Pregnancy Rate from Timed-artificial Insemination and Natural Service of Oestrus Synchronized Kedah-Kelantan and Crossbred Cows in Two Management Systems

Azizah[1]*, A., Ariff[2], O.M., Ahmad Nazri[3], A.R., Wahid[4], H., Ahmad[5], J. and Saadiah[1], J.

[1]Strategic Livestock Research Centre, MARDI Serdang, Selangor, [2]Department of Preclinical Veterinary Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor, [3]Ruminant Section, Livestock Commodity Development Division, Department of Veterinary Services Malaysia, Putrajaya, [4]Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor, [5]Strategic Livestock Research Centre, MARDI Kluang Station, Johor

*Corresponding author: aziamri@mardi.gov.my

Abstract

Improved reproductive efficiency is the outcome of herd management and could involve a decision to choose between artificial insemination (AI) and natural service (NS). The objective of the present study was to determine the time of ovulation and pregnancy rate (PR) following timed AI (TAI) and NS, and frequency of TAI of two different management systems in oestrus synchronized Kedah-Kelantan (KK) and KK crossbred (CB) cows. Cows were assigned to two management systems: pen (A, n=80) and pasture (B, n=70). The cows were synchronized with controlled internal drug release (CIDR) device for 7 d, and injected intramuscularly with 500 µg of synthetic prostaglandin (PGF) analogue 2 d prior to CIDR withdrawal. Seventeen KK and 47 CB cows were scanned for follicular mapping to determine the ovulation time. Cows were later bred in A using NS (n=20) or TAI at 56 h (ATAIS56, n=20), 65 h (ATAIS65, n=20) and 72 h (ATAIS72, n=20) and in B using TAI at 56 h (BTAIS56, n=16), 65 h (BTAIS65, n=18) and 72 h (BTAIS72, n=16) following CIDR withdrawal. KK and CB cows were then compared for number of AI services conducted: one service (S56S, n= 20) and two services (S56D, n= 20) of TAI. Mean ovulation time of the cows studied was 85.1±1.6 h. Cows managed on pasture and inseminated at a fixed time of 65 h showed higher (22%, p>0.05) PR compared with the other groups studied. The percentage of pregnant cows managed on pasture was higher (p>0.05) for cows bred with TAI at 65 h than at 56 h and 72 h and all groups under NS kept in the pen. There was no significant difference between TAI and NS groups managed on pasture. Pregnancy rate of cows with one-service TAI (25%) was higher than those with two-service TAI (20%) done at 56 h and 96 h. It can be concluded that ovulation occurs at 85.1 h after CIDR withdrawal, and the pregnancy rate is not affected by the management system and the number of services of TAI.

Key words: Pregnancy rate, timed artificial insemination, natural service, management systems, Kedah-Kelantan cows

Introduction

In ordinary dairy and beef cattle practices, the time of insemination varies with the time of standing oestrus shown by the cow. The technique usually follows the morning/evening (am/pm) breeding rule (Trimberger, 1948; Selk, 2002), which is considered to be the most favourable practice used in the artificial insemination (AI) programme for breeding cattle in many countries including Malaysia.

AI is capable of controlling a breeding programme with the choice of semen from genetically superior bulls and in reducing cost of operation by not having to purchase and maintain bulls. The AI method also protects cows from venereal diseases and hence, leads to an improvement in management and increase in productivity (Valergakis *et al.*, 2007). The breeding has also become more flexible when the semen from different breeds can be selected based on the farm's preference.

In the Malaysian cattle industry, the impact of AI is to obtain animals with higher milk yield and growth potential, besides to produce animals which are less susceptible to diseases and parasites and having higher degree of adaptability to local climate compared to the imported animals of temperate breeds. AI has seen a decrease in more than a decade since 1990 with a modest recovery experienced in 2001 and the coverage was at 4.2% in 2009 (Raymond and Saifullizam, 2010). Therefore, to improve the genetic component of cattle population in Malaysia, the estimated AI coverage needs to increase by 10% annually (Raymond and Saifullizam, 2010). Timed Artificial Insemination (TAI) has the potential of enhancing the AI coverage by way of reducing the cost of AI.

However, several studies have reported that 50% of the oestrus-synchronized cows were observed not presenting any oestrus signs (Roelofs *et al.*, 2005) because the signs were displayed in a very short time, thus, producers had difficulty in accurately identifying cows in oestrus. Oestrus observation is one of the important factors affecting the acceptable levels of reproductive performance in dairy farms if it could be done accurately (Nebel and Jobst, 1998). Thus, most cattle producers in the tropical region such as Malaysia practice natural service (NS) using bulls as their choice for breeding cattle in order to reduce the cost of oestrus observation.

The efficiency of reproduction depends on reproduction parameters such as calving interval, dry period, first service conception, percentage of cows in lactation and milk production (Smith *et al.*, 2004). The comparison of cost effectiveness between AI and NS, which were related to the reproduction parameters such as pregnancy rates (PR), shows that AI is more profitable compared to NS even in average management condition (Valergakis *et al.*, 2007). AI has been argued of its additional cost from the extended calving interval because of poor oestrus observation and thus, NS can result in higher conception (Overton, 2005) and pregnancy rate (Niles *et al.*, 2002).

AI at predetermined time named as timed AI (TAI) or fixed timed AI without oestrus observation has been attempted (Wilson *et al.*, 2010). For TAI to work efficiently oestrus synchronization is required to facilitate animal management. Thus, the approach to TAI requires knowledge of follicular development especially on turnover of follicles as measured by ultrasonography in order to determine the ovulation time for optimum timing of TAI on farm animals (Pursely *et al.*, 1995).

The major aim of this study was to fit the protocol of oestrus synchronization on the timing of ovulation in oestrus synchronized cows that could be used in TAI protocol in local beef cattle herds. Therefore, the objective of the study was to determine the ovulation time, time of service for TAI and pregnancy rate following TAI and NS, and frequency of TAI services in oestrus synchronized Kedah Kelantan and crossbred cows managed in two different management systems.

Materials and Methods

Animal management and treatment

The study was conducted in MARDI Kluang Station, Johor from June 2010 to December 2011. The cows were raised in two management systems: pen (A, n=80) and pasture (B, n=70) and were bred using natural service (NS) or timed AI (TAI) at different intervals post CIDR removal. In the first experiment, a total of 64 cows of Kedah-Kelantan (KK, n= 17), Brahman-KK (BK, n= 18), Charolais-KK (CK, n= 18) and mixed KK crosses, (MK, n= 11) were used to monitor ovarian follicular development to determine the ovulation time in the different breedtypes. In the second experiment, 150 KK and KK- crossbred (CB) cows at varying stages of oestrous cycles, aged 3 to 5 years, and ranging from first to third calving were used and divided into eight treatments: 1) cows raised in A and bred using NS (ANS, n=20), 2) cows raised on pasture and bred using NS (BNS, n=20), 3) cows raised in A and bred using TAI at 56 h after CIDR removal (ATAI56, n=20), 4) cows raised in B and bred using TAI at 56 h after CIDR removal (BTAI56, n=16), 5) cows raised in A and bred using TAI at 65 h after CIDR removal (ATAI65, n=20), 6) cows raised in B and bred using TAI at 65 h after CIDR removal (BTAI65, n=18), 7) cows raised in A and bred using TAI at 72 h after CIDR removal (ATAI72, n=20) and 8) cows raised in B and bred using TAI at 72 h after CIDR removal (BTAI72, n=16). The cows were kept in each management system for 14 d before oestrus synchronization, and were released after rectal palpation was performed on day 60 after AI. The insemination was performed at 56, 65 and 72 h after the CIDR was removed. Cows in the NS group in the A and B management systems were kept with bulls for 5 d beginning from 12 h after CIDR withdrawal. The cows were released after rectal palpation was performed on day 60 after AI.

In the third experiment TAI at 56 h was chosen as a treatment based on its higher and consistent result compared to TAI at 65 and 72 h after CIDR withdrawl shown in the second experiment to evaluate the frequency of services of single and double doses of TAI at 56 h after CIDR removal on pregnancy rate (PR). Forty cows were used and divided into two groups: 1) cows receiving one dose of AI at 56 h after the CIDR withdrawal (56S, n=20) and 2) cows receiving two doses of AI at 56 h after the CIDR withdrawal (56D, n=20).

Synchronization of oestrus

All cows selected for the study were at random stages of oestrous cycles and each received a controlled internal drug release (CIDR; Pharmacia & Upjohn, Australia) device containing 1.38 g progesterone- for 7 d and followed with intramuscular injection of 500 µg synthetic prostaglandin analogue (PGF; Estrumate®, Schering – Plough Animal Health, Australia) on day 5 following CIDR insertion. The synchronization of oestrus protocol is shown in Figure 1.

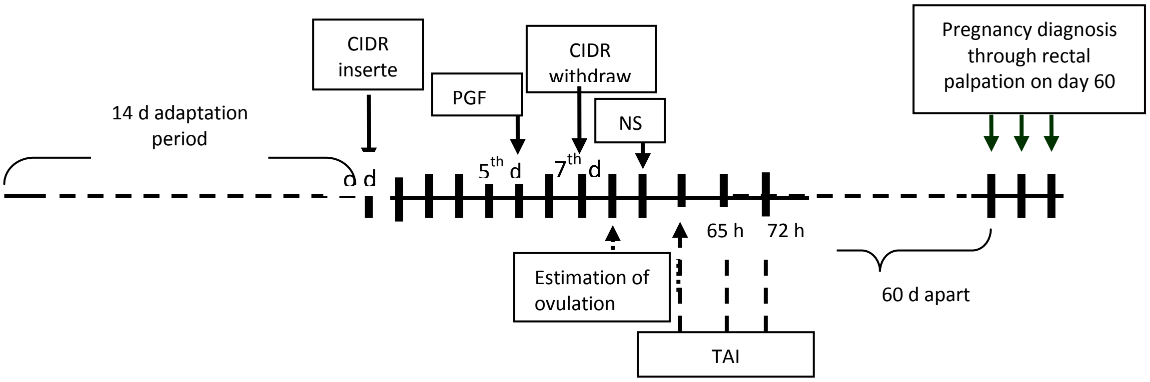


Figure 1: Experimental protocol for oestrus synchronization and breeding choice using natural service (NS) and timed AI (TAI) at 56, 65 and 72 h after CIDR removal

Ovarian ultrasonography and follicular mapping

Ovarian ultrasonography was carried out with a 7.5 MHz linear array transducer attached to the portable ultrasound (Aloka®, SSD-500). The transducer was lubricated with hypoallergenic gel and inserted into the rectum in lateral-medial and dorso-ventral planes to determine the position of the follicle in the ovaries. A follicle greater than 4 mm in diameter was obtained by freezing its image, followed by its measurement with electronic callipers at the interface of the follicular wall with ovarian stroma. For the non-spherical shape, the largest and the smallest widths were measured, and the average width was recorded. Ovarian data were combined for the two ovaries of each cow. The size and relative dimension of the follicles and corpus luteum (CL) were sketched on a follicle map.

The scanning was carried out initially from 12 h after CIDR withdrawal and was done at 6-h intervals until the dominant follicle (DF) ceased. Impending ovulation was predicted by ultrasonic evidence of increased intrauterine fluid, heterogenous uterine echo texture, a regressing CL, and a large preovulatory follicle as described by Pursely *et al*. (1995). Ovulation was confirmed by the collapse of large DFs with sizes greater than 10 mm in diameter and subsequent formation of a CL in the same location in the ovary. The time of ovulation in hour was then recorded. Time of ovulation was determined from the time when CIDR was withdrawn until the large DF collapsed and CL was obtained during the ultrasonogarphy scanning.

Management of bulls for natural service

The bulls underwent and passed the Breeding Soundness Examination prior to their selection for breeding. The average scrotal circumference size of the fertile bulls was 23.5 cm, consistency in palpation score was 2-3 and libido score was 9 out of 10 following the criteria described by Sylvia (2014). The cows in NS groups were mated to proven KK bulls in a breeding ratio of 1 bull to 20 cows 12 h after CIDR withdrawal.

Artificial insemination and pregnancy diagnosis

The time of ovulation calculated earlier was used to conduct TAI. Cows were inseminated with KK frozen thawed semen at 56, 65 and

72 h after CIDR withdrawal. Semen was deposited either in the uterine body or close to the middle part of the uterine horn ipsilateral to the ovary bearing the DF during AI. Pregnancy diagnosis (PD) was performed via rectal palpation on day 60 after AI.

Cows maintained in the intensive pen management system were fed with concentrate pellet feed and had free access to water. Concentrate pellet feed containing 15.9% crude protein and 17.6 MJ calculated gross energy was offered to the cows based on the maintenance requirement of beef cows (ARC, 1980) at a rate of 1 kg per 100kg bodyweight per day in addition to the estimated intake of feed from grasses. Cows kept in the pasture system were released in the paddock for grazing on improved signal grass (*Brachiaria decumbens*) immediately after AI, and were diagnosed for pregnancy via rectal palpation on day 60 after AI.

Statistical analyses

The ovulation time data for breedtype comparison were analyzed using analysis of variance using SAS version 9.3 (SAS Institute, Cary NC). Frequency of pregnant cows and time of service were analyzed using chi-square test for non-parametric data.

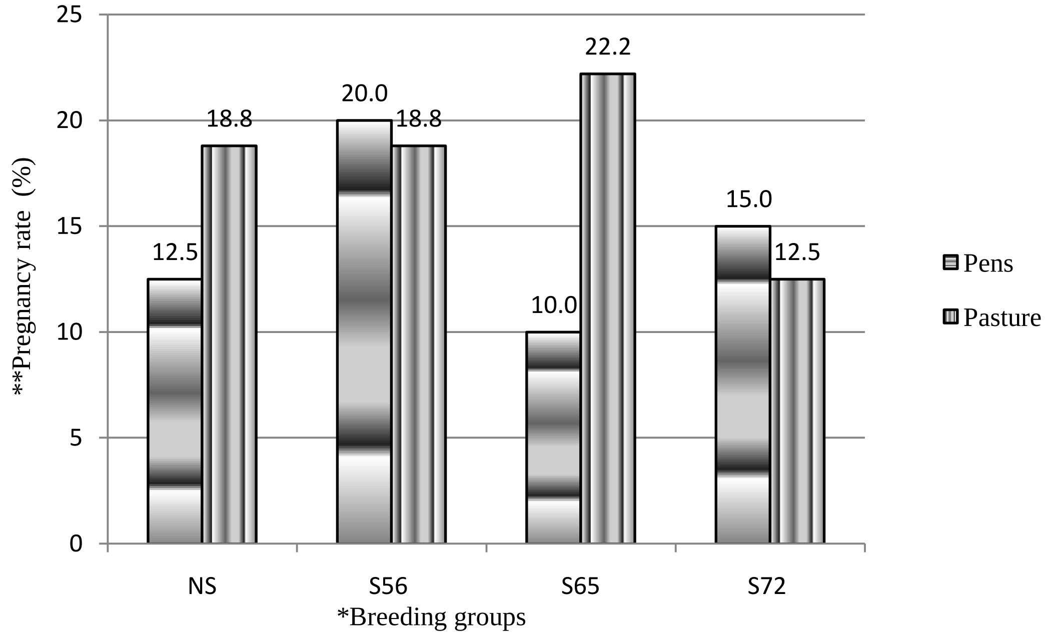
Results and Discussion

The mean ovulation time was not significantly different (p>0.05) among the four breed types (mean of 85.1±1.6 h and range of 57.7 to 119.9 h, Table 1). The mean proportion of cows which exhibited oestrus was 82.8%, ranging from 77.8% in CK to 88.23% in KK cows. The proportion of pregnant cows raised in pens in the intensive system had higher (p>0.05) PR compared to cows in the pasture group (Figure 2). This suggested that management system did not affect pregnancy rate of the cows. Cows in the group TAI56 (19.4%; Figure 2) had a higher proportion of pregnant cows compared with groups TAI65 (10.1%) and TAI72 (13.8%). Similarly, mean PR was higher (p>0.05) in the TAI56 group (19.4%) than NS group (15.7%). The results of the study showed PR of cows in the 56S group was higher (25%, p>0.05) compared with those in the 56D (20%).

Table 1: Mean ovulation time of different breedtypes of beef cattle

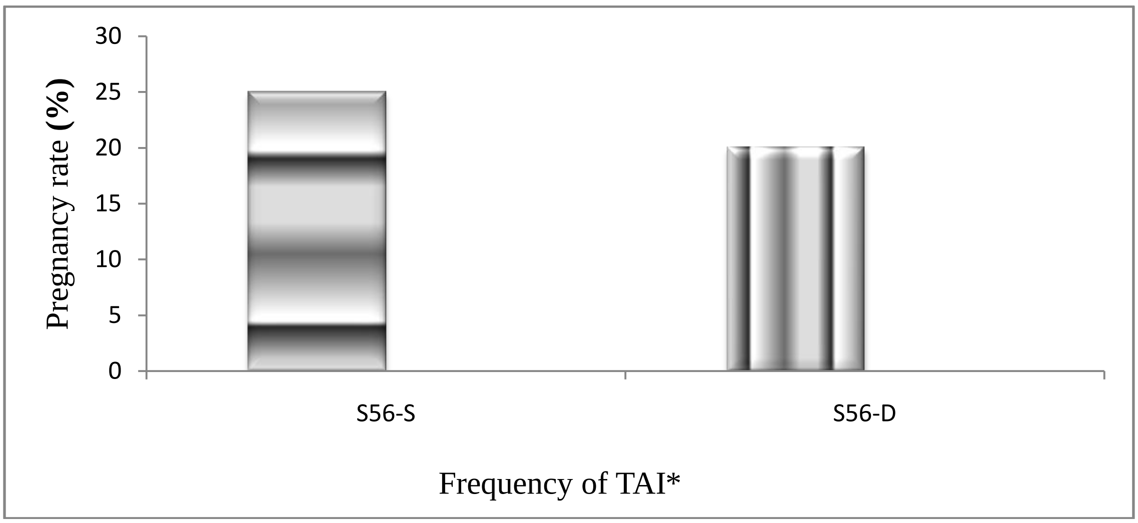
Breedtype	No.	% in oestrus	Ovulation time (h)	Range (h)
KK	17	88.23	85.3±2.9	70.2 – 119.9
BK	18	77.78	88.1±3.7	57.7 - 114.0
CK	18	83.33	84.9±3.3	63.3 - 110.2
Mixed	11	81.81	80.2±0.9	77.2 - 85.7
Mean	64	82.79	85.1±1.6	57.7 - 119.2

*No significant differences between breedtypes (p>0.05)



*Breeding groups: NS = Natural service, S56, S65 and S72 = TAI at 56, 65 and 72 h after CIDR withdrawal, respectively

Figure 2: Pregnancy rate of KK crossbred cows artificially inseminated at fixed time raised in two management systems



*S56-S = Single artificial insemination at 56 h after CIDR withdrawal, S56-D = Double artificial insemination at 56 and 96 h after CIDR withdrawal

Figure 3: Pregnancy rate of different frequencies of timed artificial insemination in KK and KK crossbred cows

The breeding practice in the present study has started a new era of breeding technique whereby the animals could be bred without observing for oestrus behaviour as was earlier proposed by Pursely *et al*. (1997). It has also refined the development of reproductive biotechnology by including the use of ultrasonography to identify the occurrence of ovulation (Pursely *et al*., 1995).

The synchronization of oestrus protocol in this study used CIDR containing 1.38 g progesterone to synchronize the oestrous cycle and increase the number of animals bearing the functional CL through increasing progesterone secretion following AI. The injection of PGF is to induce luteolysis of the corpora lutea. Following CIDR withdrawal, the amount of serum progesterone would then increase thus allowing the dominant follicle to ovulate.

KK presented a higher percentage of cows exhibiting oestrus. However, the proportion of cows which exhibited oestrus was not significantly different between the breedtypes studied ($p>0.05$). Of the 64 cows from the four breedtypes synchronised, 82.79% exhibited oestrus. Cows exhibiting oestrus prior to TAI had been reported to show significantly higher PRs (Busch *et al*., 2008), and the expression of oestrus sign was suggested to be a good and prominent indicator to prophesise the success of TAI. Cows which exhibit oestrus have greater serum estradiol concentration which is necessary to effectively prepare follicular cells for luteinisation, and induce an adequate number of uterine P_4 receptors (Zelinski *et al.*, 1980). This condition provides an adequate amount of uterine environment for pregnancy establishment and maintenance.

In developing TAI, the present study evaluated the time of ovulation in KK and CB cows. The mean time of ovulation of the four beef cattle breedtypes was 85.1±1.6 h calculated from the time of CIDR withdrawal. The mean time of ovulation was taken in order to estimate the optimum time for AI, and therefore, AI could be done before projected ovulation in such a way to yield a higher PR (Parrish and Foote, 1986). The optimum time to AI in this study was estimated between 12 to 30 h before projected ovulation or 56 to 72 h calculated from when the time CIDR was removed, which could be a recommended window of time relative to ovulation.

A few studies have demonstrated that ovulation occurred at 31.1 h after the onset of oestrus in PGF-induced protocol (White *et al*., 2002), or between 24 to 32 h after the second GnRH injection of Ovsynch protocol (Pursely *et al*., 1998). Therefore, the optimum time for AI was suggested to be between 4 to 24 h before ovulation (Yadav *et al*., 1986). Consequently, cows inseminated before oestrus had lower PR compared to cows inseminated after oestrus (Kasimanickam and Whittier, 2011).

PRs obtained in cows on TAI 56 h after CIDR withdrawl were not significantly different from those in the NS group. This finding agrees with Lima *et al*. (2008) who found no difference in PR of lactating dairy cows bred between first and second service by NS (27.36 and, 24.03%, respectively) and AI (27.06 and 29.56%, respectively) in the warm season. Nonetheless, the current finding disagrees with Niles *et al*. (2002) with PR for NS was reported to be higher than AI at observed oestrus in a heat stress condition. From this study, it can be postulated that if TAI were done at the correct receptive window relative to ovulation, the proportion of cows becoming pregnant could be higher than what was obtained in NS. This indicates that within five days of breeding time, TAI is capable of producing a higher number of pregnant cows while NS has a limitation in the number of females mated within a given time.

The results obtained from this study suggest that the time of AI can be carried out between 56 to 72 h after CIDR withdrawal. This is in agreement with the result presented by Kasimanickam and Whittier (2011) who reported that the acceptable AI pregnancy was achievable with AI up to 72 h after CIDR withdrawal with Angus cows inseminated at 67 h presented higher fixed TAI pregnancy rate (44.4%) compared to cows inseminated at 47 h (33.3%). The finding could be related to the phenomenon that when an ovum ovulates, it takes some time to arrive at ampula for fertilization process. As illustrated in Figure 2 the 56S group of 1-dose AI had 25% of the cows pregnant compared with 20% of cows pregnant in the 56D group receiving two doses of AI. No significant difference was detected in the percentage of cows pregnant between inseminated once or twice. Inseminating at 96 h after CIDR withdrawal is too late because the ovulation would have occurred and there is a possibility that the sperm would fertilize a low quality ovum. Lower PRs were also observed when AI was carried out after the time of ovulation (Pursely *et al.*, 1998). Cows bred after ovulation was beyond the optimal time for AI (Wiltbank *et al.*, 2000), and might also be attributed to the low conception rate (Demiral *et al*., 2006). The variability of TAI in these studies could be related to the random stages of oestrous cycles during synchronization, because the effect of $PGF_{2\alpha}$ depends on the stage of development of the DF (Monteiro *et al*., 2009), and the capability of PGF to induce regression of the CL.

An insemination which is conducted later than the optimum time causes the ovum to shift from high to low quality due to aging and consequently causing low fertilization rate and lower embryo quality (Dalton *et al*., 2010). Hence, the timing of insemination is a combination and balance among time of ovulation, time needed for sperm transportation and capacitation, and the longevity of sperm in the reproductive tract to maintain competency in fertilization (Kasimanickam and Whittier, 2011).

The present study also determined that the pregnancy percentage was not influenced by the micro-environment where the animals were placed. Similarly, Busch *et al*. (2008) found that PRs following TAI at 66 h were consistent at two locations over two years. However, geographic location was found to have an influence on PRs of TAI (Larson *et al*., 2006). Therefore, the short period of the present study found that the ability of cows to become pregnant was not affected by the management systems.

Lower PRs in the NS and TAI groups in this study could have been caused by several factors. Cows of *Bos indicus* sp. have been known to exhibit smaller size CL and DFs compared to *Bos taurus* cows (Sartori and Barros, 2011). Hence, ovulation of small DFs was postulated to lead to continuing reduction of circulating estradiol and progesterone serum concentration (Atkins *et al*., 2010), which then resulted in impaired fertility, higher incidence of pregnancy loss and late embryonic or fetal loss (Perry *et al*., 2005), and reduced PRs (Perry *et al*., 2005) in beef and dairy cattle.

The lower PR following TAI obtained in the present study could be due to the occurrence of anoestrous cows or body condition that could reflect inadequate supply of energy and protein to maintain above average body condition score. Lower nutrition levels could affect the follicles' ability since larger diameter ovulatory follicles had been found to improve fertility (Perry *et al*., 2007) and affected the characteristics of follicular fluid and environmental condition in the follicles (Iwata *et al*., 2006) which could result in lower PRs. Other factors such as failure to conceive, early embryonic death (Sartori and Barros, 2011), and post-thaw process, semen quality and types of semen used(Kasimanickam and Whittier, 2011), seasons or genetic background (Osemi, Misztal and Tsurute, 2005) may also contribute to low PRs.

Conclusion

The present study has shown that mean ovulation time among KK and KK crossbred cattle was 85.1±1.6 h and the appropriate time to perform AI was between 56 to 72 h after CIDR withdrawal. The proportion of pregnant cows was not influenced by the management system in which they were raised. Therefore, it is recommended that the time for insemination among KK and KK crossbred cattle to be between 56 to 72 h after CIDR withdrawal.

References

Agricultural Research Council (ARC). 1980. The nutrient requirements of ruminant livestock. Commonwealth Agricultural Bureaux, Slough, 351.

Atkins, J.A., Smith, M.F., Wells, K.J. and Geary, T.W. 2010. Factors affecting preovulatory follicle diameter and ovulation rate after gonadotropin-releasing hormone in postpartum beef cows. Part I: Cycling cows. J. Animal Science, 88: 2300-2310.

Busch, D.C., Schafer, D.J., Wilson, D.J., Mallory, D.A., Leitman, N.R., Haden, J.K., Ellersieck, M.R., Smith, M.F. and Patterson, D.J. 2008. Timing of artificial insemination in postpartum beef cows following administration of the CO-synch + controlled internal drug release protocol. J. Animal Science,. 86: 1519-1525.

Dalton, J.C., Nadir, S., Noftsinger, M. and Saacke, R.G. 2010. Insemination related factors affecting fertilization in estrus-synchronized cattle. Proc. Applied Reproductive Strategies in Beef Cattle, San Antonio, Texas, pp 193-205.

Demiral, O., Un, M., Abay, M., Bekyurekand, T. and Ozturk, A. 2006. The effectiveness of cosynch protocol in dairy heifers and multiparous cows. Turkish J Vet. Anim. Sci. 30: 213-217.

Iwata, H., Inoure, J., Kimura, K., Kuge, T., Kuwayama, T. and Monji, Y. 2006. Comparison between the characteristics of follicular fluid and the developmental competence of bovine oocytes. Anim. Reprod. Sci. 91: 15-23.

Kasimanickam, R.K., and Whittier, W.D. 2011. Effect of estrus-AI interval on timed AI pregnancy rates in beef cows inseminated with fresh extended or frozen thawed semen. Vet. World 4(6): 245-247.

Larson, J.E., Lamb, G.C., Stevenson, J.S., Johnson, S.K., Day, M.L., Geary, T.W., Kesler, D.J., Dejarnette, J.M., Schrick, F.N., DiCostanzo, A. and Arseneau, J.D. 2006. Synchronization of oestrus in suckled beef cows for detected oestrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin $F_{2\alpha}$, and progesterone. J. Animal Science, 84: 332-342.

Lima, F., De Vries, A., Thatcher, M.J., Risco, C.A. and Thatcher, W.W. 2008. Direct comparison of natural service vs. timed AI: Reproductive efficiency and economics. Proc.45th Florida Dairy Production Conference, Gainesville, April 29, 2008 pp 54-65.

Monteiro, F.M., Meloa, D.S., Ferreira, M.M., Carvalho, L.M., Sartoreli, E., Ederhardt, B.G., Gde Nogueira, P. and Barros, C.M. 2009. LH surge in Nelore cows (*Bos indicus*), after induced estrus or after ovarian superestimulation. Anim. Reprod. Sci. 110(1-2): 128-138.

Nebel, R.L. and Jobst, S.M. 1998. Evaluation of systematic breeding programs for lactating dairy cows: A review. J. Dairy Science, 81: 1169-1174.

Niles, D.C.A., Risco, C.A. and Thatcher, M.J. 2002. Seasonal evaluation of artificial insemination and natural service pregnancy rates in dairy herds. Compend Contin Educ. 44: S44-S48.

Overton, M.W. 2005. Cost comparison of natural service sire and artificial insemination for dairy cattle reproductive management. Theriogenology, 64: 603-613.

Parish, J.J. and Foote, R.H. 1986. Fertility of cooled and frozen rabbit sperm measured by competitive fertilization. Biol. Reprod. 35: 253- 257.

Perry, G.A., Smith, M.F., Roberts, J.A., MacNeil, M.D. and Geary, R.W. 2007. Relationship between size ovulatory follicle and pregnancy success in beef cattle. J. Animal Science, 85: 684-689.

Perry, G.A., Smith, M.F., Lucy, M.C., Green, J.A., Parks, T.E., MacNeil, M.D., Roberts, A.J. and Geary, T.W. 2005. Relationship between follicle size at insemination and pregnancy success. Proceedings of the National Academy of Science of the United States of America 102(14): 5268 -5273.

Pursely, J.R., Mee, M.O. and Wiltbank, M.C. 1995. Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology, 44: 915-923.

Pursely, J.R., Kosorok, M.R. and Wiltbank, M.C. 1997. Reproductive management of lactating dairy cows using synchronization ovulation. J. Dairy Science, 80: 301-306.

Pursely, J.R., Silcox, R.W. and Wiltbank, M.C. 1998. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronisation of ovulation in lactating dairy cows. J. Dairy Science, 81: 2139-2144.

Raymond A.K. and Saifullizam A.K. 2010. The cattle artificial insemination industry in Malaysia. Mal. J. Vet. Res.1(1): 1-7.

Roelofs, J.B., Van Eerdenburg, F.J.C.M., Soede N.M. and Kemp, B. 2005. Various behavioral signs of estrus and their relationship with time of ovulation in dairy cattle. Theriogenology, 63: 1366–1377.

Sartori, R. and Barros, C.M. 2011. Reproductive cycles in *Bos indicus* cattle. Anim. Reprod. Sci. 124: 244-250.

SAS/STAT® 9.3 User's Guide. 2011.

Selk, G. 2002. Artificial insemination for beef cattle. Retrieved April 8, 2009, from www.thecattlesite.com/articles/721/artificial-insemination-for-beef-cattle/print

Smith, J.W., Ely, L.O., Gilson, W.D. and Graves, W.M. 2004. Effects of Artificial Insemination vs Natural Service Breeding on Production and Reproduction Parameters in Dairy Herds. The Prof. Anim. Sci. 20: 185-190.

Sylvia, J.B. 2014. The Merck Veterinary Manual. Breeding Soundness Examination of Bulls. Retrieved October 1, 2014, from www.merckmanuals.com/vet/management_and_nutrition/breeding_soundeness_examination_of_bulls.html

Trimberger, G.W. 1948. Breeding efficiency in dairy cattle from artificial insemination at various intervals before and after ovulation. Nebr. Agric. Exp. Sta. Res. Bull. 153: 3-25.

Valergakis, G.E., Arsenos, G. and Banos, G. 2007. Comparison of artificial insemination and natural service cost effectiveness in dairy cattle. Animal, 1: 293-300.

White, F.J., Wettemann, R.P., Looper, M.L., Prado, T.M. and Morgan, G.L. 2002. Seasonal effects on estrous behavior and time of ovulation in non-lactating beef cows. J Anim. Sci. 80: 3053-3059.

Wilson, D.J., Mallory, D.A., Busch, D.C., Leitman, N.R., Haden, J.K.., Schafer, D.J., Ellerseick, M.R., Smith, M.F. and Patterson, D.J. 2010. Comparison of short-term progestin-based protocols to synchronize estrus and ovulation in postpartum beef cows. J. Animal Science, 88: 2045-2054.

Wiltbank, M.C., Pursley, J.R. and Vasconcelos, J.L.M. 2000. What is the optimal time for AI? Proc. 18th Tech. Conf. on Artificial Insemination and Reproduction. Milwaukee, WI. Pp 83.

Yadav, M.C., Walton, J.S. and Leslie, K.E. 1986. Timing of the onset and duration of ovulation in superovulated beef heifers. Theriogenology, 26: 509-521.

Zelinski, M.B., Hirota, N.A., Keenan, E.J. and Stormshak, F. 1980. Influence of exogenous estradiol-17 beta on endometrial progesterone and estrogen receptors during the luteal phase of the ovine estrous cycle. Biol. Reprod. 23: 743-751.