

Determination of Follicular Wave Development in Oestrus Synchronised Beef Cows

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Abstract

A study was conducted to determine the follicular wave development in terms of dominant follicles size, number of follicular waves and follicles existing at pre- and post-ovulation stage, and length of oestrous cycle in oestrus synchronised beef cows. Thirty cows consisting of 3 breedtypes: KK (n=10), Brakmas (n=10), and Charoke (n=10), were inserted with controlled internal drug releasing (CIDR) device containing 1.38 g progesterone for 7 days and given intramuscular injection of a synthetic prostaglandin analogue of 25 mg prostaglandin two days prior to CIDR withdrawal. It was shown of BK, CK and KK cows had 45%, 35% and 20% 3-follicular waves pattern, respectively. BK cows had higher number of cows having 3-follicular waves ($P < 0.05$) compared with KK and KK had a higher ($P < 0.05$) percentage of cow having 2-follicular waves pattern, compared with BK and CK cows. In pre ovulation stage, the number of follicles at emergence was significantly higher ($P < 0.05$) in KK (4.4 ± 0.6) followed by CK (2.6 ± 0.4), and BK (3.2 ± 0.4). However there was no significant difference ($P > 0.05$) observed in terms of mean diameter of follicle at emergence stage in the three breedtypes. The mean diameter of ovulatory follicular development was significantly larger ($P < 0.05$) in BK (14.2 ± 0.1 mm), followed with the other two breedtypes, CK (12.2 ± 0.1 mm) and KK (11.8 ± 0.5 mm), and was also significantly faster to become dominant ($P < 0.01$) compared to the other two breedtypes. In post ovulation stage or at first follicular wave development, the number of follicles at emergence of KK, BK and CK was 3.8 ± 0.9 , 3.2 ± 0.5 and 2.7 ± 0.7 , respectively. The first dominant follicle reached maximum diameter of 11.4 ± 0.08 mm; 13.1 ± 0.08 mm and 13.1 ± 0.07 mm in KK, BK and CK, respectively with no significant difference detected among the breeds studied ($P > 0.05$). Similarly, there was also no significant difference ($P > 0.05$) of the dominant follicle diameter was observed in the second and third (ovulatory follicle) follicular waves patterns among the three breeds of cows studied. The mean diameter of ovulatory follicle in KK was 10.2 ± 0.1 mm, BK 11.6 ± 0.10 mm and CK 10.7 ± 0.1 mm, in KK, BK and KK cows, respectively. There was no significant difference ($P > 0.05$) found among the three breeds of cows studied for mean length of oestrous cycle. In conclusion, the results of the present study indicated there was no difference on the follicular wave development in terms of dominant follicles size in pre-ovulation, first, second and third follicular wave development; follicles existing at post-ovulation stage, and length of oestrous cycle in KK, BK and CK cows. However, in terms of the number of follicular waves, BK and CK cows had a higher proportion of 3- and 2-follicular waves

development than KK cows. and the number of follicles existing at pre-ovulation stage in KK and BK cows were predominantly higher CK cows.

Keywords: Beef cows, follicular development, oestrus synchronization, Kedah-Kelantan cows, CIDR.

Introduction

Most studies on follicular dynamics have been conducted on the *Bos taurus* or the European breeds of cattle (Savio *et al.*, 1988; Sirois and Fortune, 1988; Ginther *et al.*, 1989), while reports on Zebu cattle (*Bos indicus*) are rather limited to the studies in Brazil (Figueiredo *et al.*, 1997; Rhodes *et al.*, 1995; Pinheiro *et al.*, 1998). These studies used different protocols for oestrus synchronization. The same synchronization protocol has been proven to react differently, depending on the type of cows, age, breed and type of hormones used for ovarian super stimulation. The addition of a controlled internal drug release (CIDR) device into the Ovsynch protocol, which combined gonadotropin releasing hormone (GnRH) and prostaglandin (PGF), has improved the rate of conception following timed-artificial insemination (TAI) in postpartum Japanese Black beef cows (Kawate *et al.*, 2004). Fertility to TAI using combination of CIDR, PGF and GnRH, has been used in beef cattle (Colazo *et al.*, 2003), and has been proven to increase plasma progesterone (P₄) concentration (Echternkamp and Thallman, 2011), proportion of cows in oestrus, and pregnancy rate using TAI by 10.3 percentage units (Lamb *et al.*, 2010). These studies were conducted to improve the synchronization protocols of oestrus and ovarian super stimulation in order to adopt TAI (Fricke *et al.*, 1998; Lamb *et al.*, 2010; Mallory *et al.*, 2011; Echternkamp and Thallman, 2011).

Synchronizing the oestrous cycle involves manipulation of the ovarian

activity so that the time of ovulation can easily be predicted. Using this method, over 90% of cattle can be induced to enter oestrus within 24 hours (Cavaliere *et al.*, 2003), and a greater proportion of the anoestrus prepubertal animals was induced to ovulate following the treatment (Fike *et al.*, 1997). Meanwhile, the administration of GnRH during the oestrous cycle resulted in LH (Lutenising hormone) release (Chenault, 1990), causing ovulation or luteinization of dominant follicles in the ovary, and synchronizing recruitment of a new follicular wave (Thatcher, 1989) and follicular development (Wolfenson, 1994).

In cattle and sheep, administering PGF during luteal phase for oestrous synchronization reduces the P₄ level and length of the oestrous cycle, and thus, enhancing the formation of follicular growth and the occurrence of ovulation earlier than its expected time (Pursely, 1997). The development of oestrus synchronization method and TAI could best be preceded by understanding the physiology of bovine oestrous cycle. Therefore, the objective of the study was to determine the differences of follicular wave development in terms of dominant follicles size, number of follicular waves and follicles existing at pre- and post-ovulation stage, and length of oestrous cycle in oestrus synchronised beef cattle.

Materials and Methods

Animal Management and Treatment

The study was conducted at MARDI Research Station, Kluang, Johor. Thirty cows of three local zebu cattle (*Bos indicus*)

breedtypes were Kedah-Kelantan (KK, n=10), Brakmas (BK, n=10), and Charoke (CK, n=10) were used in this study. The cows ranged from the first to third parity and were 2 to 5 years of age. The body weight ranged from 250 to 350 kg and the cows had an average body condition score of 4 (1=emaciated, 4=moderate, 8=overweight). The KK is a local Zebu breed, whereas BK was developed from crossing Brahman bulls and KK cows, while CK was developed by crossing Charolais sires to KK cows. Cows were managed in a semi-intensive system, whereby cows were released for grazing in the morning and maintained indoor for the rest of the day in individual pens and fed with cattle pellet and had free excess to water. The feed containing 15.9 % Crude Protein and 17.6 MJ calculated Gross Energy (GE) was offered to cows based on the maintenance requirement of beef cows (ARC, 1980) at a rate of 1 kg per 100 bodyweight per day in

addition to the estimated intake of feed from grasses. The study was conducted for two consecutive oestrous cycles.

Synchronization of oestrus

Cows received CIDR (Pharmacia & Upjohn, Australia) containing 1.38 g P₄ for 7 days. Intramuscular injection of a synthetic PGF analogue of 25 mg prostaglandin (PGF; Estrumate®, Schering – Plough Animal Health, Australia) was given on Day 5 after the CIDR insertion. The cows were synchronized on two consecutive occasions, 14 days apart; the second CIDR was inserted during the induced luteal phase as a modification of the method described by Saumande and Humblot (2005). Cows were observed for signs of oestrus after CIDR withdrawal (Day 8) in the second part of synchronization (Figure 1).

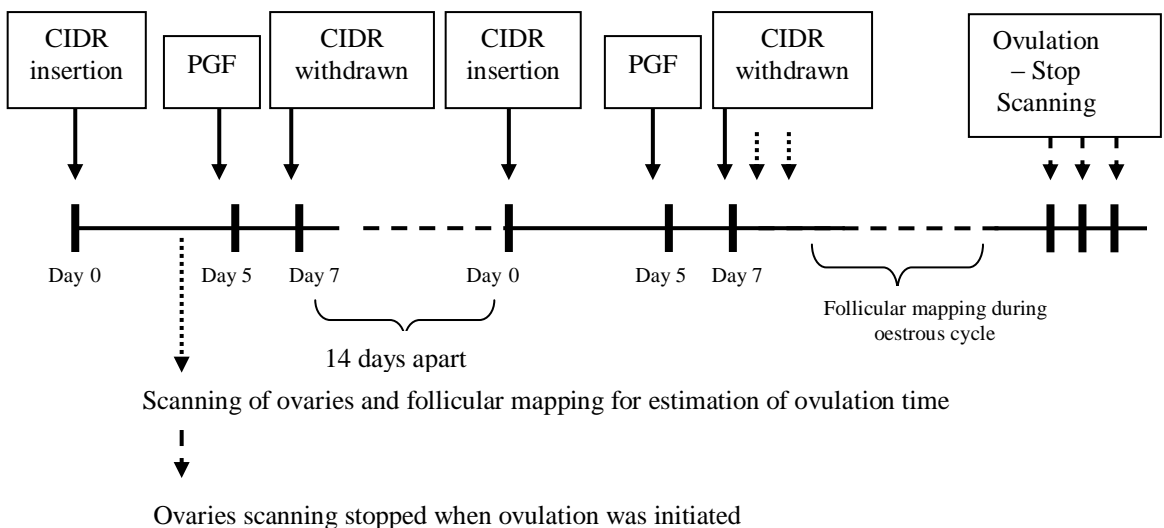


Figure 1: Experimental protocol for oestrus synchronization, scanning of ovaries and follicular mapping

Ovarian ultrasonography and follicular mapping

Prior to ultrasonography, the rectum of the cows were emptied of feces and the position of ovaries was localized and identified by rectal palpation. The ovaries were located either slightly underneath the uterus or to the side of the uterus at a variable distance.

Both ovaries were scanned using a 7.5 MHz linear array transrectal transducer that was attached to a portable ultrasound device (Aloka®, SSD-500) to visualize the image of ovaries onto a monitor. Scanning was carried out from six hours at the end of oestrus and was repeated every six hours until ovulation occurred. The ovaries were further scanned every two days for the determination of follicular wave pattern development.

The diameter of the follicle was obtained by freezing the image, followed by its measurement using electronic calipers at the interface of the follicular wall with an ovarian stroma. The number of follicles on both ovaries greater than or equal to 4 mm in diameter was counted, measured and mapped. For the non-spherical shape, the largest and the smallest widths were measured, while the average width was also recorded. Meanwhile, the size and relative dimension of follicles and corpus luteum (CL) were sketched on a follicle map.

The detection of a follicle diameter of ≥ 4 mm was identified as a dominant follicle, and that day was taken as the first day of a wave, following the method of Ginther *et al.* (1989). A dominant follicle was the largest follicle which occupied the most space of ovarian stroma during its growth phase. Ovulation was confirmed by the disappearance of large antral dominant follicles of size greater than 10 mm in diameter, as evident from the formation of a CL in the same location on the ovary. Thus,

the day of ovulation which occurred at the beginning of an interovulatory interval, was designated as Day 0.

Blood collection and progesterone assays

During each ultrasound examination, 10 ml of blood was sampled from all cows through jugular venipuncture into plain tubes (Vacutainer®, Becton Dickinson Limited, England) using a hypodermic disposable needle of 1.2 x 38 millimeter (mm) size to determine P₄ concentration. Blood was kept at room temperature for an hour and stored at 4°C for 24 hours. Serum was obtained from all the samples through centrifugation at 700g for 20 minutes. This was followed by decanting and keeping the serum in small bottles before it was frozen at -20°C. Later, the serum was transported on dry ice for radioimmunoassay (RIA) of P₄ performed using the developed kit assembled by the Animal Production Unit (Diagnostic Products Corporation, Los Angeles, CA 90045). The sensitivity of the assay was 0.02 nanogram millilitre⁻¹ (ng/mL). The inter- and intra-assay coefficients of variation for progesterone were 6.8% and 12.6%, respectively.

Statistical analyses

Statistical analysis for detection of breed differences in follicular dynamics, concentration, the growth and regression of dominant follicles, and the emergence of the new follicular wave were analyzed using analysis of variance (ANOVA). A proportion of the cows with specific number of waves of follicular development were tested using chi square analysis. Meanwhile, data on P₄ concentration were also analyzed as repeated measures. All

analyses were conducted using SPSS version 17.0.

Results and Discussion

The results of the follicular wave development characteristic, after CIDR withdrawal pre- and post-ovulation of KK, BK and CK cows, are presented in Tables 1

to 6. In Table 1, it was shown 45%, 35% and 20% of BK, CK and KK breeds respectively having 3-follicular waves pattern. BK appeared to have higher number of cows having 3-follicular waves pattern compared to CK ($p>0.5$), and KK ($p<0.05$). However KK had a higher number of cows having 2-follicular waves, followed by CK ($p>0.5$), and BK ($p<0.05$).

Table 1. Mean follicular wave development in Kedah-Kelantan (KK), Brakmas (BK) and Charoke (CK) cattle.

| Breeds | No. of waves | 2-follicular waves ¹ | 3-follicular waves ¹ |
|--------|--------------|---------------------------------|---------------------------------|
| | No. of cows | n (%) | n (%) |
| KK | 10 | 6 (60%) ^a | 4 (20%) ^a |
| BK | 10 | 1 (10%) ^{bc} | 9 (45%) ^{bc} |
| CK | 10 | 3 (30%) ^{ab} | 7 (35%) ^{ab} |
| Total | 30 | 10 (33.33%) | 20 (66.67%) |

^{abc}Means within rows and columns are significantly different ($p<0.05$)

¹Percentage (%) of cows in each waves = Number of cows in each waves / Number of cows in each breed x 100

Table 2 shows the follicular wave development characteristic following CIDR withdrawal during the pre-ovulation of the KK, BK and CK cows. The number of follicles at emergence was significantly higher ($p<0.05$) in KK (4.4 ± 0.6), compared to CK (2.6 ± 0.4), but was similar to BK (3.2 ± 0.4). Nonetheless, there was

significant differences were observed in the mean diameter of follicle at emergence stage. However, the mean diameter of ovulatory dominant follicle after the CIDR withdrawal appeared significantly bigger ($p<0.05$) in BK (14.2 ± 0.1 mm), followed with the other two breeds, CK (12.2 ± 0.1 mm) and KK (11.8 ± 0.5 mm).

Table 2: Mean and standard error (mean \pm se) for the follicular wave development characteristics following CIDR withdrawal during the pre-ovulation of the Kedah-Kelantan (KK), Brakmas (BK) and Charoke (CK) cattle breeds

| Parameters/Breeds | KK | BK | CK |
|---|---------------------|--------------------|-----------------|
| No. of cows | 10 | 10 | 10 |
| No. of follicles at emergence | 4.4 ± 0.6^a | 3.2 ± 0.4^{ab} | 2.6 ± 0.4^b |
| Diameter of follicles at emergence (mm) | 4.9 ± 0.1 | 5.0 ± 0.1 | 5.4 ± 0.1 |
| Diameter of dominant ovulatory follicles (mm) | 11.8 ± 0.5^{ac} | 14.2 ± 0.1^b | $12.2 \pm 0.^c$ |

^{abc}Means with different superscripts within rows are significantly different ($p<0.05$)

Table 3 shows the first follicular wave development characteristic during post

ovulation after the CIDR withdrawal. The number of emerging follicles was not

significantly different ($p > 0.05$) in the three breeds studied. The number of follicles at emergence post ovulation of KK, BK and CK was 3.8 ± 0.9 , 3.2 ± 0.5 and 2.7 ± 0.7 , respectively. The new follicles emerged post ovulation on days 1.5 ± 0.2 , 1.4 ± 0.3 and 1.2 ± 0.3 respectively following CIDR removal with mean size of 5.0 ± 0.01 , 5.4 ± 0.02 and 4.9 ± 0.0 mm for BK, CK and KK, respectively, with no significance

difference ($p > 0.05$) was detected among the breeds studied. The first dominant follicle took 8.9 ± 0.7 days, 7.5 ± 1.1 days and 5.1 ± 0.7 days in KK, CK and BK, respectively, to become dominant ($p > 0.05$). The first dominant follicle reached maximum diameter of 11.4 ± 0.08 , 13.1 ± 0.08 and 13.1 ± 0.07 mm in KK, BK and CK, respectively, with no significant difference detected among the breeds studied ($p > 0.05$).

Table 3: Mean and standard error (mean \pm se) for the first follicular wave development characteristics following CIDR withdrawal post-ovulation of the Kedah-Kelantan (KK), Brakmas (BK) and Charoke (CK) cattle breeds

| Parameters/Breeds | KK | BK | CK |
|--|------------------|---------------------|------------------|
| No. of cows | 10 | 10 | 10 |
| No. of follicles at emergence | 3.8 ± 0.88^a | 3.2 ± 0.51^{ab} | 2.7 ± 0.70^b |
| Day of follicles emergence (days) | 1.2 ± 0.03 | 1.5 ± 0.02 | 1.4 ± 0.03 |
| Diameter of follicles at emergence (mm) | 4.9 ± 0.02 | 5.0 ± 0.01 | 5.4 ± 0.02 |
| Diameter of dominant follicles (mm) | 11.4 ± 0.08 | 13.1 ± 0.08 | 13.1 ± 0.07 |
| Day of follicles become dominants (days) | 8.9 ± 0.70^a | 5.1 ± 0.74^b | 7.5 ± 1.09^a |

^{ab}Means with different superscripts within rows are significantly different ($p < 0.05$)

The second follicular wave of follicular development is tabulated in Table 4. The second dominant follicle emergence was detected on days 8.3 ± 2.1 , 8.07 ± 0.7 and 9.2 ± 2.0 in KK, BK and CK, respectively. The duration in emergence among the three breeds did not vary significantly ($p > 0.05$). The mean diameter of non-ovulatory and ovulatory dominant follicle of second wave of follicular development were 11.4 ± 0.01 , 12.8 ± 0.08 and

12.9 ± 0.08 mm in KK, BK and CK, respectively, and there was no significant difference ($p > 0.05$) of the dominant follicle diameter among the three breeds of cattle observed. Similarly, the day of follicle emergence was not significantly different between breeds ($p > 0.05$). It was found that the follicle developed into a dominance much faster in BK ($p < 0.05$, 13.8 ± 1.1 days) followed by KK (15.0 ± 1.2 days) and CK (17.8 ± 0.5 days).

Table 4: Mean and standard error (mean \pm se) for the second follicular wave development characteristics during the estrous cycle of Kedah-Kelantan (KK), Brakmas (KK) and Charoke (CK) cattle breeds

| Parameters/Breeds | KK | BK | CK |
|--|----------------------|-------------------|-------------------|
| No. of cows | 10 | 10 | 10 |
| Day of follicles emergence (days) | 8.3 ± 2.09 | 8.0 ± 0.69 | 9.2 ± 2.00 |
| Diameter of dominant follicles (mm) | 11.4 ± 0.01 | 12.8 ± 0.01 | 12.9 ± 0.01 |
| Day of follicles become dominants (days) | 15.0 ± 1.19^{ab} | 13.8 ± 1.09^b | 17.8 ± 0.49^a |

^{ab}Means with different superscripts within rows are significantly different ($p < 0.05$)

The dominant follicle in the second follicular wave contained non-ovulatory and ovulatory follicles

The mean diameter of third follicular wave dominant follicle (ovulatory follicle) was not significantly different among the three breeds of cows (Table 5). The mean ovulatory follicle in KK was 10.2±0.1 mm, BK 11.6±0.10 mm and CK

10.7±0.1 mm. It was also found that the mean length of oestrous cycle were 20.3±0.8 , 20.4±0.6 and 20.9±0.5 days in KK, BK and CK breeds, respectively with no significant difference (p>0.05) among the three breedtypes of cows.

Table 5: Mean and standard error (mean ± se) for the third follicular wave (ovulatory follicle) development characteristics during the estrous cycle of the Kedah-Kelantan (KK), Brakmas (BK) and Charoke (CK) cattle breeds

| Parameters/Breeds | KK | BK | CK |
|---|------------|-------------|------------|
| No. of cows | 4 | 9 | 7 |
| Day the follicles became dominants (days) | 18 ± 0.1 | 19.11 ± 0.6 | 18 ± 1.0 |
| Diameter (size) of the dominant follicle (mm) | 10.2 ± 0.1 | 11.6 ± 0.1 | 10.7 ± 0.6 |
| Length of the oestrous cycle (days) | 20.3 ± 0.8 | 20.4 ± 0.6 | 20.9 ± 0.5 |

All means within rows are not significantly different (p>0.05)

Figures 2a and 2b represent the turnover of dominant follicles producing 3- and 2- follicular wave patterns development in the present study. Figure 2a shows the first and the second dominant follicles developed in a similar pattern. However, the first dominant follicles initially achieved the maximum diameter on days 1 - 3,

whereas the second dominant follicles were on days 5 - 9. The third dominant or ovulatory follicles appeared on day 11 and ovulated on day 22. Figure 2b illustrates that the first dominant follicles achieved the maximum diameter on days 8 - 9. Meanwhile, the ovulatory follicles emerged on days 7 - 8, and ovulated on days 19 - 20.

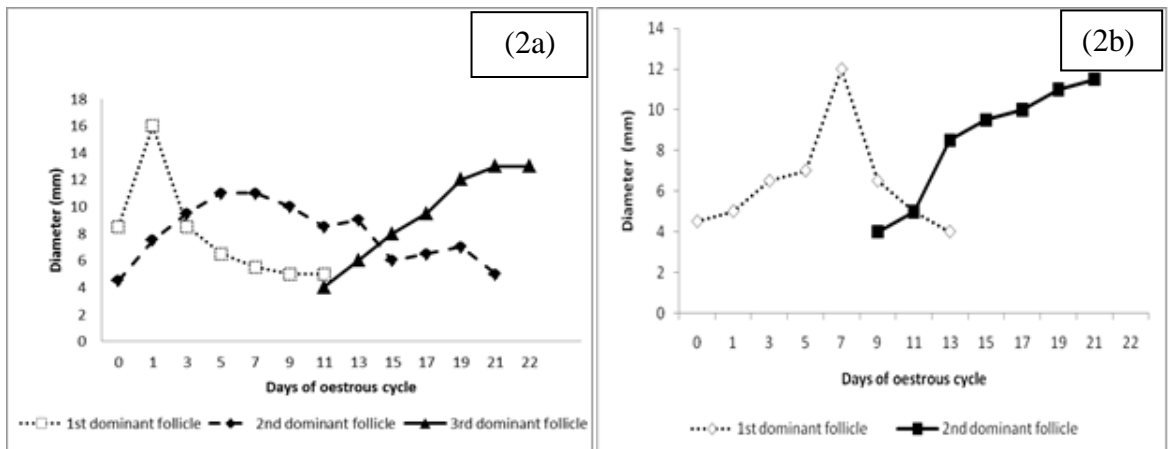


Figure 2: Growth pattern of the dominant follicles during the oestrous cycle of cows with (2a) 3-dominant follicles (3-follicular wave patterns) and (2b) 2-dominant follicles (2-follicular wave patterns).

The growth rate of the dominant follicle in the present study was similar for all the three cow breedtypes as shown in Table 6. The regression rate of BK was $2.3 \pm 0.04 \text{ mm day}^{-1}$ and was significantly

faster compared to KK and CK ($p \leq 0.05$). The regression rate of dominant follicle of BK determined was 1.52 mm day^{-1} faster than the other two breedtypes in the study.

Table 6: Mean growth and regression rates, and progesterone concentration (mean \pm se) within the waves of follicular development in the Kedah-Kelantan (KK), Brakmas (BK) and Charoke (CK) cattle.

| Parameters/Breeds | KK | BK | CK |
|------------------------------------|---------------------|------------------|------------------|
| No. of cows | 10 | 10 | 10 |
| Progesterone concentration (ng/ml) | 2.74 ± 0.6^{ac} | 1.13 ± 0.2^b | 1.12 ± 0.2^b |
| Follicles growth rate (mm/day) | 1.2 ± 0.5 | 1.7 ± 0.1 | 1.3 ± 0.1 |
| Follicles regression rate (mm/day) | 1.1 ± 0.1^b | 2.3 ± 0.1^a | 1.4 ± 0.1^b |

^{abc}Means with different superscripts between columns within rows are significantly different

The pattern of progesterone concentrations of KK, BK and CK cows as presented in Figure 3 showed the P_4 achieved higher peak of concentration on days 4, 8.2 and 4.5 for the first follicular wave, and days 12, 14 and 16.5 for the second follicular waves in BK, KK and CK breedtypes, respectively. Figure 4 illustrates when the size of dominant follicle achieved maximum diameter, the amount of P_4 regressed. This can be observed from the

Tables 3 and 4, as the diameter of dominant follicle became maximum on days 5.1, 8.9 and 7.5 in first follicular wave, and on days 13.8, 15 and 17.8 in second follicular wave for BK, CK and KK breeds, respectively, the peak of P_4 shown in Figure 3 started to regress and declined. However, the P_4 concentration in one oestrous cycle was significantly higher in KK ($2.7 \pm 0.6 \text{ ng ml}^{-1}$; $p < 0.05$) compared to the other two breedtypes of cows.

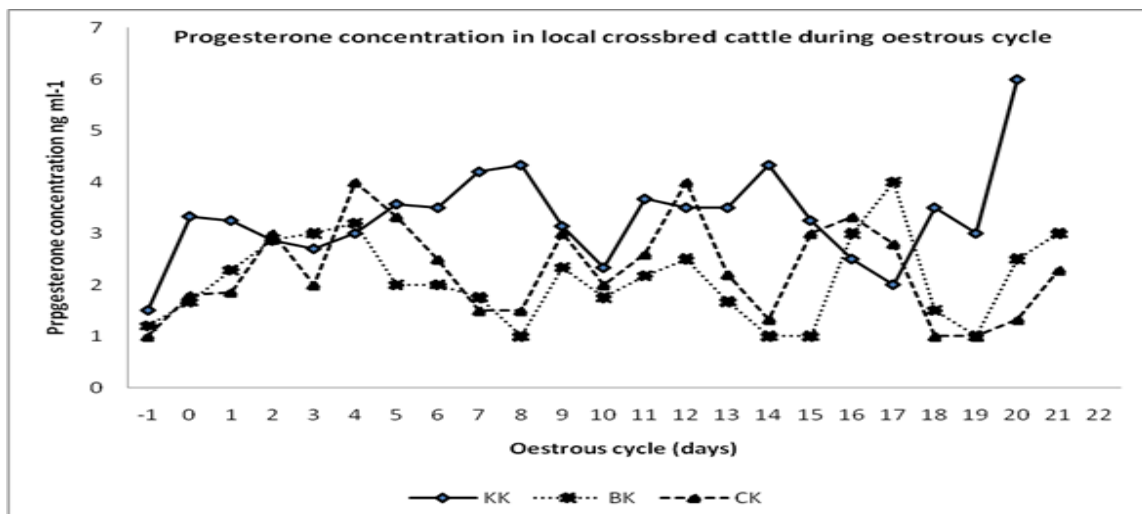


Figure 3: Progesterone concentration (ng/ml) of Kedah Kelantan (KK), Brakmas (BK) and Charoke (CK) during the oestrous cycle.

The study determined the wave pattern of the follicular characteristic in three Malaysian breeds of beef cattle, namely, KK, BK, and CK. The oestrous cycle of the three breeds of cattle evaluated indicated that BK had a higher proportion (45%) of 3-follicular waves, followed by CK (35%) and KK (20%). As for the 2-follicular waves, it was highest in KK (60%), followed by CK (35%) and BK (10%). Nonetheless, there was no evidence of these breeds having four or more waves of follicular development. This finding is similar to that of the Rathi cattle, whereby no four or more follicular waves were found (Gaur and Purohit, 2007).

Evidence of a higher proportion of the 2-follicular waves (78.6%) compared to 3-follicular waves (21.4%) of follicular development was also reported by Gaur and Purohit (2007). Nevertheless, a higher incidence of 3-follicular waves of follicular development and a small proportion of cows having 4- or more follicular waves during the estrus cycles were found in Gir cattle (Vianna *et al.*, 2000). The present study found that BK had a higher proportion of 3-follicular waves follicular development. However, KK had a higher incidence of 2-follicular waves follicular development. Similarly, many studies conducted in Europe reported a higher prevalence of cycles with 3-follicular waves (Savio *et al.*, 1988; Sirois and Fortune, 1988; Viana *et al.*, 2000) and 2-follicular waves (Pierson and Ginther, 1988; Taylor and Rajamahendran, 1991) of follicular development patterns.

To date, this is the first study that determines the wave patterns of follicular growth in Malaysian cattle. Majority of the Brakmas cattle in the study tended to have 3-follicular waves pattern (45%). On the contrary, various studies conducted on Brahman cattle have shown that a 2-follicular wave pattern was recorded more frequently (Alvarez *et al.*, 2000; Figueiredo

et al., 1997; Zeitoun *et al.*, 1996). The difference seen could probably be due to the seasonal effects (Zeitoun *et al.*, 1996). Climatic changes could be one of the factors that influenced the variation in the follicular wave reported in these studies. The characteristics features of the climate of Malaysia are uniform temperature, high humidity and copious rainfall. Situated in the equatorial doldrum area, the rainfall pattern over the southwest costal area (i.e. where Kluang was located), maximum rainfall occur in October-November while secondary rainfall occur in March-May. February is the month with the minimum rainfall and the June-July minimum rainfall is indistinct. Elsewhere, the discrepancies in the occurrence of 2-follicular waves pattern in the Brahman cows observed were 56, 38 and 84% conducted in summer (July and August) in Florida (Alvarez *et al.*, 2000), spring (May) and Fall (October) in Texas (Zeitoun *et al.*, 1996); and winter (July and August) in Brazil (Figueiredo *et al.*, 1997), respectively. The differences in the number and follicle size, due to seasonal effects, have also been reported by Lammoglia *et al.* (1996).

It has been documented that the number of follicular waves, during the oestrous cycle, was regulated by the length of the luteal phase (Ginther *et al.*, 1989; Fortune *et al.*, 1991; Lucy *et al.*, 1992), and cows with 3-follicular waves had been observed to have longer luteal phase, which would thus lead to a longer duration of the oestrous cycle (Ginther *et al.*, 1989; Fortune *et al.*, 1991; Lucy *et al.*, 1992). Although BK showed a higher frequency of the 3-follicular waves, it did not affect the length of its oestrous cycle. Similarly, Savio *et al.* (1988) found that the length of the oestrous cycle was not influenced by the number of follicular waves which occurred during the oestrous cycle. In the study, the growth rate of the dominant follicle of BK was shown to

be similar to the other two breeds of KK and CK. The results of the present study postulated a possibility that the regression rate of the dominant follicle in the BK occurred faster compared to those of the other breeds in the current study. In addition, it was showed on the first of follicular wave, BK was detected significantly faster to become dominant among the breedtypes. Thus, it led to the duration of each wave occurring almost at a similar time and did not influence the length of the estrous cycle. Other factors, such as temperature and rainfall, could also be the predisposing factors that influenced the wave occurrence of follicular development affecting the length of the estrous cycle.

After post-CIDR ovulation, the follicle was capable to develop on its own until it reached 4mm in size (Garverick *et al.*, 2002). However, it was shown to be dependent on follicle stimulating hormone (FSH) when it reached a size greater than 4mm and composed the cohort that participated in the follicular wave (Garverick *et al.*, 2002). The FSH-dependent follicle capable of promoting oestradiol led the follicle to maximize its size to a larger diameter (Lucy *et al.*, 2007). FSH was postulated to have caused the emergence of the follicular wave. The increased concentration of FSH, during and after LH, triggered the follicular recruitment. In the study, it could be postulated that the FSH surge occurred during postovulatory and midcycle of the first wave leading to the formation of atretic dominant follicles. Thus, it would have initiated the first and second follicular waves. Similarly in the 3-follicular wave pattern, the third FSH arises when the second dominant follicle becomes atretic.

It is important to note that ovarian follicular growth is indicated by the number of follicles in all the categories that develop during the oestrous cycle. The study has

shown that the total mean size of the first, second and third dominant follicles of KK and KK crossbreds was 12.5 ± 0.04 , 12.4 ± 0.08 to 12.9 ± 0.06 mm, respectively. The dominant follicle was selected from a cohort of follicles, and it developed to attain dominance. The dominant follicle has the ability to suppress the growth of other smaller subordinate follicles (Mihm, 2002; Hendriksen *et al.*, 2003; Quirk *et al.*, 2004) and has the capability to ovulate with the influence of hormonal conditions (Fortune, 1993), and thus is termed the ovulatory follicle. It was observed in this study that the dominant follicle inhibited the subordinate follicle from growing until the next anovulatory or ovulatory dominant follicle, irrespective of 2- or 3-follicular wave of follicular development, and this is similar to the finding of Kulick *et al.* (2001). The average first dominant follicle in the present study was slightly bigger as compared to that of Angus (11.4mm), but smaller compared to that of Brahman (15.3mm) and Senepol (13.9mm) (Alvarez *et al.*, 2000). The results of this study support the findings of Bo *et al.* (2003) who concluded that the maximum diameter of the dominant follicles in *Bos indicus* was smaller than that of the *Bos taurus* cattle. In the present study, the ovarian follicular development and growth were found to be bigger in BK than in either CK or KK, as indicated by the diameter of the first ovulatory dominant follicles after the CIDR removal.

In the first-wave follicular development of the study, the mean day of new follicular wave emergence was at 1.4 ± 0.27 days, with a size diameter of 5.1 ± 0.02 mm and the total mean number of days for follicle emergence was 3.2 ± 0.40 days. In the Rathi cattle, however, the 2- and 3-follicular waves of follicular development emergence were at 2.1 ± 0.4 and 4.1 ± 1.0 days, respectively. A previous study by Utt

et al. (2003) revealed that the follicular wave emergence, after CIDR withdrawal, was 4.8 days when the cows were treated with CIDR for 7 days, and 25 mg of PGF was given a day prior to CIDR withdrawal. In the Angus cow which had been treated with GnRH or estradiol-17 β and P4 at CIDR insertions, the intervals from the CIDR withdrawal to follicular wave emergence were apparently at days 6.6 and 4.7 (Utt *et al.*, 2003). In the present study, the preovulatory follicular wave development post-CIDR removal was shorter (i.e. from days 1.2 – 1.5). This was probably due to the different protocols and gonadotrophin used in the study, or it could be also related to the different stages or sizes of the dominant follicles developing during the synchronization (Lucy, 2007). The study therefore, has met the objectives in determining the existence of follicular wave development in terms of dominant size follicles in each follicular wave of synchronized Kedah-Kelantan (KK) and KK crossbred cows with progestin and PGF.

The results of the present study concur with the hypothesis that the growth and development of follicle followed a wave-like pattern, and there were differences in the development of the first, second and third follicular waves in terms of the number of follicles emergence and dominance following the induction of synchronization of estrus in KK, BK, and CK cattle. Nevertheless, it is important to note that the growth pattern of follicle is dependent on the number of follicular waves developed in certain duration to attain the normal length of the estrous cycle. Therefore, the study on follicular development is particularly important to understand and identify the turnover of follicular development during the estrous cycles and to refine the synchronization protocol for subsequent procedures, such as

timed-AI or transvaginal oocyte aspiration (ovum pick-up).

Conclusions

In conclusion, the results of the present study indicated there were no differences on the follicular wave development in terms of dominant follicles size in pre-ovulation, first, second and third follicular wave development, follicles existing at post-ovulation stage and length of oestrous cycle in KK and its crossbreds of BK and CK. However, in terms of the number of follicular waves, BK and CK had higher proportion of 3- and 2-follicular waves development patterns, respectively, and the follicles existing at pre-ovulation stage showed KK and BK were predominantly higher in the number of follicles at emergence compared with CK following synchronisation.

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