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Ovarian follicular wave patterns, oestrus interval and ovulation rate in oestrus synchronised Kedah-Kelantan cows

(Corak gelombang folikel ovari, selang tempoh estrus dan kadar ovulasi lembu betina Kedah-Kelantan yang estrus diselaraskan)

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Abstract

The relationship between 2- and 3-follicular wave patterns on progesterone hormone profiles, oestrus interval, ovulation time and pregnancy rate in Kedah- Kelantan (KK) cows were studied. A total of 30 primiparous and multiparous KK cows were inserted intravaginally with controlled internal drug releasing device (CIDR®) for 7 days, followed by intramuscular injection of 25 mg prostaglandin 2 days prior to CIDR® removal. The study indicated that the oestrus cycle length and proportion of pregnant cows were not significantly different between the 2- and 3-follicular wave patterns. However, follicle emergence in the 3-wave pattern was 4.1 days later than the 2-wave pattern while the dominant follicle in the 3-wave pattern took 4.3 days earlier to achieve the maximum diameter. The dominant follicle took a respective 6.4 and 5.4 days for 2-and 3-wave patterns to become dominant at days 9.8 and 9.5 respectively. The progesterone concentration at the time of emergence of the first dominant follicle (DF) was lower in 3-wave pattern compared to 2-wave pattern. Oestrus intervals, ovulation time and pregnancy rates were not significantly correlated in the 2- and 3-wave patterns. There was no relationship between ovarian follicular wave patterns, ovulation time and pregnancy rates during the oestrus cycle in synchronised KK cows.

Introduction

Kedah-Kelantan (KK) is an indigenous cattle breed of Malaysia and is mainly reared for meat production. The mature KK cows weigh 200 – 400 kg with the age of first calving varying from 24 – 62 months. Studies on follicular dynamics have been reported mostly in European cattle breeds (Savio et al. 1988; Sirois and Fortune 1988; Ginther et al. 1989; Roche and Boland 1991; Badinga et al. 1994). However, there is a scarcity of information on follicular changes in Zebu cattle or *Bos indicus* (Figueiro et al. 1997; Vianna et al. 2000), particularly the KK cattle. The existence of follicular development differences among the breeds

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of cattle necessitate the establishment of a common ovulation parameter and management procedures in the breeding of *Bos indicus* cows (Vianna et al. 2000).

A phenomenon of follicular waves involves the follicular growth in the ovary. Lucy et al. (1992) defined follicular growth as a series of cohorts of mature follicles that are able to ovulate under the influence of pituitary gonadotropic stimulation. A wave of follicular development (FD) in cattle is characterized by the synchronous growth of a number of small follicles followed by selection of a dominant follicle (DF) and a subsequent regression of the subordinate follicles (Sirois and Fortune 1988).

Synchronising of oestrus cycle involves manipulation of ovarian activity so that the time of ovulation can be predicted. Using this particular method, over 90% of cows can be induced to enter oestrus within 24 h (Cavalieri et al. 2003). A greater proportion of anoestrus of prepubertal animals can also be induced to ovulate following hormonal treatment (Fike et al. 1997). Meanwhile, an administration of gonadotropin releasing hormone (GnRH) during the oestrus cycle resulted in release of luteinising hormone (LH) (Chenault et al. 1990), which caused ovulation or luteinization of the DF in the ovary, synchronized recruitment of a new follicular wave (Thatcher et al. 1989) and FD (Wolfenson et al. 1994).

The number of waves can affect the length of oestrus cycle in cows (Vianna et al. 2000). The 3-wave pattern has an extra 2 days (Ginther et al. 1989) or 3 days (Wael 2003) oestrus cycle length compared to cows with 2-wave pattern. The length of the oestrus cycle's interval is dependent on the ability of the dominant follicle to ovulate following LH surge. The DF is influenced by the physiological maturity stage at the time of ovulation. However, the DF itself is dependent on the preovulatory follicle size (Perry et al. 2007), composition of the ovary follicular fluid, and the nuclear maturation and developmental competence of enclosed oocytes (Iwata et al. 2006). Therefore, the occurrence of ovulation depends on the size of preovulatory follicles (Perry et al. 2005), the size of follicles and the follicle environment which affects the quality of the oocytes (Iwata et al. 2006), pregnancy rates and late embryonic or foetal survival (Perry et al. 2007).

Progesterone (P_4) is very important for the maintenance of pregnancy (McDonald et al. 1952) and stimulation of endometrial secretion (Geisert et al. 1992). In most studies, cows were considered anoestrus if the serum concentration of progesterone was less than 1 ng/ml. However, cows with serum progesterone concentrations of more than 1 ng/ml were considered to be cycling due to the presence of an active corpus luteum (Perry et al. 2007; Echternkamp and Thallman 2011).

The relationship between follicle size at insemination and pregnancy success has been determined (Perry et al. 2005). It has been speculated that cows with GnRH-induced ovulation with follicles less than 11.5 mm in diameter secreted less progesterone due to the smaller size of corpus luteum (Vasconceloes et al. 2001). Postpartum cows with follicle size less than 11 mm had decreased pregnancy rates and high rate of late embryonic mortality (Perry et al. 2005). However, ovulatory follicles of 12.1 mm in diameter was less likely to support pregnancy compared to ovulatory follicles of more than 5.2 mm which tended to support pregnancy after insemination (Perry et al. 2005).

Therefore, a study on DF can lead to the development of breeding tools in the reproduction of cows. Many other benefits can be derived from better understanding of DF development, such as treating anoestrus cows (Larson et al. 2006; Busch et al. 2008; Wilson et al. 2010) or cows with cystic ovaries (Crane et al. 2006), or improving the protocols for timed artificial insemination (TAI), superovulation and embryo transfer. The objective of the present study was to examine the relationship between 2- and 3-wave follicular development patterns on oestrus interval, ovulation time and pregnancy rate in synchronized KK cows.

Materials and methods

Animal management and treatment

The study was conducted at MARDI Research Station, Kluang, Johor. A total of 30 KK cows, ranging from the first to third parity and 2 – 4 years of age with body weight ranging from 250 – 350 kg and average body condition score of 4 (1 = Emaciated, 4 = Moderate, 8 = Overweight) were used in the study. All the cows were maintained in similar feeding and management conditions. The cows were released for grazing in the evening, but were kept individually and fed with pellet containing 15.9% crude protein and 17.6 MJ GE (mega joule gross energy) at a rate of 1 kg per 100 kg of body weight per day. The cows also had free access to water in the pen every time when ultrasonography for follicular mapping was conducted.

Synchronisation of oestrus

The cows were on adaptation period to the feed and pen for 14 days before the synchronisation was carried out. Each cow was inserted intravaginally with a controlled internal drug releasing device (CIDR®, Pharmacia & Upjohn, Australia) containing 1.38 g P_4 for 7 days followed by intramuscular injection of 25 mg prostaglandin (PGF, Estrumate, Schering-Plough Animal Health, Australia) on day 5 after CIDR® insertion. The cows were observed for oestrus through visual observation after CIDR removal on day 8 and they were regarded as standing oestrus when they stood up while being mounted by another herd mate.

Ovarian ultrasonography and follicular mapping

The position of both ovaries was localized and identified through rectal palpation and scanned using a 7.5 MHz linear array transrectal transducer (Aloka CO., LTD, Japan) that was attached to the portable ultrasound (Aloka®, Echo System, SSD-500, Japan). The ultrasonography was carried out 6 h after the removal of CIDR, and was repeated at 6 h intervals until the DF ceased. The ovaries were further scanned every 2 days for the determination of follicular wave development. The number and size of follicles greater than 4 mm in diameter were counted, measured and mapped from both ovaries onto a follicle map. The DF of a wave is one that grows at least 8 mm and exceeds the diameter of all other follicles in the wave as described by Ginther et al. (1989). The measurement was done at both sides of the interface follicular wall by taking the mean of two diameter measurements of each follicle. The data collected from both ovaries were combined for each animal.

The maximum diameter and the growth rate of the DF of the first, second or third follicular wave were also recorded. The growth rate (mm/day) of follicles was determined by dividing the increment in size of the diameter of each follicle by the total number of days taken for the observation. A follicular wave was identified to be anovulatory or ovulatory of the largest follicle (≥10 mm) when it attained a diameter of its subordinate follicles, and each DF was partitioned into growing, static and regressing phases.

Ovulation was predicted by the loss of dominance and it also coincided with the emergence of a new follicle wave. The day of ovulation, taken as the emergence of new follicles was designated as day 0. The time of ovulation was calculated by subtracting the day the DF was not observed with the day when the last DF was observed on the ultrasonography monitor. The 2- and 3-wave interovulatory intervals were classified as the interval between first wave with a dominant anovulatory follicle and the second wave with a dominant ovulatory follicle, whereas the first and second waves with a dominant anovulatory follicle and the third wave with dominant ovulatory follicle respectively (Wael 2003).

Blood collection and progesterone assays

During each ultrasound examination, 10 ml blood was collected from each cow through jugular venipunture into plain tubes (Vacutainer®, Becton Dickinson Limited, England) using a hypodermic disposable needle (4.57 mm x 0.38 mm) for progesterone hormone analyses. The blood was then kept at room temperature for 1 h and stored in a refrigerator at 4 °C for 24 h. Serum was obtained from all blood samples by centrifugation at 700 g for 20 min. The serum was decanted and kept in small bottles before it was frozen and stored in a freezer at –20 °C. The serum samples were analysed for their progesterone concentration using radio immunoassay (RIA) kits (Diagnostic Products Corporation, Los Angeles, CA 90045) and a gamma counter unit. The RIA kits contained progesterone antibody-coated tubes and buffered deionized iodine 125 (^{125}I). The sensitivity of the assay was determined as 0.02 ng/ml. The inter- and intra-assay coefficients of variations for progesterone were 6.5% and 12.9%

Artificial insemination and pregnancy diagnosis

Artificial insemination (AI) was carried out using frozen KK semen supplied by the National Institute of Veterinary Biodiversity (IBVK), Department of Veterinary Services, Jerantut, Pahang, Malaysia. The semen was deposited at the posterior part of the cervix, which was on the body of uterus. Pregnancy diagnosis (PD) was performed on day 42 post AI via rectal palpation.

Statistical analysis

The variables being tested were cow's follicular wave (FW) activities and hormone P_4 concentration. The follicular wave is described as the pattern of follicular development. The follicular wave activities related to the follicular parameters were tested using independent t-test. The data were tested for normality and homogeneity of variance before a t-test of mean comparison was conducted using SPSS version 19.0. The proportion of cows with different follicular wave activities and number of pregnant cows were tested using chi square test. Data on the concentration of progesterone, which were positive skewers, were normalized by log transformation. The non-parametric correlation of Spearman was used to determine the relationship between the number of follicular waves, oestrus interval, time of ovulation and number of pregnant cows. A confidence level of 95% or more is considered to be statistically significant.

Results and discussion

Results presented in *Table 1* shows the duration of follicular patterns, and mean duration of the follicular waves from post-CIDR removal to 1-wave, 1-wave to 2-wave, and 2-wave to 3-wave which were similar between the two types of follicular wave (p >0.05). The proportion of cows having 3-wave pattern was higher than those with 2-wave. The total mean duration of the 2- and 3-wave follicular development pattern from CIDR removal to 1-wave, 1-wave to 2-wave, and 2-wave to 3-wave were 5.9, 7.15 and 7.25 days respectively.

The day of follicle emergence, the number of emerging follicles and the day the follicles achieved the maximum diameter were similar between the 1-, 2- and 3-wave interovulatory intervals (*Table 2*). Although the follicle emergence in 3-wave pattern was 4.1 days later than in 2-wave pattern, it appeared that the DF of 3-wave pattern achieved the maximum diameter 4.3 days earlier (p >0.05) than the DF of 2-wave pattern indicating the growth rate in 3-wave was faster than in 2-wave. Thus, the findings in this study showed that the growth rate of ovulatory follicles was 1.2 ± 0.01 mm/day in 2-wave pattern compared to 1.5 ± 0.19 mm/day in the 3-wave pattern.

In addition, it was also observed that the size of DFs at post-CIDR removal of the first and second follicular waves interovulatory intervals (*Table 3*) did not

Table 1. Mean duration between waves of follicular development patterns in Kedah-Kelantan cows

Parameter	Follicular Pattern*		Total means**
	2-wave	3-wave	
Number of cows (n)	6	24	
Duration from Post-CIDR removal to the 1st wave (d)	6.4 ± 1.32	5.4 ± 2.36	5.9
Duration from the 1st to 2nd wave (d)	6.3 ± 2.04	8.0 ± 1.41	7.15
Duration from the 2nd to 3rd wave (d)	7.2 ± 0.75	7.3 ± 0.33	7.25

*Means are not significantly different between the 2- and 3-wave of follicular development at *p* >0.05 for all the parameters
**Total means = means (2-wave + 3-wave)/2

Table 2. Mean day, number of emerging follicles, and maximum diameter of follicles during follicular development in the Kedah-Kelantan cows

Follicular parameters (n, %)	Follicular Pattern*		Differences between two parameters**
	2-wave (n = 6, 20%)	3-wave (n = 24, 80%)	
Day of follicle emergence (1st wave)	2.0 ± 0.41	1.2 ± 0.25	+ 0.8
No. of emerging follicles (1st wave)	4.5 ± 0.65	5.5 ± 0.65	– 1.0
Day follicles achieved maximum diameter (1st wave)	9.8 ± 1.11	9.5 ± 1.77	+ 0.3
Day of follicle emergence (2nd wave)	9.3 ± 2.60	13.4 ± 1.86	– 4.1
Day follicles achieved maximum diameter (2nd wave)	16 ± 1.73	11.7 ± 2.86	– 4.3
Growth rate (mm/day)***	1.2 ± 0.01	1.5 ± 0.19	– 0.3

*Means are not significantly different between the 2- and 3-wave of the follicular development at *p* >0.05 for all the parameters
**Differences between two parameters = Mean (2-wave – 3-wave)
***Growth rate (mm/day) = dividing the increment of size in the follicular diameter with the total number of days taken for the observation

Table 3. Mean dominant follicle size (mm) of each follicular development pattern in the Kedah-Kelantan cows

	Follicular pattern		Total means
	2- wave	3- wave	
Size of dominant follicle (post-CIDR removal)	10.7 ± 0.09	11.3 ± 0.09	11
Size of dominant follicle (1st wave)	11.9 ± 0.17	11.1 ± 0.07	11.5
Size of dominant follicle (2nd wave)	12.3 ± 0.14	10.9 ± 0.19	11.6
Size of dominant follicle (3rd wave)	–	10.7 ± 0.11	

Means are not significantly different between the 2-and 3-wave of follicular development at *p* >0.05 for all the parameters
Total means = means (2-wave + 3-wave)/2

Table 4. Mean ± SEM of progesterone concentration (ng/ml) of the interovulatory interval of the two follicular development patterns in the Kedah-Kelantan cows

Patterns	1-wave Interovulatory interval			2-wave Interovulatory interval		
	Emergence	Selection	Dominant	Emergence	Selection	Dominant
2- wave	1.1 ± 0.19	1.5 ± 0.27	1.6 ± 0.55	2.5 ± 0.79	1.8 ± 0.36	2.5 ± 0.57
3-wave	0.6 ± 0.24	2.1 ± 0.63	2.4 ± 1.27	3.7 ± 3.29	2.8 ± 2.11	2.5 ± 0.85

Means are not significantly different between the 2- and 3-wave follicular development at $p > 0.05$ for all the parameters

differ ($p > 0.05$) with the progesterone concentration (*Table 4*, $p > 0.05$) for both the 2- and 3-wave intervals. The mean diameter of dominant follicles for 2- and 3-wave patterns ranged between 10.7 and 12.3 mm. The progesterone concentration at the time of emergence of the first DF was lower in 3-wave pattern (0.6 ng/ml) compared to 2-wave pattern (1.1 ng/ml). However, the amount of progesterone in the 3-wave pattern increased to 2.1 ng/ml on the day of selection of the first wave of interovulatory interval which continued to increase towards the end of the interovulatory follicles of the second DF.

The results showed that the differences in oestrus cycle interval and time of ovulation between 2- and 3-wave of FD were not significant ($p > 0.05$). The mean of oestrus interval and the time of ovulation were 20.2 days and 89.05 h respectively (*Table 5*). Nonetheless, the proportion of pregnant cows was not significantly different ($p > 0.05$) between the groups of cows with 2- or 3-wave pattern.

The first DF or anovulatory follicle of 2-wave was detected on day 2 post-ovulation, whereas the second DF or ovulatory follicle was detected on day 9 (*Figure 1a*). The first DF had emerged on day 1, while the second and third DFs emerged almost at the same time (*Figure 1b*). The second DF attained the maximum diameter on day 13, but the third DF continued to grow and ovulate approximately on day 22.

Studies on FD in cows and heifers indicated two (Pierson and Ginther 1988; Taylor and Rajamahendran 1991a; Taylor and Rajamahendran 1991b; Gaur and Prohit 2007), three (Savio et al. 1988; Sirois and Fortune 1988) and four (Vianna et al. 2000; Bleach et al. 2004) follicular waves during the oestrus cycles. In the present study on KK, 3-wave pattern (80%) was shown to be more predominant. Similarly, studies on native cows (Wael 2003) and Rathi cattle (Gaur and Purohit 2007) revealed that 71.4% and 21.42% of the cows showed predominant 3-wave pattern respectively. The average length of the interovulatory and oestrus intervals in the study was a day longer in the 3-wave follicular development (*Table 1*). Thus, the findings of the present study were similar to earlier ones which had reported on the mean length of the oestrus cycles which showed no significant difference between 2- and 3-wave patterns (Savio et al. 1988). On the contrary, earlier studies by Ginther et al. (1989) and Wael (2003) showed that the 3-wave pattern was 2.4 and 2.7 days longer respectively in the oestrus cycle length when compared to the 2-wave pattern.

The present study also indicated that follicle growth followed a wave-like pattern. The turnover of new follicle development could be observed in each follicular wave during the oestrus cycles. The follicles increased in size until they were 4 – 5 mm in diameter within 1.2 days in the 3-wave pattern and 2 days in the 2-wave pattern. The day the follicle reached the size of

Table 5. Mean oestrus interval, ovulation time and pregnancy rate of the Kedah-Kelantan cows with 2- and 3-wave follicular development patterns

Pattern of follicular development	N (%)	Oestrus interval (days)	Ovulation time (h)	Pregnant (n, %)
2-wave	6 (20%)	19.7 ± 0.42	85.9 ± 4.80	4 13.3%
3-wave	24 (80%)	20.7 ± 0.43	92.2 ± 3.13	14 46.7%
p value		0.09	0.30	0.66

Means are not significantly different between the 2- and 3-wave follicular development at *p* >0.05 for all the parameters

$$\text{Pregnancy rates} = \frac{\text{No. of animals pregnant in each wave}}{\text{Total number of synchronised animals}} \times 100$$

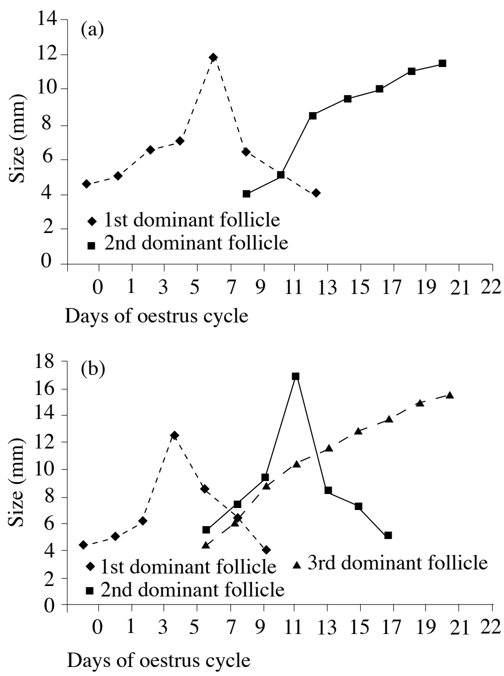

Figure 1. The growth pattern of the dominant follicle during the oestrus cycle of cows with (a) 2-dominant follicles of 2-wave, and (b) 3-dominant follicles of 3-wave

4 – 5 mm was taken as the day of follicle selection because of its FSH-dependency (Lucy 2007). The follicles containing receptors for follicle stimulating hormone (FSH) produce oestradiol which helps in maximising their size to become dominant (Fortune et al. 2004). The FSH enhances the follicles to increase in size at every stage of the oestrus cycle to reach the maximum diameter.

In the first wave of interovulatory interval, the 3-wave pattern achieved the maximum diameter 0.3 day earlier than the 2- waves although at the beginning of follicle emergence, the amount of progesterone for the 3-wave was slightly lower than the 2-wave (*Table 5*). However, starting from the stage of selection of the first wave of interovulatory interval until the second wave of interovulatory interval the amount of progesterone increased from 2.1 to 3.7 ng/ml. Hence, the amount of progesterone reflected the duration of DF to become dominant at respective sizes of 11.9 mm and 11.1 mm for 2- and 3-wave patterns. The DF took a respective 6.4 and 5.4 days for 2-and 3-wave patterns to become dominant at days 9.8 and 9.5 respectively.

In the second follicular wave, the duration from the first to the second DF was 8.0 days in 3-wave pattern compared to 6.3 days in the 2-wave pattern (*Table 1*). This was related to the delayed emergence of the second wave of interovulatory interval of 13.4 days in 3-wave pattern and 9.3 days in 2-wave pattern. However, the time the follicles became dominant was 4.3 days faster in 3-wave than 2-wave pattern. The size of the second wave follicles from 2- and 3-wave patterns were 12.3 mm and 10.9 mm (*Table 3*) which were achieved in 16 and 11.7 days (*Table 2*) respectively.

In the present study, a predominance of the 3-wave pattern was observed to be without an increase of the FW's interval length, which was similar to the 2-wave pattern. The mean length of the oestrus cycles of the 3-wave pattern was not significantly different than the 2-wave pattern (*Table 1*). The present result also agrees with the findings of Savio et al. (1988) who had previously reported that there were no significant differences in the mean length of the oestrus cycle between the 2- and 3-wave FD patterns. Meanwhile, the maximum follicular diameter, the duration of the wave and progesterone concentration of 2- and 3-wave patterns were similar to that reported by Wael (2003).

The findings of the current study have shown that the second and third DF existed almost at the same time, although the growth rate of the ovulatory follicle of the 2-wave pattern was lower than that of the 3-wave pattern. This could be due to the competition between the second and third DF to become dominant. The ability of the DF to grow was dependent on the surrounding granulosa cells to secrete oestradiol at a concentration (Fortune et al. 2004) sufficient to compromise with the appropriate hormonal reaction (Fortune 1993) through negative feedback regulation. This was followed by the production of FSH and the development of the LH receptors to produce higher LH to trigger preovulatory surge to induce ovulation.

The serum of progesterone concentration started to increase from emergence towards the end of oestrus from 1.1 – 2.5 ng/ml, and 0.6 – 3.7 ng/ml for both 2- and 3-wave patterns respectively (*Table 4*). In most studies, cows were considered anoestrus if the serum concentration or progesterone was less than 1 ng/ml (Echternkamp and Thallman 2011). The amount of progesterone produced by the cows in this study indicated that cows were cycling. However, the size of preovulatory follicles was smaller and the amount of progesterone was low. An earlier study reported that *Bos indicus* cows have small size corpus luteum (Vianna et al. 2000) that was also observed in KK cows. Thus, the present study agreed with Perry et al. (2005) who indicated that the ovulatory follicle of 12.1 mm in diameter was less likely to support pregnancy.

The present study also found that the follicular pattern did not correlate (r) with the oestrus interval ($p = 0.09$; r = 0.32), ovulation time ($p = 0.39$; r = 0.39) and the proportion of pregnant cows ($p = 0.12$; r = 0.55). This indicated that cows with 2- or 3-wave patterns were similar in terms of interval between two consecutive oestrus cycles, time of ovulation at the point after CIDR removal and the proportion of pregnant cows.

It was also revealed that the development of 2- or 3-wave patterns was not related to the proportion of pregnant cows. This study supported the finding of Bleach et al. (2004) who found that pregnancy rates were not significantly different among cows with different follicle development patterns. This was probably associated with smaller or greater ovulatory follicle size (Perry et al. 2007), the maturity characteristics of follicular fluids in the ovary and environmental conditions in the follicles (Iwata et al. 2006) which could affect pregnancy rates, late embryonic or foetal survival (Perry et al. 2007).

This further indicates that the oestrus interval, ovulation time and pregnancy rates in KK cows were not influenced by the turnover of the follicular waves and the relationship between the 2- and 3-wave pattern characteristics. These findings may be used in developing a protocol for oestrus synchronisation in TAI KK cows. Further research on KK cows is required in identifying the relationship between follicle size and pregnancy success rate in order to improve pregnancy rates and embryonic survival in KK cows.

Conclusion

Cows with 2- or 3-wave follicular development patterns did not differ in the interval between two consecutive oestrus cycles, time of ovulation, characteristics of DF size, number of follicles at the time of emergence on the first, second and third follicular wave interovulatory intervals. The results also revealed that the proportion of pregnant cows was not significantly different between cows with 2- or 3-wave follicular development patterns. Thus, the results of the present study could be used as guidelines in developing a protocol for TAI. In conclusion, there was no relationship between 2- and 3-wave follicular patterns, ovulation time and pregnancy rates during the oestrus cycle in oestrus synchronised KK cows.

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Abstrak

Pertalian antara corak 2- dan 3-gelombang folikel dengan profil hormon progesteron, tempoh estrus, masa pengovulan dan kadar kebuntingan dalam lembu betina Kedah-Kelantan(KK) telah dikaji. Sejumlah 30 ekor lembu betina KK yang terdiri dari tahap pariti pertama hingga ketiga telah dimasukkan alat lepas dadah dalaman terkawal (CIDR®) secara intravagina selama 7 hari, diikuti dengan suntikan 25 mg prostaglandin secara intraotot dua hari sebelum penyingkiran CIDR®. Kajian menunjukkan tempoh kitaran estrus dan kadar kebuntingan tidak berbeza antara 2- dan 3- gelombang folikel. Sementara itu, kemunculan folikel dalam 3-gelombang adalah 4.1 hari lebih lewat daripada 2-gelombang. Folikel dominan dalam 3-gelombang mencapai diameter maksimum 4.3 hari lebih cepat daripada 2-gelombang. Folikel dominan pada corak 2- dan 3-gelombang masing-masing mengambil masa 6.4 dan 5.4 hari untuk menjadi dominan, dan terbentuk pada hari yang ke-9.8 dan 9.5. Kepekatan progesteron semasa kemunculan pertama folikel dominan pada corak 3-gelombang lebih rendah berbanding dengan 2-gelombang. Tempoh estrus, masa pengovulan dan kadar kebuntingan lembu betina KK tidak dipengaruhi oleh corak pembangunan folikel 2- atau 3-gelombang. Corak gelombang folikel ovari tidak mempunyai pertalian yang signifikan ke atas masa pengovulan dan kadar kebuntingan lembu betina KK yang estrus diselaraskan.

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