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Interval Between Oestrous Characteristics and Pregnancy Rate in Synchronised Kedah-Kelantan and Crossbred Cows

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Abstract: The study was conducted to determine the differences in the interval between removal of Controlled Internal Drug Release (CIDR) and standing oestrus (C_ES) interval between CIDR removal and Ovulation (C_OV) interval between oestrus and Ovulation (ES_OV), predetermined Artificial Insemination (AI) and Pregnancy rate following prostaglandin (PGF) treatment and CIDR removal at standing oestrus in Kedah-Kelantan (KK) and KK crossbred cows. A total of 35 KK (n = 11), Brakmas (BK: n = 10) and Charoke (CK: n = 14) cows were inserted with CIDR containing 1.38 g Progesterone (P4) for 7 days and followed with intramuscular injection of 500 μg Cloprosterol of PGF synthetic analogue 2 days prior CIDR removal. The oestrous behaviour was observed for 72 h at 2 h intervals beginning 12 h after CIDR removal. AI was carried out at 6 and 12 h after standing oestrus was detected. Mean interval between C_ES, C_OV and ES_OV were 52.97±6.16, 94.42±16.41 and 53.75±25.36 h, respectively. The number of follicles >5 mm in diameter at the time of CIDR removal was significantly lower in CK (p = 0.04) compared to KK. The results of POF size and number of follicle size ≥5 mm either at CIDR removal or prior ovulation and P4 concentration at day 0, 7 and 14 were found to be not significantly different at p = 0.05. The pregnancy rate was higher (p<0.05) in CK (43.8%) followed by KK (31.3%) and BK (25%). The study provides results which could be used in the development of TAI on oestrous synchronisation protocol of KK and KK crossbred cows.

Key words: Beef cattle, synchronisation, oestrus, ovulation, artificial insemination, pregnancy diagnosis and crossbred cows

INTRODUCTION

Kedah-Kelantan (KK) was a local Malaysian Zebu cattle beef breed. The breeds were known as hardy, resistance to heat stress and parasite. In Malaysia, attempts have been made to improve the local Kedah-Kelantan (KK) cattle through crossbreeding with improved breeds such as Brahman (Ariff, 1992). Brakmas (BK) was developed from the crossing of Brahman and KK breeds and Charoke (CK) from crossing of Charolais and KK breeds. So far, none of the earlier research has published that involved the three breeds of *Bos indicus* cattle in the environmental that developed from tropical country such as Malaysia. Oestrous synchronisation is applied when it is desirous to bring a group of female animals into a restricted ovulatory phase. The oestrous synchronisation can be used at any stage of the oestrous cycle. Many studies have optimized the induction of follicular wave emergence in cattle by tightly sequencing the events of synchronisation of oestrus, ovulation and Artificial Insemination (AI) (Barros *et al*., 2000; Williams *et al*., 2002; Richardson *et al*., 2002). The use of progestin based protocols is a tight synchrony of oestrus (Richard *et al*., 1986) and it can be employed for Timed Artificial Insemination (TAI) (Echternkamp and Thallman, 2011). However, its use alone can cause reduction in synchrony of follicular growth (Williams *et al*., 2002). The situation normally can be

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resolved by administering Prostaglandin (PGF) after the end of treatment period with a progestin (Larson *et al*., 2006). Ovulation occurs in response to several physiologic, biochemical and biophysical mechanisms.

The physiological mechanism is controlled by the hypothalamus which triggers the release of Gonadotropin Releasing Hormone (GnRH) to cause the Luteinisation Hormone (LH) surge (Yoshioka *et al*., 2001). Ovulation can be determined by a sudden disappearance of a dominant follicle following examination by ultrasonogaphy (Pursley *et al*., 1995).

AI has been used extensively to disseminate superior genes in many livestock species, particularly dairy cattle. AI is used to increase the rate of gamete dissemination in order to improve and maximize the utilization of genetically superior males in a local cattle breed. Many studies have been conducted toward developing a TAI program in dairy cattle since 1970s (Lauderdale, 2009). However, each type of synchronisation protocol is not suitable due to numerous factors such as an oestrus animal, the oestrus cycling status of the animals, availability of the labour and various management practices. Therefore, the objective of the present study was to determine the effect of Controlled Internal Drug Release (CIDR) device and Prostaglandin (PGF) treatment on the variations in the intervals between the onset of oestrus and ovulation and pregnancy rates in KK and KK crossbred cows.

MATERIALS AND METHODS

Animal selection and management: A total of 35 KK and KK crossbred cows of Brakmas (BK) and Charoke (CK) breedtypes were used in this study. The cows were selected from a breeding herd managed at MARDI Research Station, Kluang, Johor (Lat: 1°56'57" and Long: 103°21'56"E) situated about 107 m above sea level with ambient temperature of 24-32°C and relative humidity of 87-96% with trace means rainfall amount <0.1 mm. The cows were divided into three groups; A: KK, n = 11; B: BK, n = 10 and C: CK, n = 14. The cows ranged from 2-5 years of age with body weight of 200-350 kg and Body Condition Score (BCS) of 4-5 (scale 1 (severely thin) to 9 (obese); Richard *et al*., 1986). During the study, the cows were raised in a semi-intensive system involving daytime grazing in fertilized paddocks cultivated with improved pasture of Signal grass, *Brachiaria decumbens*. The cows were kept indoor in holding pens at night and during ultrasonography for follicular mapping. Commercial pellet feed containing 15.9% crude protein and 17.6 MJ calculated Gross Energy (GE) were fed to the cows at a rate of 1 kg per 100 kg bodyweight per day.

Synchronisation of oestrus: All cows were on adaptation period for 14 days before synchronization. All cows at random stages of oestrous cycle were inserted intravaginally with CIDR device containing 1.38 g P4 (Pharmacia and Upjohn, Australia) and left for 7 days. This was followed with an intramuscular injection of 500 µg Cloprostenol of PGF synthetic analogue (Estrumate®; Schering-Plough Animal Health, Australia) 2 days prior to CIDR removal (Fig. 1).

Observation of oestrous behaviour characteristics: Observation for oestrous behaviour in the treated cows was carried out at 2 h intervals for 72 h beginning 12 h after CIDR withdrawal. During oestrous behaviour observation, 5-6 cows in each treatment were kept in a holding pen of 2.74×1.83 m size. Oestrous behaviour was monitored through visual observation by two trained personnel. The cows were determined to be in standing oestrus when they stood and allowed to be mounted by their herd mates and were discontinued when no successful mounts were observed. The day and time of CIDR removal and cows were in oestrus was recorded. At this moment, data of interval from removal of CIDR to standing oestrus (C_ES) were obtained.

Ovarian ultrasonography: Both ovaries were scanned using 7.5 MHz linear array transducer (Aloka Co., Ltd. Japan) attached to an ultrasound scanner (Aloka®, Echo System, SSD-500, Japan). Scanning of ovarian follicular was carried out 6 h after oestrus at 6 h intervals until the dominant follicle ceased. Follicles of size greater than or equal to 5 mm in diameter were grouped into 4 phases: I (days 1-4), II (days 5-10), III (days 11-17) and IV (days 18-20) as described by Ireland *et al.* (1979). Ovulation was determined by the disappearance of large follicles of sizes >10 mm in diameter and subsequent presence of a Corpus Luteum (CL) in the same location of the collapsed follicles.

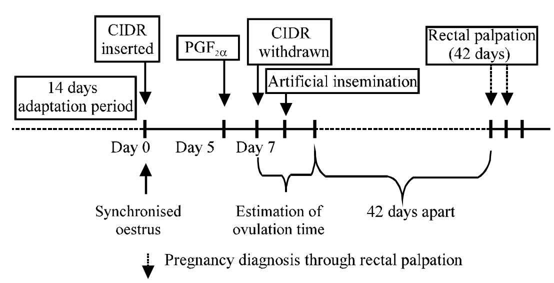

Fig. 1: Experimental protocol for synchronisation of oestrus, estimation of ovulation, artificial insemination and pregancy diagnosis

The size of Preovulatory Follicles (POF) and time of ovulation were recorded. The POF can be identified as largest follicles at the completion of luteolysis (Fortune and Quirk, 2002). Data of interval from removal of CIDR to ovulation (C_OV) was calculated by subtracting the time occurrence of ovulation with the time when CIDR was pulled out. Whereas the interval from standing oestrus to Ovulation (ES_OV) were determined by taken the differences of observation between ovulation and oestrus. Data of interval from removal of CIDR to Ovulation (C_OV) and interval from standing oestrus to Ovulation (ES_OV) were determined.

Artificial insemination and pregnancy diagnosis: AI was carried out twice for each cow at 6 and 12 h after standing oestrous was observed. The semen was deposited at anterior part of the cervix as outlined by Lopez-Gatius (2000). After AI, the cows were maintained in a holding yard for a week and then released into the paddocks for grazing until PD via rectal palpation was performed on day 42 after AI.

Progesterone assays: Blood samples were collected via jugular venipuncture into 10 mL Vacutainer tubes (Fisher Scientific, Pittsburgh, PA) on days 0, 7 and 14. Blood was allowed to clot at room temperature for 1 h and 4°C for 24 h, respectively. The blood was then centrifuged at 700 g for 25 min. Serum was collected and stored at -20°C until assayed. P4 assay was performed by radioimmunoassay using the developed kit manufactured by the Animal Production Unit (Coat-a-count; Diagnostic Products Corporation, Los Angeles, CA). Measurement of P4 was made on days 0, 7 and 14 in order to assess the presence of a functional CL (Saumande and Humblot, 2005; Echternkamp and Thallman, 2011).

Statistical analyses: Data on intervals among oestrous characteristics were analyzed as one-way ANOVA with breedtypes as the independent variable. Data on pregnancy rate among breedtypes were compared using χ^2-test. The relationship among oestrous characteristics was determined using Pearson correlation. The statistical package SPSS Version 17 was used for all statistical analyses.

RESULTS AND DISCUSSION

Three, four and six animals did not show any oestrous characteristic in KK, BK and CK groups, respectively. However, the POF and time of ovulation of those cows were obtained using ultrasonography. The results of intervals between the onset of oestrus and ovulation in KK, BK and CK are shown in Table 1. Mean intervals for C_ES, C_OV and ES_OV were 52.87, 94.06 and 54.38 h, respectively. No significant difference of interval between C_ES (p = 0.80), C_OV (p = 0.06) and ES_OV (p = 0.19) of the breedtypes was detected. However, interval between C_OV was longer in KK compared to the other two breedtypes.

The results of POF size, number of follicle size ≥5 mm at CIDR removal and prior ovulation and P4 concentration at day 0, 7 and 14 are shown in Table 2. The POF size at CIDR removal was not significantly different (p = 0.5) among the three breedtypes of cattle with mean and range of POF size of 10.90 and 6.7-15.5 mm, respectively. There was also no breedtype differences for mean POF size prior to ovulation with the mean of 11.74 mm and a range of 8.5-17.5 mm (p = 0.35). The number of follicles at CIDR removal of size ≥5 mm was significantly lower (p = 0.04) in CK compared to KK cows. However, the number of follicles of size ≥5 mm prior ovulation was not significantly different among the breedtypes (p = 0.99). The mean number of follicles of size ≥5 mm was 4.27 with a range of 1.3-8.0.

Figure 2 shows the mean number of follicles of size ≥5 mm at phase I-IV during the oestrous cycle. There were no significant differences among breedtypes for mean number of follicles of size ≥5 mm (p = 0.23). Mean P4 concentrations on day 0, 7 and 14 were found to be not significantly different at 2.07 ng mL^{-1} (p = 0.49), 2.82 ng m L^{-1} (p = 0.14) and 4.33 ng mL^{-1} (p = 0.06), respectively.

The proportion of cows pregnant as determined by rectal palpation is shown in Table 3. Pregnancy rate was higher in CK (42.9%) followed by KK (23.8%) and BK cows (33.3%). Interval C_OV was significantly correlated (r = 0.44, p = 0.01) with interval ES_OV. However, there was no significant correlation (p>0.05) among POF size size of follicle ≥5 mm, C_ES and C_OV.

The study provided estimates of intervals of oestrous characteristics, namely intervals between C_ES, C_OV and ES_OV and mean number of follicles on different phases of ooestrous cycles and percentage of cows pregnant in KK and KK crossbred cows. Oestrus was estimated to occur at 52.97 h and ovulation at 94.42 h from

Table 1: Mean and Standard Deviation (mean±SD) for intervals between the onset of oestrus and ovulation in Kedah-Kelantan (KK), Brakmas (BK) and Charoke (CK) cows

		Interval (days)		
Breeds	n	CIDR-Oestrus	CIDR-Ovulation	Oestrus-Ovulation
KK	11	51.98±5.78	103.46±15.14	61.11±21.19
BK	10	52.75±3.09	87.78±15.22	58.91±34.21
CK	14	53.87±7.66	90.93±15.87	43.13±18.81
Mean	35	52.97±6.61	94.42±16.41	53.75±18.81

Means within columns are not significantly different (p>0.05)

Table 2: Mean number of follicles, size of preovulatory follicles, P4 concentration at different day 0, 7 and 14 and time of ovulation in Kedah-Kelantan (KK), Brakmas (BK) and Charoke (CK) cows

At CIDR removal	P	KK	BK	CK	Means
Size of POF (mm)	0.53	10.32±2.23	11.60±3.17	10.79±2.38	10.90±2.59
No. of follicles (≥5 mm)	0.04	4.40±0.60[a]	3.20±0.360[ab]	2.60±0.37[b]	3.40±0.44
Prior ovulation					
Size of POF (mm)	0.35	11.31±2.04	12.2±2.720	11.71±1.90	11.74±2.22
No. of follicles (≥5 mm)	0.99	4.17±2.93	4.25±1.890	4.40±3.44	4.27±2.75
Progesterone (ng mL^{-1})					
Day 0	0.15	3.33±2.52	1.67±0.520	1.80±0.45	2.07±1.27
Day 7	0.14	4.20±2.49	1.75±0.960	1.50±0.71	2.82±2.14
Day 14	0.06	4.33±1.53	1.00±0.000	1.33±0.58	2.57±1.90

[a-c]Means within the rows with different superscripts are significantly different (p≤0.05); POF = Preoulatory Follicles

Table 3: Pregnancy rates obtained from rectal palpation for Pregnancy Diagnosis (PD) of observed oestrus in Kedah-Kelantan (KK), Brakmas (BK) and Charoke (CK) cows

Number of cows	KK (n = 9)	BK (n = 10)	CK (n = 14)
Pregnant (%)*	5 (23.8)	7 (33.3)	9 (42.9)
Not pregnant (%)	4 (33.3)	3 (25.0)	5 (41.7)

*Pregnant (%) = (Number of animal pregnant on each breed/Total number of animal pregnant)×100

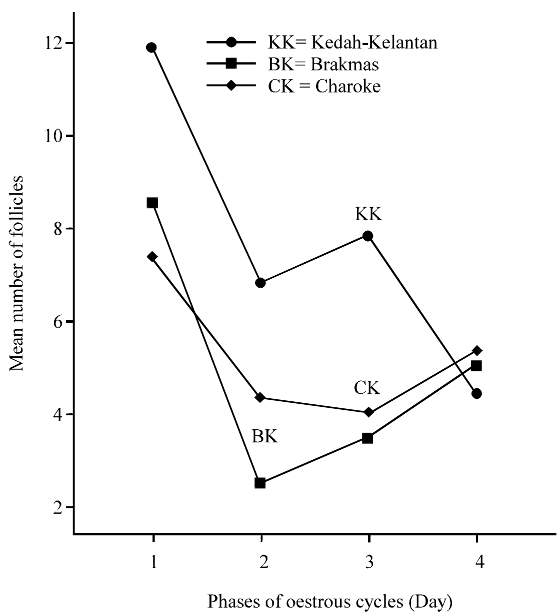

Fig. 2: Mean number of follicles on different phases of oestrous cycle: I (days 1-4), II (days 5-10), III (days 11-17) and IV (days 18-20)

the time of removal of CIDR. However, KK has showed a longer time to ovulate among the three breedtypes of cows studied. The mean interval from standing heat to ovulation was 53.75 h with no significant difference detected among the breedtypes involved in the present study.

Many earlier studies have shown that the intervals between removal of insert (CIDR or Crestar) and ovulation were shorter than that found in the present study.

Monteiro *et al.* (2009) reported the interval between removals of CIDR to Oestrus in Nelore cows was 45.5 h which was earlier than the finding in the present study. The interval between oestrus and ovulation had been reported to range from 38.5 h (Saumande and Humblot, 2005) to 72.3 h (Monteiro *et al.*, 2009).

The longer interval between C_OV, C_ES, ES_OV found in the present study could probably be due to the differences in parity, age and oestrous cycling status of the cows. The wide variation in the types of luteolytic agents used for oestrous synchronisation (Richardson *et al.*, 2002; Leitman *et al.*, 2008; Monteiro *et al.*, 2009) and stress of handling on the animals during treatment (Dobson and Smith, 2000; Monteiro *et al.*, 2009) have also been suggested as predisposing factors to cause variation in ovulation rate. A reduction of hypothalamic GnRH secretion will influence the amplitude and pulsatility of LH release from the anterior pituitary which compromise the ovarian follicular growth reduce the peripheral concentration of oestradiol in the blood circulation. This leads to some variability of the follicular maturation and insufficient preovulatory LH surge which cause lower ovulation rate (Monteiro *et al.*, 2009).

The P4 concentration both on day 7 and 14 was higher in KK compared to the other two breedtypes studied. However, P4 concentration obtained in the present study was lower compared to that reported by Echternkamp and Thallman (2011). The P4 concentration in *Bos indicus* cattle was reported to be lower (Randel, 1984; Segerson *et al.*, 1984) due to the smaller size of CL (Alvarez *et al.*, 2000) compared to *Bos taurus* cattle. Although, it was observed that the functional CL existed on day 0 (Echternkamp and Thallman, 2011), 7 and 14 (Saumande and Humblot, 2005) but the occurrence of functional CL may also, depend on the condition of the animals, size of CL and the capability of CL to secrete P4.

The information of the interval between oestrus to preovulatory LH surge and the interval between oestrus and oestradiol concentration and information regarding the physiological condition differences between the heifers and cows is required in order to improve the synchronisation of oestrus in animals for TAI. The separation between heifers and cows is equally important to develop an effective synchronisation protocol. In the present study, the use of PGF alone could not provide acceptable synchrony because the effect of PGF depends on the stage of development of the dominant follicle at the time when PGF was given to induce regression of the CL (Monteiro *et al*., 2009). Studies have shown that the addition of gonadotropin at the earlier part of the synchronisation protocol would improve the synchrony of follicular waves of cows with different physiological condition or having reproductive problem such as cystic ovary (Crane *et al*., 2006) which may not be suitable for the heifers. The results of the study might be used as guidelines for future study in the development of protocol suitable for either heifers or cows in TAI of KK and KK crossbred cows.

CONCLUSION

The oestrous synchronisation protocol used in the present study enabled the synchronisation of oestrus in the three breedtypes studied. Using of the protocol, standing oestrus occurred at 52.97 h and the time of ovulation was 94.42 h, calculated from removal of CIDR.The mean interval from oestrus to ovulation was 53.75 h with no difference observed among the breedtypes involved. However, KK was shown to take a longer time to ovulate among the breedtypes studied. The protocol could produce higher pregnancy rate in CK (42.9%; $p<0.05$) followed by KK (23.8%) and BK (33.3%). It was shown the protocol could be used as an oestrous synchronisation protocol in KK and KK crossbred cows although, it appeared that there could be breedtype differences when considering the use of protocol for timing the AI in relation to oestrous observation. The results of the study therefore provide the basic information in the development of an oestrous synchronisation protocol that could be used in TAI for KK and KK crossbred heifers and cows.

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