

## **The effects of different timing of artificial insemination, the association of uterine tone and site of semen deposition on pregnancy rate following oestrus synchronisation in beef cattle**

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### **Abstract**

The pregnancy rate following induced ovulation at different timing of artificial insemination (TAI), and the association of uterine horn tone and site of semen deposition on pregnancy rate in Kedah-Kelantan (KK) and crossbred beef cows were studied. The cows were divided into three groups, each artificially inseminated either at 56 h, 65 h or 72 h following the removal of controlled internal drug release devices (CIDR<sup>®</sup>). The uterine tone was evaluated on a 4-point scale and pregnancy diagnosis was performed through rectal palpation. The percentage of cows that exhibited oestrus was not significantly different ( $p > 0.05$ ) between the groups studied. Group S56, S65 and S72 exhibited oestrus rates of 75, 73.7 and 70% respectively. Group S56 had a higher pregnancy rate compared to the other two groups. A total of 49.2, 37.3 and 13.6% cows were observed to have intermediate, extreme and slight condition of uterine tone respectively. The correlation coefficient indicates no relationship exists between TAI and the site of semen deposition, and pregnancy rate. However, a negative correlation exists between TAI and uterine tone condition. Thus, TAI can be performed between 56 h and 72 h after CIDR<sup>®</sup> removal, and uterine tone can be as indicator on the success of AI to pregnancy rate in KK and crossbred cows.

Keywords: beef cows, uterine tone, semen deposition, timed artificial insemination, pregnancy rate

### **Introduction**

The latest development of breeding protocols in cows focuses on the methods that effectively synchronise oestrus at various stages of the oestrous cycle. The method facilitates the cows to come to oestrus at a predetermined time, and enables timed artificial insemination (TAI) be performed on the cows. This method can be used as a management tool in beef cattle breeding operations as suggested by Seidel

et al. (1995). The conception rates following PGF2 $\alpha$  administration have been reported to be similar to those that have been obtained after spontaneous oestrus (Archbald et al. 1992). Oestrus cows have higher serum estradiol concentrations which effectively prepare follicular cells for luteinisation, and induce up regulation of uterine progesterone receptors (Zelinski et al. 1980). Thus, uterine progesterone receptors provide an adequate amount of uterine environment

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for pregnancy establishment and maintenance. It has been documented that the synchronisation protocol react differently depending on the stage of oestrous cycle of cows (Lamb et al. 2006), breed (Alvarez et al. 2000) and type of hormones used (Burns et al. 2008) for oestrus synchronisation.

Studies on insemination time were mostly based on recommendations by manufacturer of pharmaceutical and AI industries. The combination of CO-Synch + CIDR<sup>®</sup> protocol attained AI between 54 h and 66 h after the injection of PGF2 $\alpha$ . A few studies in the USA, Scandinavia and Spain, which involved similar protocol, have evaluated the effect of TAI with insemination times on pregnancy rates. The insemination time varied were from 48 h to 72 h calculated from the last injection of PG (Larson et al. 2006; Schafer et al. 2007; Bridges et al. 2009; Kasimanickam and Whittier 2011).

The success rate of AI also depends on the ability of the technician to pass the AI gun tip into the cervical canal and deposit the sperm into the body of uterus. During AI, uterine tone is assessed by the thickness of the digitally compressed myometrium and it is used as a diagnostic aid to estimate the stage of oestrus. Uterine tone is important in cows because the contractions of myometrium can accelerate sperm transport and storage in the oviduct to increase the chances of fertilisation (Dobrowoski and Hafez 1970; Bonafos et al. 1995; Pancarci et al. 2008). Low uterine tone contraction will lead to slower sperm transport, and reduce the potential of spermatozoa to fertilise oocytes. This will result in lower fertilisation rate and thus, increases embryonic loss (Dalton et al. 2010).

Besides uterine tone, the site where semen is deposited also plays a role in the success of AI. During AI, semen is deposited on uterine body and spermatozoa move predominantly in an anterior direction. There is evidence that a large proportion of inseminated semen got discharged from the reproductive tract within 12 h

after insemination (Mitchell et al. 1985). It has been reported that the spermatozoa will flow retrograde from the uterus to the vagina irrespective whether the semen was inseminated into the uterine body or horns. The retrograde flow was increased 2-fold when the semen was deposited into the cervical (Gallahart and Senger 1989). Nevertheless, the inseminators could also contribute to the loss of spermatozoa when they are not inconsistent in depositing the semen whether in the cervix or uterine body of the female reproductive tract.

Thus, the objective of the study was to determine the effects of fixed-time AI (FTAI), the association of uterine tone and site of semen deposition on pregnancy rate following oestrus synchronisation in Kedah-Kelantan (KK) crossbred beef cows.

## Materials and methods

The study was conducted at MARDI Kluang Station, Johor. A total of 59 KK and crossbred cows ranging from first to third calving (3 – 5 years) were used in this study. During selection, rectal examination through rectal palpation was conducted to examine the genital organs of cows to characterise the internal genital tract and ovarian structures, and diagnosis genital tract pathology. Cows were divided into three groups: S56 (n = 20) single standard dose of AI at 56 h after the controlled internal drug releasing device (CIDR<sup>®</sup>, Pharmacia & Upjohn, Australia) removal; S65 (n = 19) single standard dose of AI at 65 h after the CIDR<sup>®</sup> removal; and S72 (n = 20) single dose of AI at 72 h after the CIDR<sup>®</sup> removal.

Cows were released to graze in the paddocks after AI, but were maintained in pens, fed with pellet feed and had free access to water during oestrus observation and rectal palpation on day 45 post AI.

All cows in the three groups received a vaginal insert CIDR<sup>®</sup> containing 1.38 g progesterone for 7 days followed by an intramuscular injection of 500  $\mu$ g cloprostenol, a prostaglandin synthetic analogue (PGF2 $\alpha$ ; Estrumate<sup>®</sup>, Schering

Plough Animal Health, Australia) on day 5 following CIDR<sup>®</sup> insertion.

The cows were visually observed for oestrous behaviour at 6 h interval for 72 h beginning at 12 h after CIDR<sup>®</sup> removal. During this observation period, 5 – 6 cows in each group were kept in a pen of 5.48 m x 1.83 m. The oestrous behaviour of each cow was monitored by two trained personnels. Observation was discontinued when no successful mounts were observed. Oestrus data were recorded and combined accordingly.

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 condition of uterine tone was evaluated by a 4-point scale; extreme, moderate, slight and no tone during rectal palpation before AI as previously described by Pancarci et al. (2008). The insemination depends on the TAI and therefore, the opening of the cervix among the cows varies in sizes. Thus, the site where the semen will be deposited depends on the ability of the inseminator to pass through the tips of AI gun during the insemination. The uterine tone evaluation and AI were performed by the same personnel.

The site where semen was deposited was recorded and classified based on the placement tips of AI gun to deposit the semen into three positions: anterior os (or on body of uterus), medial or posterior of the cervix. Pregnancy diagnosis (PD) was performed on day 45 post AI via palpation per rectum. Pregnancy rate was defined as

number of cows pregnant after AI, divided by the total number of cows artificially inseminated  $\times 100$ .

### Statistical analyses

The proportion of association between treatment and pregnancy rate, frequency of AI and uterine tone were tested using non-parametric cross tabulation of chi-square ( $\chi^2$ ) analysis of SPSS version 19. The relationship between uterine tone and site of semen deposition with pregnancy rate was determined using logistic regression. Differences between treatments were considered statistically significant at  $p < 0.05$ .

### Results

Table 1 presents the number and percentage of cows that exhibited oestrus following oestrus synchronisation and the number of pregnant cows at the different timing of AI in KK and crossbred beef cows. The percentage of cows that exhibited oestrus was not significantly different ( $p > 0.05$ ) among the three groups. However, group S56 had higher cows that exhibited oestrus followed by S65 and S72 with the percentage of cows exhibited oestrus rates of 75, 73.7 and 70 respectively. Figure 1 shows that group S56 (40%, 8/20) had significantly ( $p < 0.05$ ) higher pregnancy rate than groups S72 (30%, 6/20) and S65 (21.05%, 4/19). However, the pregnancy rate which was based on total number of cows that exhibited oestrus, showed that group S56 (53.33%, 8/15) had a higher proportion of pregnant cows compared to groups S72 (42.86%, 6/14) and S65 (28.57%, 4/14).

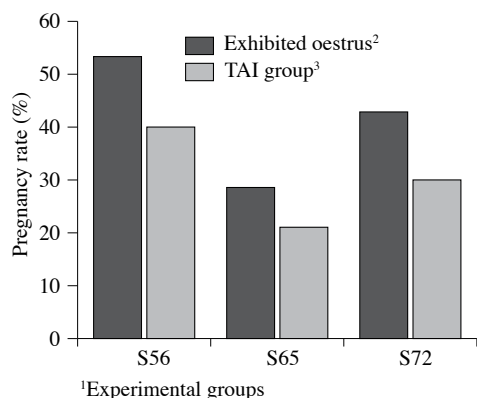
Table 1. Number of cows exhibited oestrus and pregnant at different timed AI (TAI)

Treatment Group*	Number of cows	Number of cows that exhibited oestrus	Percentage (%) of cows that exhibited oestrus
S56	20	15	75.0
S65	19	14	73.7
S72	20	14	70.0

Means are not significant among the three groups (S56, S65 and S72) at  $p > 0.05$

\*Experimental groups: S56 = TAI at 56 h after CIDR<sup>®</sup> removal; S65 = TAI at 65 h after CIDR<sup>®</sup> removal; S72 = TAI at 72 h after CIDR<sup>®</sup> removal

Table 2 shows the condition of the uterus at the time of AI. During rectal palpation, 49.2, 37.3 and 13.6% of cows were observed to have intermediate, extreme and slight condition of uterine tone respectively. None of the cows were observed to have no tone condition. Cows in groups S56 (n = 15, 25.4%) had more intermediate uterine tone, while cows in group S65 (n = 11, 18.6%) had higher proportion ( $p > 0.05$ ) of extreme uterine tone at the time of AI. During AI, 71.2% of the time, semen was deposited at the anterior



<sup>1</sup>Experimental groups:

S56 = timed-AI (TAI) at 56 h after CIDR<sup>®</sup> removal,

S65 = TAI at 65 h after CIDR<sup>®</sup> removal,

S72 = TAI at 72 h after CIDR<sup>®</sup> removal

<sup>2</sup>Pregnancy rate = (Number of cows pregnant after TAI/Total number of cows exhibited oestrus) x 100

<sup>3</sup>Pregnancy rate = (Number of cows pregnant after TAI/Total number of cows submission for TAI) x 100

Figure 1. The proportion of pregnant cows at different timing of AI, exhibited oestrus and number of cow submission for AI in Kedah-Kelantan crossbred beef cattle

os of the cervix or on the body of the uterus while 23.7% and 5.1% respectively, was deposited on the posterior and middle part of the cervix respectively (Table 3).

Table 4 shows the relationship between treatment groups, uterine tone and semen deposition at time of artificial insemination, and pregnancy rate. The correlation coefficient indicated that no relationship existed between the different timing of AI and the site of semen deposition ( $r = -0.280$ ,  $p = 0.090$ ), and pregnancy rate ( $r = -0.100$ ,  $p = 0.480$ ). However, a negative correlation existed between different timing of AI and uterine tone condition ( $r = -0.280$ ,  $p = 0.031$ ).

### Discussion

An era of cattle breeding has emerged whereby cows can be bred at pre-determined time or timed artificial insemination (TAI) (Bridges et al. 2008; Busch et al. 2008). In this study, group S56 had higher cows exhibited oestrus followed by group S65 and S72, similarly AI at 56 h attained the highest pregnancy rate followed by AI at 65 h and 72 h, although it was shown that no significant difference was observed among the groups. Based on the overall results, an assumption could be made that AI could be performed between 56 h and 72 h after CIDR<sup>®</sup> removal. Whereas, result presented by Kasimanickam and Whittier (2011) reported that an acceptable rate of pregnancy was achievable with AI of up to 72 h following CIDR<sup>®</sup> withdrawal.

The insemination time has an effect on pregnancy rates where cows inseminated at

Table 2. The distribution of uterine tone (number; %) of cows at the time of artificial insemination

Group	Fixed-time AI (after CIDR removal)	Uterine tone			Total
		Extreme	Intermediate	Slight	
S56 (n, %)	56 h	5 (8.5%)	15 (25.4%)	0 (0%)	20
S65 (n, %)	65 h	11 (18.6%)	6 (10.2%)	2 (3.4%)	19
S72 (n, %)	72 h	6 (10.2%)	8 (13.6%)	6 (10.2%)	20
	Total	22 (37.3%)	29 (49.2%)	8 (13.6%)	59

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

Table 3. Distribution of sites of semen deposition during artificial insemination

Group	Site of semen deposition during insemination (n, %)			Total
	Anterior cervix	Medial cervix	Posterior cervix	
S56 (n, %)	16 (27.1%)	1 (1.7%)	3 (5.1%)	20
S65 (n, %)	10 (16.9%)	2 (3.4%)	7 (11.9%)	19
S72 (n, %)	16 (27.1%)	0 (0%)	4 (6.8%)	20
Total	42 (71.2%)	3 (5.1%)	14 (23.7%)	59

P = 0.230

<sup>1</sup>Group A = AI 56 h after CIDR<sup>®</sup> removal; B = AI at 65 h after CIDR<sup>®</sup> removal; C = AI at 72 h after CIDR<sup>®</sup> removal

<sup>2</sup>Percentage site of semen deposition = Number of cows with particular placement of semen deposition / Total number of cows submission for timed-AI x 100

Table 4. The relationship between treatment groups, uterine tone and gun position at the time of artificial insemination, and pregnancy rate of TAI

	Treatment group <sup>1</sup>	Uterine tone during AI <sup>2</sup>	Site of semen deposition <sup>3</sup>	Pregnancy rate
<b>Uterine tone during AI</b>				
Correlation Coefficient	-0.280	1.00	0.062	-0.141
Sig (2-tailed)	0.031*		0.640	0.334
N	59	59	59	59
<b>Site of semen deposition</b>				
Correlation Coefficient	0.23	0.06	1.00	0.02
Sig (2-tailed)	0.09	0.64		0.912
N	59	59	59	59
<b>Pregnancy rate</b>				
Correlation Coefficient	-0.10	-0.14	0.01	1.00
Sig (2-tailed)	0.48	0.33	0.92	
N	59	59	59	59

\*The correlation coefficient was significant at the level of 0.05

<sup>1</sup>Treatment Group; A = AI 72 h after CIDR<sup>®</sup> removal; B = AI at 56 h after CIDR<sup>®</sup> removal; C = AI at 65 h after CIDR<sup>®</sup> removal

<sup>2</sup>Uterine tone = Extreme, moderate, and slight

<sup>3</sup>Site of semen deposition = Anterior, medial and posterior of the cervix

66 h following PG administration were 1.32 times more likely to conceive after fixed TAI than cows that were inseminated at 54 h following PG administration (Busch et al. 2008). Bridges et al. (2008) did TAI at 60 h and 72 h to compare 7-day versus 5-day CO-Synch + CIDR protocol. In their study, cows that were treated with 5d CO-Synch + CIDR following timed AI concurrent with GnRH at 72 h after CIDR withdrawal showed improved pregnancy rates compared

to a 7d CO-Synch + CIDR programme with timed AI at 60 h after CIDR withdrawal.

The oestrous cycling status affected the oestrous response and pregnancy rates prior to treatment initiation. In the present study, it was observed that oestrus response was proportionate to the pregnancy rates in all the TAI groups. This indicates that cows exhibited oestrus prior to TAI has a relationship to the pregnancy outcome. In the study that observed by Busch et al.

(2008), hypothesised cows that exhibited oestrus prior to TAI had significantly higher pregnancy rates than cows that did not exhibit oestrus before TAI (Busch et al. 2008). Kurykin et al. (2007) found that the pregnancy rate for heifers showing strong signs of oestrus was 25.1% higher than heifers showing weak sign. This indicated that oestrus was a good indicator for the success of AI. The oestrus response was similar in cycling and anoestrous cows (Busch et al. 2008). However, the pregnancy rate of TAI was influenced by oestrus cycling status of cyclic or anoestrous cows (Wilson et al. 2010). Cows that exhibited oestrus had greater serum estradiol concentration necessary to effectively prepare follicular cells for luteinisation, and (or) induced up regulation of uterine progesterone receptors (Zelinski et al. 1980). The condition provides an adequate amount of uterine environment for pregnancy establishment and maintenance.

The correlation coefficient shows that there is no relationship between different timing of AI, site of semen deposition and pregnancy rate. However, a negative correlation existed between different timing of AI and uterine tone condition. The results indicated that when time of AI increased, the condition of uterine tone will decrease, from extreme to slight tone. However, when determining the relationship between different time of AI and uterine tone, it could be interpreted that the time for conducting AI was suitable between 56 h and 72 h after CIDR<sup>®</sup> removal. The finding is in agreement with the result presented by Kasimanickam and Whittier (2011), and Bridges et al. (2009) who reported that the acceptable AI pregnancy rate was achievable with AI up to 72 h after CIDR<sup>®</sup> removal.

The uterine tone plays a major role in performing AI for pregnancy success in cows. The ability of the myometrium to contract will accelerate the transportation of spermatozoa to oviducts and facilitate fertilisation (Dobrowski et al. 1970; Bonafos et al. 1995; Pancarci et al. 2008). The

deposited frozen-thawed semen contains lower concentration and have shorter life span. Thus, the uterine tone should be in the extreme condition in order to accelerate the spermatozoa to arrive on time at the fertilisation site (Dalton et al. 2010). A decrease in uterine tone leads to a slower sperm transport, and thus produces aged spermatozoa at the fertilisation site. Fertilisation by aged and low potential spermatozoa will result in embryonic loss (Dalton et al. 2010).

In this study, the site of semen deposition did not correlate with pregnancy rate. Similarly, an assessment of three sites semen deposition on heifer pregnancy rate did not differ significantly whether the sex-sorted sperms were deposited near the utero-tubal junction, in the middle of the horn or into the uterine body (Kurykin et al. 2007). The pregnancy rate achieved was 43.1% and did not differ between the sites of sperm deposition (Kurykin et al. 2007). Therefore, based on the overall results, it is concluded that AI can be performed between 56 h and 72 h after CIDR<sup>®</sup> removal.

### **Conclusion**

The use of fixed-time artificial insemination governs an improvement of oestrus synchronisation for the ease of cattle management and can reduce labour costs which are associated with oestrus detection. The protocols used in the present study required further refinement before they can be adopted for breeding of local beef cows. Perhaps, it could be further tested in terms of animal types, stages of oestrous cycle or additional GnRH. The protocols, however, have a potential to be used for oestrus synchronisation and fixed-TAI programme for use in beef cattle production systems. Thus, it can be concluded that TAI can be performed between 56 h and 72 h after CIDR<sup>®</sup> removal and uterine tone can be used as a diagnostic aid during AI in KK crossbred beef cows.

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### Abstrak

Kadar kebuntingan (PR) selepas ovulasi teraruh dan permanian beradas secara tepat masa (TAI) dilakukan pada masa yang berbeza, dan hubungan antara tonus tanduk rahim dengan tapak meletakkan air mani ke atas PR pada lembu pedaging Kedah-Kelantan (KK) dan kacukannya telah dikaji. Lembu dibahagikan kepada tiga kumpulan di mana TAI masing-masing dilakukan pada 56, 65 dan 72 jam selepas alat lepas dadah dalaman terkawal (CIDR<sup>®</sup>) dikeluarkan. Tonus rahim dinilai pada 4-titik skala diagnosis kebuntingan dilakukan melalui palpasi rekta. Peratus lembu betina yang mempamerkan kelakuan estrus tidak berbeza secara signifikan ( $p > 0.05$ ) antara kumpulan yang dikaji. Kumpulan S56 menunjukkan lembu betina yang mempamerkan estrus yang tinggi diikuti oleh S72 dan S65 masing-masing dengan peratusan 75, 73.6 dan 70%. Kumpulan S56 mempunyai PR yang tinggi berbanding dengan dua kumpulan lain. Sebanyak 49.2, 37.3 dan 13.6% lembu betina masing-masing mempunyai tonus uterus yang sederhana, melampau dan sedikit. Korelasi koefisien merumuskan tiada perhubungan yang bererti di antara masa AI yang berbeza dan tempat meletakkan semen dan kadar kebuntingan. Walau bagaimanapun, negatif korelasi wujud antara keadaan tonus uterus dengan masa AI yang berbeza. Kesimpulannya, kajian ini menunjukkan bahawa TAI boleh dilakukan di antara 56 – 72 jam selepas penyingkiran CIDR<sup>®</sup>, dan tonus rahim boleh dijadikan sebagai petunjuk dalam menentukan kejayaan AI kepada PR dalam lembu pedaging kacukan KK.