [2, 3]. In order to improve Aceh cattle productivity through implementation of reproduction technology, the information on follicle dynamics in Aceh cattle is needed. Follicle dynamics during estrus cycle in *B. indicus* is similar to *Bostaurus* [4] however Aceh cattle follicle dynamics has never been reported yet.

Basic knowledge of follicular dynamic is very beneficial to monitor cattle fertility [5], to be the basis of reproductive manipulation improvement [6] and to clarify incidences that take part in ovulation and estrus synchronization more thoroughly, in order to heighten the response of superovulation. The benefit of the research on ovaries function itself has given great contribution in understanding ovaries distinction based on follicular dynamics [7]. Several studies on water buffalo showed low efficiency during artificial insemination and embryo transfer [8, 9]. Follicular dynamic knowledge is the base of improving fertility in estrus synchronization and increase superovulation response [10]. Some factors have been known having negative effects on ovaries function, such as nutrition, weather, physiology, health status and environmental pollutions [11].

Follicular dynamics in cattle describe as the form of follicle growth waves. A follicle growth wave consists of a simultaneous growth of a group of follicle, in which one of them will become the dominant follicle and reach the largest size and suppress the growth of other smaller follicle [12]. Several researches reported variating number of follicle waves. In Brahman heifers, there are two, three and even four follicle waves, each sizing 26.5, 66.7 and 6.8% [13]. *Bosindicus* cattle with four follicle growth waves are only observed in 7% of heifers and 27% of mature cattle [14].

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## MATERIALS AND METHODS

About 10 clinically healthy female Aceh cattle with body condition score of 3-4 in the scale of 5, aged 5-8 years, weight approximately 150-250 kg, have had two regular cycle, not in gestation and having corpus luteum (CL) were used. The cattle were kept in open pen with divider and provided with feed and water container. The cattle were fed forage two times a day and concentrate one time a day, water was provided ad libitum.

**Research Procedure:** On the first day of research (H1) estrus synchronization was conducted to lyse the CL and at the same time the estrus was evaluated. Estrus synchronization method was done by prostaglandin induction (Lutalyse™, Pharmacia & Upjohn Company, Pfizer Inc., 5 mg/ml) intramuscularly during luteal phase. Estrus behaviour observation was conducted in the morning and late afternoon visually, starting from H2 until the time estrus symptom disappear (H2-H4). Finally on H5, estrus observation was done once in the morning until estrus behavior occurred again.

**Ultrasonography:** The observation of follicular dynamics was conducted by using ultrasonography (USG) technique (MINDRAY DP3300VET, Shenzhen Mindray Bio-Medical Electronic Co. Ltd., China) with electronic linear endorectal transducers 5 MHz (50L60EAV, Shenzhen Mindray Bio-Medical Electronic Co. Ltd., China). Cattle were placed in restraining box and USG equipment was placed in relatively safe while easy to operate by the operator. Feces were taken out of cattle's rectum and then proceed by manual exploration of reproduction tract topography before USG was conducted. The surface of the transducer was covered by lubricants while wrapped in thin plastic to avoid irritating rectal mucosa and to obtain good USG imaging. Transducer was inserted into cattle's rectum and directed cranially along rectum ventral area following the reproduction tract [15]. The parameters observed were CL diameter, numbers and diameters of follicles on cattle's ovaries as measured by USG instrument internal caliper in the form of the gap between two end points of the longest axis in millimeter (mm). The follicles were classified into small follicle with 3-5 mm in diameter (Class I), medium follicle with 6-9 mm (Class II) and large follicle with = 10 mm in diameter (Class III) [16].

**Data Analysis:** The qualitative and quantitative data of follicular dynamics were presented descriptively using Microsoft Office Excel 2007.

## RESULTS

Observations towards follicular dynamics during estrus cycles showed that there were 3 follicular waves occurred. The average value of Class I; II; and III were10.54±2.74 mm; 5.68±2.18 mm; and 2.42 ± 1.10 mm, respectively.

The observation result of follicle classification in Aceh cattle during synchronization until estrus day (H0) showed decrease number of small follicle from H3 (13.30±2.00)to H2 (7.70±1.89), then increase in H-1 (9.40±1.96) to H0 (9.90±1.20). Increased number of medium size follicle already observed from H-4 (3.10±1.29) to H-2 (5.20±2.62) whereas for large follicle from H-3 (3.40±1.17) to H-1(4.60±1.78).

Based on observation result from H0 (Estrus) to H-18, in the first wave, the number of small follicle increased in H1 (7.70±2.41) and continued to increaseto H3 (10.80±4.32) then decreased on H4 (10.30±2.58) and rose up again and reached its peak on H5 (14.50±1.78), then decreased again on H6 (10.80±1.81). The number of medium follicle increased from H0 (3.20±1.87) and reached its peak on H3 (7.00±2.11) then went down again until H6 (4.40±1.78). The number of large follicle decreased since H1 (3.70±1.43) until H6 (1.70±1.25) as shown on Figure 1.

On the second wave, the number of small follicle decrease from H7 (13.10±4.23) to H8 (7.40±1.90) then increased until its peak on H11 (12.10±3.00). Medium follicle numbers increased since H7 (6.30±2.58) and then decreased until H9 (5.50±2.12) then rose up again to its peak on H11 (6.90±1.97). Large follicle increased since H8 (1.50±0.85) and reached its peak on H10 (1.60±0.70) and then went down until H11 (0.80±0.63).

On the third wave, there was a decrease in small follicle on H12 (10.20±2.82) and it increased on H14 (12.10±4.86) then went down again on H15 (9.00±3.83) and rose up again on H17 (11.50±2.88). On the other hand, there was an increase in medium follicle starting from H12 (7.00±2.72) which reached its peak on H13 (7.70±3.27), decrease until H14 (5.00±1.94) followed by an increase to its peak on H17 (6.60±2.46). Large follicle continued to increase from H12 (1.30±0.95) up to H18 (2.00±0.94).

Largest follicle growth in the first wave (1$^{st}$DT) had started from H0 (5.50±1.29 mm) and reached its peak between H5 (10.00±1.83 mm) then underwent atresia since H6 (9.25±1.50 mm) until the end of the cycle. The second largest follicle (1$^{st}$ SF) increased from H0 (4.50±1.29 mm) and reached its peak on H5 (8.25±2.06 mm) then atresia until H7 (6.27±0.50 mm) with deviation day on H5 (8.25±2.06 mm).

Table 1: Characteristics of follicular dynamics of Aceh cattle

| | Follicular waves | | |
|---|---|---|---|
| Follicular parameters | I | II | III |
| Wave onset (day) | H0 | H8 | H14 |
| Wave duration (day) | 8 | 6 | 4 |
| Max DF diameter (mm) | 10, 00±1, 83 | 8, 75±0, 50 | 13, 75±1, 71 |
| Day of max diameter (day) | H5 | H12 | H18 |
| Atresia onset (day) | H6 | H13 | H18 |
| Largest max SF Diameter (mm) | 8, 25±2, 06 | 7, 75±1, 26 | 11, 00±1, 41 |

Keys:DF=Dominant follicle, Max.=maximum, SF=subordinate follicle

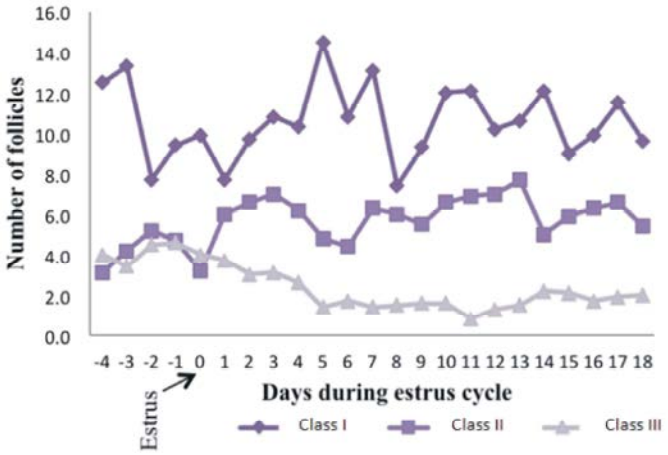


Fig. 1: Follicle size classification in Aceh cattle

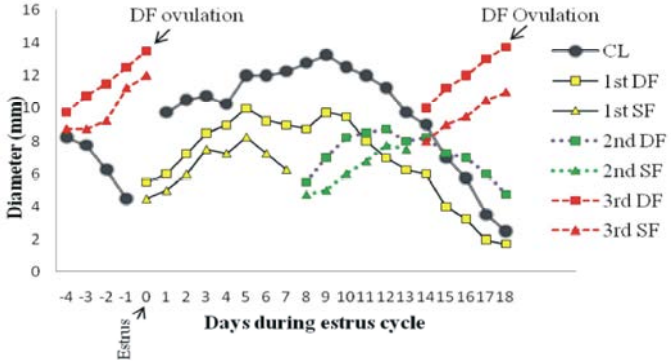


Fig. 2: The cycle of follicular dynamics and CL regression in Aceh cattle

On the second wave, the largest follicles (2nd DF) increased from H8 (5.50±1.73 mm) and reached its peak on H12 (8.75±0.50 mm) and then turned atresia from H13 (8.00±0.82 mm) until the end of the cycle. The second largest follicle (2nd SF) increased on H8 (4.75±0.96 mm) and reached its peak on H12 (7.75±1.26 mm) then atresia, with deviation day also on H12 (7.75±1.26 mm).

On the third wave, a very fast growth of largest follicle (3rd DF) was clearly displayed from H14 (10.00±1.63 mm) until it turned into dominant follicle ready for ovulation on H18 (13.75±1.71 mm). The second largest follicle (3rd SF) increased from H14 (8.00±0.82 mm) and reached its peak on H18 (11.00±1.41 mm) with deviation day also on H18 (111.00±1.41 mm) as presented on Figure 2.

## DISCUSSION

The decreasing number of follicle during first wave was caused by a number of small follicles turned atresia. It was in line with Johnson and Everit [17] that high concentration of progesterone produced by CL during luteal phase naturally inhibits dominant follicle ovulation, which caused the follicle to undergo atresia.

On the third wave, large follicle kept on increasing from H12 until H18. Thus, indicating the dominance of progesterone hormone in luteal phase resulted in a decrease of follicle growth rate due to the hormone has negative feedback effect on gonadotropin hormone secretion [18].

Based on the observation of follicle classification, it was found that Aceh cattle follicle wave happened 3 times in 1 estrus cycle. Although in this research steroid hormone concentration was not measured, but the result of the research induced an assumption that progesterone level in blood plasma reflects CL function status during estrus cycle [19]. Based on follicle morphological observation during the research found that the follicle growth is a dynamic process controlled by local and systemic process [20].

Several research reports on follicular dynamics on estrus cycle, for example Ratih cattle (*Bosindicus*) have 2 (78.57%) and 3 (21.42%) waves in their estrus cycles [21] and PFH cattle have 2 follicle waves in their estrus cycle [19].

Adam [22] reported that >95% of cattle estrus cycle consists of two to three follicle cycle. Moreover, generally cattle with 2 follicle cycles have 19-20 days of estrus duration, while cattle with 3 follicle waves have 22-23 days. However, Aceh cattle showed different pattern that is 18 days of estrus cycle duration and 3 follicle waves. The number of follicle waves occurred is similar to FH cattle [23] Japanese black cows [24] and PO cattle [15].

Normal estrus cycle consists of 2 or 3 follicle waves, which comprised of period of emergence, growth, deviation, dominance, atresia, or ovulation. Each wave involves growth of large, medium and small follicle. Deviation period comprised of reduction or termination of second largest follicle (subordinate = SF) and the largest follicle becomes the dominant and its size increased. This deviation period is a selection mechanism that control a number of ovulated follicles into a single follicle [25].

The increase of follicle diameter in Aceh cattle one day before estrus synchronization using PGF2$\alpha$ showed that there was growth in dominant follicle started from H-4 (9.75±0.96 mm) until estrus H1 (12.50±1.91 mm) and it became a follicle ready for ovulation H0 (13.50±1.91 mm) and followed by the second largest follicle that began to undergo atresia on H0 (12.00±1.63 mm). Estrus occurs 1-3 days after synchronization. This result is similar to Melia [15] who reported estrus arise 2-3 days after therapy. Cattles synchronized by PGF2$\alpha$ will display estrus simultaneously on relatively the same days [23, 26, 27].

The growth of largest follicle in first wave (1$^{st}$ DF) has started since H0 (5.50±1.29 mm) and reached its peak between H5 (10.00±1.83 mm) and then start to become atretic on H6 (9.25±1.50 mm) until the end of the cycle. The second largest follicle (1$^{st}$ SF) increased from H0 (4.50±1.29 mm) and reached its peak on H5 (8.25±2.96 mm) then turned atresia on H7 (6.27±0.50 mm) with deviation day on H5 (8.25±2.06 mm). During this wave, the growth and the follicle atresia process is slower compared to other waves, presumably because of the presence of inhibition factor from progesterone hormone to gonadotropin hormone secretion [20].

In the second wave, the largest follicle (2$^{nd}$ DF) increased from H8 (5.50±1.73 mm) and reached its peak on H12 (8.75±0.50 mm) and began to be atresia on H13 (8.00±0.82 mm) through the end of the cycle. The second largest follicle (2$^{nd}$ SF) increased from H8 (4.75±0.96 mm) and reached its peak on H12 (7.75±1.26 mm) and turned atresia, with deviation day also on H12 (7.75±1.26 mm). The cause of small follicle atresia was the role of CL and dominant follicle. It was proven that when dominant follicle in ovaries naturally disappear then it followed by growth of the next new small follicles [28]. It is in line with the statement of Adams [22] that the presence of dominant follicle will impede the occurrence of the next follicle wave.

In the third wave, there was an apparent rapid growth of the largest follicle (3$^{rd}$ DF) and it was clearly shown on H14 (10.00±1.63 mm) until it reaches readily ovulated dominant follicle on H18 (13.75±1.71 mm). The second largest follicle (3$^{rd}$ SF) increased from H14 (8.00±0.82 mm) and reached its peak on H18 (11.00±1.41 mm) with deviation day also on H18 (11.00±1.41 mm). This indicated the dominant follicle inhibits further growth of small follicles on both ovaries, because intraovary regulations occur during the next development of follicle [29]. Ovulation occurrence within the wave happened because CL regression caused progesterone level to go down which resulted in the diminishing negative feedback inhibition on gonadotropin. As stated by Senger [26], ovulation cannot happen in a condition where progesterone level is dominant.

The results of other researches showed that there are no difference in diameter of large follicle (Dominant follicle=DF) approaching estrus, whether after synchronization or naturally, each values are 13.50±1.91 mm and 13.75±1.71 mm respectively. It has been reported by Senger [26] in FH cattle with 1.43±0.06 cm and 1.47±0.06 cm each and by Melia [15] on PO cattle with 1.43±0.06 cm and 1.47 ±0.06 cm each. This indicates

that PGF2α as luteolytic hormones for estrus synchronization does not change the size of successfully ovulated follicle.

The length of estrus cycle in Aceh cattle was found to be a little short, with the average of estrus cycle in all observed individuals being 18 days (17.8±0.4 day) with successfully ovulated follicle's diameter ranging between 11-17 (13.75±2.50 mm). According to Perez *et al*. [23] average ovulated follicle diameter in FH cattle which has repeated mating is 1.78 ±0.36 cm. Moreover, Perez *et al*. [23] also stated that FH cattle with 2 follicle waves has average follicle diameter approaching ovulation smaller than 1.45±0.2 cm. [15] reported that PO cattle with 3 follicle waves has average follicle diameter approaching ovulation of 1.47±0.06 cm. The average follicle diameter approaching ovulation in Aceh cattle was smaller than PO cattle and FH cattle with 3 follicle waves.

According to Perez *et al*. [23], cattle with 3 follicle waves has average 1st DF (Anovulatory) diameter bigger than other follicle, but smaller than cattle with 2 follicle waves. The claim is different with the result found in this research, Aceh cattle with 3 follicle waves have average 1st DF diameter smaller than its 2nd DF average diameter. The result of Aceh cattle is similar to what was found on PO cattle as reported by Melia [15].

Even though in our study, there were 3 waves found, but as a comparison, more than 3 waves have been observed by many cattle researchers around Europe [18, 30] and on *Bosindicus* cattle [21, 31]. In this study no 4 or more waves were found like in Gir cattle [31] which have a small proportion of cattle showing 4 waves or more during estrus cycle.

## CONCLUSION

Follicular dynamics in Aceh cattle consists of 3 follicle waves. The diameter of large follicle approaching estrus in cattle that has been treated for estrus synchronization is 13.50±1.91 mm. The duration of estrus cycle in Aceh cattle is 18 days (17.8±0.4 days) with the length of ovulation after synchronization is 4 days (3.8±0.8 days).

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