

Comparison of Estrus Response and Pregnancy Rates of Beef Cows Synchronized with Progesterone and Prostaglandin-Based Protocols

¹A.M. Khumran, ¹Y. Rosnina, ¹M.O. Ariff, ¹H. Wahid, ¹G. Dhaliwala,
¹N.P. Khanh, ¹K.C. Yap, ¹M. Fahmi and ²M.E. Azmil

¹Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine,
University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

²Pusat Ternakan Haiwan, Pantai Timur Tanah Merah, Kelantan, Malaysia

Abstract: The aim of this study was to compare the estrus response and pregnancy rate between the indigenous beef cows of Malaysia; Kedah-Kelantan (KK) and the exotic beef cows; Brangus (BR) following progesterone and prostaglandin-based estrus synchronization treatments. A total of 40 KK and 30 BR open cows were selected and each breed group was randomly divided equally into two. Cows in KK1 and BR1 were treated with estradiol benzoate (Cidirol, 1 mg, im) each at the time CIDR[®] was inserted (Day 0). Cloprostenol (Estrumate, 250 µg, im) was administered at the time of CIDR[®] removal on Day 9 while 1 mg of Estradiol Benzoate (EB) was injected on Day 10. On the other hand, KK2 and BR2 cows received intramuscular injections of 500 and 250 µg of cloprostenol, on Day 0 and 11, respectively. All cows were then observed for estrus signs and scanned per rectum for ovulation followed by AI upon detection of estrus. Pregnancy status was diagnosed 45 days after AI. Both treatments were effective in inducing observable estrus in all groups with synchrony of ovulation resulting in CL development and pregnancy. In the progesterone-based treatment groups, 84.2% of KK1 and 78.8% of BR1 responded. In the prostaglandin-based treatment groups, KK2 responded with the highest proportion (80.0%) compared with BR2 (50.0%). However, there was no significant difference in rate of ovulation (84.2 vs. 64.3%; 70.0 vs. 42.9%) and pregnancy (31.6 vs. 14.3%; 45.0 vs. 21.4%) among all the four experimental groups. The interval to ovulation from the last treatment time varied significantly among all the treatment groups with a higher variation observed in BR, ranging from 48 h when treated with CIDR to 84 h after treatment with PGF_{2α}. These variations could be explained by the difference in ovarian status at the time of treatment. In conclusion, the result of this data showed KK cows had a better rate of ovulation and pregnancy than BR cows in both treatments though not statistically significant. It can therefore be gathered that KK and BR responded effectively to estrus synchronization and produce acceptable pregnancy rates by both progesterone and prostaglandin-based protocols for breeding and genetic improvement.

Key words: Beef cows, estrus, synchronization, CIDR, PGF_{2α}

INTRODUCTION

Beef cattle production is a crucial industry to a nation's economic development. As part of mixed crop-livestock farming, beef production supports small-scale farmers by providing them with employment, sustainable income and social security (Boettcher and Perera, 2007). In addition, beef meat is also an important source of animal protein which is essential for bodybuilding and growth. Beef is also a good source of certain vitamins like B12, B6 and niacin, minerals like zinc, potassium and iron. Thus, its demand became a necessity for human consumption. Meat consumption therefore increases with human population and so its demand. The

growing population of the world must be matched with beef production that is capable of meeting people's demand and at the same time ensuring the conservation of such species for their traits. For instance, one of the consequences for Asia's developing economy is the rise in the demand of food arising from animal agriculture (Boettcher and Perera, 2007). Statistics have shown that the developing economy of Malaysia has caused increase in beef consumption to about 4.7 kg/person/year in 2005 (Warr *et al.*, 2008). Furthermore the self-sufficiency in beef production of Malaysia has remained at <29% as of 2010 (DVS, 2011). In an effort to meet the demand of the growing beef market, the government has established many agencies and programs that support the beef

industry (Sharif and Mohamed, 2005). Exotic breeds (such as Brahman, Charolais and Brangus) known to be of high commercial traits were imported to boost the production (Sivarajasingam and Kumar, 1993). Brangus for example, is known for its high resistance to disease, quality carcass and fertile females (Briggs and Briggs, 1980). Better selection also arises with better reproductive abilities in a given environment, since these breeds were imported, they need to demonstrate adaptability to Malaysia's hot and humid climate by competing with the local breed like Kedah-Kelantan (KK) which has excellent adaptation to the local climate and high resistance to parasite infestation (Panandam *et al.*, 2004). Therefore the objective of this study was to evaluate the estrus response and pregnancy rate between the exotic Brangus (BR) and the indigenous Kedah-Kelantan (KK) cows that were estrus synchronized with either CIDR or PGF_{2α}.

MATERIALS AND METHODS

Experimental animals: A total of 70 animals were used in this study. Forty KK cows (weight from 250-590 kg, age 3-11 years, parity 1-9) and 30 BR cows (weight from 300-694 kg, age 3-9 years, parity 1-6). Their Body Condition Score (BCS) between 2 and 6 based on the 1 = thin, 9 = obese scale (Eversole *et al.*, 2009). All the cows were apparently healthy and were certified open when scanned with a transrectal B-mode ultrasound scanner (Sa Filho *et al.*, 2010), fitted with a 5 MHz linear array transducer rectal probe. The experiment was carried out between October and December, 2010. The first part of the experiment which involved KK cows was conducted at a government farm located at Tanah Merah (5o°48'38.20"N, 102°00'30.55"E), Kelantan. The second part which involved BR was conducted at University's farm (2°9'18.36"N, 101°43'49.61"E), University Putra Malaysia (UPM).

Housing and feeding: All cows were kept in a 10 ha paddock with unrestricted access to water and grasses, mainly *Brachiaria decumbens*. They were also fed with commercial feed which contained approximately 16% crude protein, 22.5% crude fiber, 1.5% calcium, 2.6% crude fat, 10.6% moisture and 0.49% phosphorous. The cows were also provided with mineral licks.

Estrus synchronization: The first batch of cows (KK1, n = 20 and BR1, n = 15) were inserted intravaginally with CIDR (Pfizer, New Zealand Ltd.) and left in place for 9 days. The 1st day of insertion was considered as day 0. In addition, an intramuscular injection of 2 mL Cidiro1® (Bomac Laboratories Ltd. Auckland, New Zealand)

containing 1 mg of EB per mL was administered also. Intramuscular injection of 1 mL Estrumate® (Schering-Plough Animal Health, NSW, Australia) containing 250 µg mL⁻¹ cloprostenol was administered at the time of CIDR removal on day 9 and 1 mL intramuscular injection of Cidiro1® on day 10. For the second batch of cows (KK2, n = 20 and BR2, n = 15) 500 µg of cloprostenol was injected intramuscularly on day 1 and 250 µg of cloprostenol on day 11 (Gungor *et al.*, 2009).

Estrus detection: Estrus detection by visual observation (Lyimo *et al.*, 2000), commenced 24 h after removal of CIDR inserts in KK1 and BR1 cows and 24 h after the second cloprostenol injection in KK2 and BR2 cows. Estrus detection was performed twice daily for 5 days from 0600-0900 h and 1800-2100 h. Each time a cow displayed an estrus sign a designated number of points was recorded for that particular estrus cow (Table 1). A cow is considered in estrus when she scored >95 points (Roelofs, 2005). Onset of estrus was defined as the first observation time the cow expressed an estrus behavior. End of estrus was defined as the last observation time a cow expressed an estrus behavior.

Artificial insemination: A recto-vaginal insemination was carried out using the a.m. to p.m. rule. Cows observed to be in estrus in the morning were inseminated in the evening of the same day while cows that were observed in estrus in the evening were inseminated the next morning. Artificial Insemination (AI) was carried out by an experienced personnel with the semen deposited onto the body of uterus. All cows were inseminated once, each with a 0.25 mL straw of frozen thawed KK semen.

Determination of ovulation: The ovaries of all the cows were scanned twice daily with each scanning session after an estrus observation period in order to determine time of ovulation (Roelofs *et al.*, 2004). Time of ovulation was defined as the first scanning time that the dominant follicle disappears. The transrectal ultrasound scanning was repeated 6 days after ovulation to locate the CL (Roelofs *et al.*, 2004).

Table 1: Scoring scale for observed estrus signs

Estrus signs	Points
Flehmen	3
Restlessness ^a	5
Sniffing of the vulva of another cow	10
Mounted but not standing	10
Resting with chin on the back of another cow	15
Mounting other cows (attempt)	35
Mounting head side of other cow (attempt)	45
Standing heat	100

^aCan only be recorded once during observation time (Roelofs *et al.*, 2004)

Determination of pregnancy: All cows were scanned 45 days after AI in order to determine pregnancy rate. A B-mode ultrasound rectal scanner (Aloka SSD-500, Japan) (Sa-Filho *et al.*, 2010) fitted with a 5 MHz linear array transducer rectal probe was used for the diagnosis.

Statistics: The data on response of the cows to estrus synchronization as well as rates of ovulation and pregnancy were categorical, therefore analyzed by Pearson's χ^2 -test and the results were presented in percentages. Due to the small number of cows that showed signs of estrus, the data of the animals that express particular sign of estrus was rather described. Since, the data on the Onset of Estrus (OE), Duration of Estrus (DE) and Time to Ovulation (TO) did not satisfy the assumptions of Levene's and/or Shapiro-Wilk tests, it was then analyzed by non-parametric Mann-Whitney test and results were presented as median and ranges. All analyses were accomplished using the John's Macintosh Project, Version 9 (JMP9) Software which is a division of Statistical Analysis System (SAS) JMP Version 9, SAS Institute Inc. Cary, NC 1989-2011. Analysis were carried out with 95% confident interval. Therefore, p-values <0.05 were considered significant difference.

RESULTS AND DISCUSSION

Estrus response: Three animals were excluded from the present study. One cow from group BR2 sustained an injury and two cows, one each in groups KK1 and BR1 had lost their CIDR. Table 2 shows the number of cows that expressed signs of estrus during at least one observation period. Out of the total 67 females that were

Table 2: Rate of estrus response following estrus synchronization by CIDR and PGF_{2α} methods

Groups ^a	CIDR		PGF _{2α}	
	Estrus response (%)	Groups	Estrus response (%)	Response by breed
KK1	16/19 (84.2)	KK2	16/20 (80)	32/39 (82.1)
BR1	11/14 (78.8)	BR2	7/14 (50)	18/28 (64.3)
Response by treatment	27/33 (81.8)		23/34 (67.6)	50/67 (74.6)

^aThere is no difference among the groups

Table 3: Signs of estrus as displayed by the different groups of cows

Groups	Signs of estrus of displayed by groups (%)									
	Mucous discharge from the vulva	Flehmen	Restlessness	Sniffing of the vulva of another cow	Mounted but not standing	Resting with chin on the back of another cow	Mounting other cows (attempt)	Mounting head side of other cow (attempt)	Standing heat	
KK1	56.25	31.25	37.50	50.00	31.25	0.00	50.00	0.00	62.50	
KK2	37.50	68.75	12.50	62.50	37.50	0.00	50.00	6.25	50.00	
BR1	54.55	36.36	9.09	45.45	36.36	0.00	54.55	9.09	54.55	
BR2	57.14	100.00	14.29	100.00	57.14	28.57	28.57	0.00	42.86	

Due to the small number of cows that showed signs of estrus, the data in Table 6 above were not analyzed

treated with either CIDR or PGF_{2α}, 74.6% of the cows responded. Approximately 81.8% of the cows treated with CIDR responded while only 67.6% of the cows treated with PGF_{2α} responded. Out of 39 KK cows, 82.1% showed signs of estrus and 64.3% of BR showed signs of estrus out 28 cows. Table 2 also shows that BR did not respond well with PGF_{2α} compared with CIDR.

Signs of estrus: On the whole, 60% of the cows expressed sniffing of vulva of other cows as a sign of estrus while 54% displayed flehmen and standing heat. Resting with chin on the back of another cow was observed in only 2% of the cows and 4% attempted to mount head side of other cows (Table 3). The cows in the BR2 group that responded to the PGF_{2α} estrus synchronization treatment expressed signs of flehmen and sniffing of vulva of other cows. In addition, about 29% of the BR2 cows showed resting with chin on the back of another cow in contrast to the remaining 3 groups that did not show that sign. Standing to be mounted was expressed highest in KK1 followed by of KK2, BR1 and lastly, of BR2 (Table 3).

Onset of estrus: As shown in Table 4, there is no significant difference on the time to onset of estrus between KK1 (median = 25.5) and BR1 (33). Nevertheless, there was difference between KK2 and BR2. Similarly, there is no difference between KK1 (25.5) and KK2 (32) cows and between BR1 and BR2.

Duration of estrus: In general, the duration of estrus ranges from 5-61 h with the lowest median time of 13 h in BR2 and as high as 33 h in KK1 (Table 4). This data showed that the duration of estrus varied significantly between KK1 (9-61) and BR1 (12-25) and between KK1 (9-61) and KK2 (5-35). However, the duration of estrus did not differ significantly between KK2 and BR2 (p>0.05) and between BR1 (12-25) and BR2 (2-45).

Time of ovulation: Table 4 shows that all the groups varied significantly in time of ovulation. Cows in BR2 has the highest median value of 84 h with range from 60-84 h

after the last treatment followed by KK1 which took 72-96 h. The least time taken to ovulation was observed in groups KK2 and BR2.

Comparison of ovulation and pregnancy rates among the four treatment groups: In contrast to the time of ovulation, there was no significant difference in the rate of ovulation among all groups (Table 5). However, the KK breed has higher rate of ovulation in both treatments compared with the BR groups. In general, ovulation rate was higher with CIDR compared with PGF_{2α} treatment. Table 5, also shows the pregnancy rate of the 4 groups of cows after AI. Like the rate of ovulation, pregnancy rate was not significantly different among all groups in this study. Kedah-Kelantan cows have the highest pregnancy rate regardless of the type of estrus synchronization treatment when compared with BR cows. Overall, it was observed that 38% of KK and 17% of BR were confirmed pregnant at the end of the experiment. In CIDR treated groups, pregnancy was approximately two times higher in the KK (31%) cows than in the BR (14%) cows. Even in the PGF_{2α}-based Estrus Synchronization Method, pregnancy rate was higher in the KK cows (45%) than in BR cows (21%). However, the pregnancy rate in these two breeds of cows is considered low.

Results obtained in this study indicated that KK responded better to estrus synchronization than BR cows in both CIDR and PGF_{2α} treatments and more importantly synchrony of estrus was effectively influenced by both protocols, consistent with that reported by Voh *et al.* (2004). Voh *et al.* (2004) described that estrus was effectively synchronized in N'dama and Bunaji cattle following PRID and PGF_{2α} protocols. The characteristics

of estrus signs expressed by the cows in the present study could not be analyzed due to the small number of cows that displayed estrus signs. However, the four groups did not seem to differ much in terms of breed or treatment except in the pre-ovulatory periods which include onset of estrus, duration of estrus and time of ovulation that varied significantly between individual cows as well as among groups without a clear characteristic pattern. This type of variation has been reported by some researchers (Roelofs, 2005; Roelofs *et al.*, 2004; Saumande and Humblot, 2005) and was suggested to be of ovarian control depending on the reproductive status of the animal at the time of treatment. Saumande and Humblot (2005) found highly significant correlation coefficients between intervals to ovulation, 17 beta-Estradiol (EDL-17β) concentration and follicular growth characteristics that are suggestive of ovarian control periods from estrus to LH peak to ovulation. Roelofs *et al.* (2004) noted that the interval to ovulation has the largest variation in his study and that it was affected by the size of dominant follicle during luteolysis which is the source of natural variation of pre-ovulatory follicular phase duration. Verduzco *et al.* (2006) found that the time of onset of estrus varied significantly after norgestomet ear implant withdrawal from all the three groups of multiparous Brahman cows investigated.

Ovulation is critical to fertility of a female animal. Thus, estrus synchronization protocol must cause ovulation in cows whether they are cycling or in anestrus (Dobbins *et al.*, 2009). Comparison of results from the present data revealed that breed and treatment did not affect ovulation rate among the groups. This present finding is similar to an earlier report (Portillo *et al.*, 2008). The degree of synchrony of ovulation by both CIDR and PGF-based treatments as indicated in the present result, suggests that both protocols influenced good synchrony of ovulation in KK and BR cows. Moreover, most of the signs of estrus displayed by cows during estrus are accurate predictors of ovulation time. Except for standing heat, none of the signs is accessible automatically. Therefore, excessive labor input is demanded in order to monitor those signs (Roelofs, 2005). Additionally, Roelofs (2005) also reported that most signs of estrus can be displayed outside time of estrus but mounting behaviors are hardly displayed by cows when not in estrus. Therefore, mounting behavioral signs especially standing estrus is the most appropriate

Table 4: Time of onset, duration of estrus and time of ovulation produced by cows from the different treatment groups

Groups	CIDR		PGF _{2α}		
	Range (h)	Median (h)	Groups	Range (h)	Median (h)
Onset of estrus					
KK1	15-46	25.5	KK2	21-46	32.0 ^a
BR1	21-38	33.0 ^a	BR2	33-57	46.0 ^{b,y}
Duration of estrus					
KK1	9-61	33.0 ^{a,x}	KK2	5-35	14.0 ^a
BR1	12-25	23.0 ^a	BR2	12-45	13.0
Time of ovulation					
KK1	72-96	72.0 ^{a,x}	KK2	60-72	66.0 ^{b,y}
BR1	48-72	60.0 ^{b,x}	BR2	60-84	84.0 ^{b,y}

^{a,b}Within columns denotes significantly different (p<0.05); ^{x,y}Within rows denotes significantly different (p<0.05)

Table 5: Ovulation and pregnancy rates among the different experimental groups

Groups ^a	CIDR		PGF _{2α}		
	No. of cows ovulated (%)	No. of cows pregnant (%)	Groups	No. of cows ovulated (%)	No. of cows pregnant (%)
KK1	16/19 (84.2)	6/19 (31.6)	KK2	14/20 (70.0)	9/20 (45.0)
BR1	9/14 (64.3)	2/14 (14.3)	BR2	6/14 (42.9)	3/14 (21.4)

^aThere is no difference among the groups

for the determination of time of ovulation even though it is not displayed by all of the cows (Van Eerdenburg *et al.*, 2002; Galina and Orihuela, 2007; Roelofs *et al.*, 2010). The proportion of cows that displayed standing estrus in the present study was encouraging and the scores were similar to those obtained in earlier studies (Roelofs, 2005; Krininger *et al.*, 2003). Roelofs (2005) concluded that standing heat is the best predictor of time of ovulation. It is expressed in high proportion of cows; more precisely when more than two are in estrus simultaneously.

The chi-square analysis of the data in this study indicated no significant effect of treatment on pregnancy rate and similar result was reported by Gungor *et al.* (2009). Gungor *et al.* (2009), found that different seasons (Winter and Summer) significantly affected conception rate rather than the different estrus synchronization treatments. Results from the present study differed from an earlier report (Voh *et al.*, 2004) whereby a significantly better pregnancy rate was obtained in cows treated with PGF_{2α} than those treated with Progesterone Internal Drug Release (PIDR) devices. Similarly, in the present study, cows treated with PGF_{2α} had higher pregnancy rate than cows treated with CIDR. In another study (Sa-Filho *et al.*, 2010), it was found that CIDR+PGF_{2α} treatment yielded a significantly higher pregnancy rate than the single PGF_{2α} treated non-suckling primiparous *Bos indicus* beef cows with estrous cycles having been initiated following calving. This is probably due to the cow's corpus luteum which has a reduced response to PGF_{2α} between days 1 and 4 of the estrous cycle. Therefore, pregnancy rate can be improved by two injections of PGF_{2α}, 11 days apart (Bridges *et al.*, 2005; Weems *et al.*, 2006). In another study (Neglia *et al.*, 2003), PIDR was found to be associated with lower pregnancies (although not significant) but rather tend to induce ovulation in greater proportion in non-cyclic Italian Mediterranean buffalo cows. Therefore, CIDR can be considered a protocol of choice in circumstances where high proportions of buffalo cows are non-cyclic at the time of estrus synchronization.

The pregnancy rates expressed by the cows in the present study were lower compared with an earlier study (Kasimanickam *et al.*, 2009) who reported up to 52 and 54% pregnancy rates after CIDR + dinoprost (PGF) and CIDR + cloprostenol treatments, respectively in Angus-cross cows. In another study (Voh *et al.*, 2004), pregnancy rates of 68% for PIDR and 53% for PGF_{2α} were obtained. The reasons for the lower pregnancy rates in the present experiment compared with those of others previous reports are not clear but numerous factors that are thought to be associated with reduced pregnancy rates include environmental, nutritional and physiological status of the animals before and at time of breeding (Smith and Somade, 1994). Buddenberg *et al.* (1989) also

observed that weight, age and average daily gain of the animals affected their reproductive performance.

Physical assessment revealed that the BCS of 30% of the cows used for the present study were <5. Yelich *et al.* (1995) noted that BCS can influence subsequent reproductive performance of cows in a breeding season. Recently, Eversole *et al.* (2009) reported that conception rate drastically compromised production in cows with BCS ≤ 4. All cows in the present study were kept on a cow-calf management system with 40% of the population lactating. This could be an additional constraint to their fertility because of the heavy nutritional requirement on the lactating dam. According to Rosby (2007), early weaning beef management practice greatly relieves grassing pressure on the dams by reducing their nutrient requirements. Yavas and Walton (2000) reported earlier that restricted daily suckling to once daily for 60-90 min beginning on day 21 postpartum has shortened postpartum anestrus in beef cows. Alvarez-Rodriguez and Sanz (2009) supported the idea who noted that when beef calves were separated 60 m away from their dams with only once daily-restricted suckling (without visual, tactile and olfactory contact between them) for 30 days, the postpartum intervals to first estrus and ovulation were effectively reduced.

CONCLUSION

The findings of this study revealed that there is no significant difference in estrus response and pregnancy rate between KK and BR cows that were estrus synchronized with either progesterone or PGF-based estrus synchronization protocols under the same environmental condition. However, variations exist in the time of onset and duration of estrus which are thought to be affected by individual ovarian status at time of treatments and consequently influenced their time of ovulation. From the present study, KK tend to respond better than BR to both treatments in terms of estrus response and pregnancy rate. This is probably due to the small number of cows involved in this study or probably, BR has not been able to acclimatize to the climatic condition of Malaysia. Nevertheless, both CIDR and PGF_{2α} successfully induced estrus synchronization and produced acceptable pregnancy rates in KK and BR.

ACKNOWLEDGEMENTS

Researchers wish to thank Taman Pertanian University (TPU), Government farm Pusat Ternakan Haiwan, Pantai Timur, Tanah Merah, Kelantan and Institute of Veterinary Biotechnology Jerantut, Pahang for providing the animals and frozen semen.

REFERENCES

- Alvarez-Rodriguez, J. and A. Sanz, 2009. Physiological and behavioural responses of cows from two beef breeds submitted to different suckling strategies. *Appl. Anim. Behav. Sci.*, 120: 39-48.
- Boettcher, P.J. and B.M.A.O. Perera, 2007. Improving the Reproductive Management of Dairy Cattle Subjected to Artificial Insemination: A Summary. International Atomic Energy Agency, Vienna, Austria, ISBN: 92-0-114806-2, pp: 1-7.
- Bridges, G.A., G.E. Portillo, J.W. de Araujo, W.W. Thatcher and J.V. Yelich, 2005. Efficacy of either a single or split treatment of PGF_{2α} after a 14 day melengestrol acetate treatment to synchronize estrus and induce luteolysis in *Bos indicus* × *Bos taurus* heifers. *Theriogenology*, 64: 344-362.
- Briggs, H.M. and D.M. Briggs, 1980. *Modern Breeds of Livestock*. 4th Edn., Macmillan Publishing Co., New York, USA.
- Buddenberg, B.J., C.J. Brown, H.P. Peterson and A.H. Brown, 1989. Factors influencing pregnancy rate and calving date in four breeds of beef cows on an Ozark mountain range. *Anim. Reprod. Sci.*, 18: 243-252.
- DVS, 2011. Malaysia: Self-sufficiency in livestock products (%), 2000-2010. Department of Veterinary Services Malaysia. http://www.dvs.gov.myc/document_library/et_file?uuid=d41d4a2a-ab2b-44d2-9f97-228767acd357&groupId=28711.
- Dobbins, C.A., D.R. Eborn, D.E. Tenhouse, R.M. Breiner, S.K. Johnson, T.T. Marston and J.S. Stevenson, 2009. Insemination timing affects pregnancy rates in beef cows treated with CO-Synch protocol including an intravaginal progesterone insert. *Theriogenology*, 72: 1009-1016.
- Eversole, D.E., M.F. Browne, J.B. Hall and R.E. Dietz, 2009. *Body Condition Scoring Beef Cows*. Virginia Cooperative Extension, Blacksburg Virginia, pp: 400-795.
- Galina, C.S. and A. Orihuela, 2007. The detection of estrus in cattle raised under tropical conditions: What we know and what we need to know. *Horm. Behav.*, 52: 32-38.
- Gungor, C., M. Ulubilir, M. Saler and S.G. Karakoc, 2009. The comparison of two different seasons oestrus synchronization results with fixed-time AI protocols. *J. Anim. Vet. Adv.*, 8: 373-378.
- Kasimanickam, R., M.L. Day, J.S. Rudolph, J.B. Hall and W.D. Whittier, 2009. Two doses of prostaglandin improve pregnancy rates to timed-AI in a 5-day progesterone-based synchronization protocol in beef cows. *Theriogenology*, 71: 762-767.
- Krinninger, C.E., J. Block, Y.M. Al-Katanani, R.M. Rivera, C.C. Chase and P.J. Hansen, 2003. Differences between Brahman and Holstein cows in response to estrus synchronization, superovulation and resistance of embryos to heat shock. *Anim. Reprod. Sci.*, 78: 13-24.
- Lyimo, Z.C., M. Nielen, W. Ouweltjes, T.A.M. Kruip and F.J.C.M. van Eerdenburg, 2000. Relationship among estradiol, cortisol and intensity of estrous behavior in dairy cattle. *Theriogenology*, 53: 1783-1795.
- Neglia, G., B. Gasparrini, R. di Palo, C. de Rosa, L. Zicarelli and G. Campanile, 2003. Comparison of pregnancy rates with two estrus synchronization protocols in Italian mediterranean buffalo cows. *Theriogenology*, 60: 125-133.
- Panandam, J.M., H.H. Abdelwahid, A. Majidi and Y.W. Kit, 2004. Genetic characterisation of indigenous Kedah Kelantan cattle and its crossbred breed types. *Mol. Biol.*, 3: 56-58.
- Portillo, G.E., G.A. Bridges, J.W. de Araujo, M.V. Shaw, F.N. Schrick, W.W. Thatcher and J.V. Yelich, 2008. Response to GnRH on day 6 of the estrous cycle is diminished as the percentage of *Bos indicus* breeding increases in angus, brangus and brahman angus heifers. *Anim. Reprod. Sci.*, 103: 38-51.
- Roelofs, J., F. Lopez-Gatius, R.H.F. Hunter, F.J.C.M. van Eerdenburg and C. Hanzen, 2010. When is a cow in estrus? Clinical and practical aspects. *Theriogenology*, 74: 327-344.
- Roelofs, J.B., 2005. When to inseminate the cow? Insemination, ovulation and fertilization of the dairy cattle. Ph.D. Thesis, Wageningen University, Wageningen, The Netherlands.
- Roelofs, J.B., E.G. Bouwman, S.J. Dieleman, F.J.C.M. van Eerdenburg, L.M.T.E. Kaal-Lansbergen, N.M. Soede and B. Kemp, 2004. Influence of repeated rectal ultrasound examinations on hormone profiles and behaviour around oestrus and ovulation in dairy cattle. *Theriogenology*, 62: 1337-1352.
- Rosby, R., 2007. Early weaning beef calves. *Vet. Clin. N. Am.-Food A*, 23: 29-40.
- Sa-Filho, O.G., C.C. Dias, G.C. Lamb and J.L.M. Vasconcelos, 2010. Progesterone-based estrous synchronization protocols in non-suckled and suckled primiparous *Bos indicus* beef cows. *Anim. Reprod. Sci.*, 119: 9-16.

- Saumande, J. and P. Humblot, 2005. The variability in the interval between estrus and ovulation in cattle and its determinants. *Anim. Reprod. Sci.*, 85: 171-182.
- Sharif, Z.A. and W. Mohamed, 2005. Beef production for Malaysian entrepreneurs. Malaysian Agricultural Research and Development Institute (MARDI), Kuala Lumpur.
- Sivarajasingam, S. and A.R. Kumar, 1993. Comparative performance of Malaysian, Australian and New Zealand F1 Friesian crossbreds. *Livest. Prod. Sci.*, 36: 299-310.
- Smith, O.B. and B.S. Somade, 1994. Nutrition reproduction interactions in farm animals. International foundation seminar on animal production, Niamey, Niger, pp: 1-33.
- Van Eerdenburg, F.J.C.M., D. Karthaus, M.A.M. Taverne, I. Mercis and O. Szenci, 2002. The relationship between estrous behavioral score and time of ovulation in dairy cattle. *J. Dairy Sci.*, 85: 1150-1156.
- Verduzco, A., C.S. Galina, I. Rubio and M. Maquivar, 2006. Average response to estrus and timing of ovulation in *Bos indicus* cattle synchronized alternatively with a synthetic progestagen. *J. Anim. Vet. Adv.*, 5: 924-929.
- Voh, A.A., A. Larbi, S.A.S. Olorunju, K. Agyemang, B.D. Abiola and T.O. Williams, 2004. Fertility of ndama and bunaji cattle to artificial insemination following oestrus synchronization with PRID and PGF_{2α} in the hot humid zone of Nigeria. *Trop. Anim. Health Prod.*, 36: 499-511.
- Warr, S., G. Rodriguez and J. Penm, 2008. Changing food consumption and imports in Malaysia. Opportunities for Australian agricultural exports (Australian Bureau of Agricultural and Resource Economics ABARE research report) Canberra, Australia, pp: 1-29.
- Weems, C.W., Y.S. Weems and R.D. Randel, 2006. Prostaglandins and reproduction in female farm animals. *Vet. J.*, 171: 206-228.
- Yavas, Y. and J.S. Walton, 2000. Postpartum acyclicity in suckled beef cows: A review. *Theriogenology*, 54: 25-55.
- Yelich, J.V., H.S. Mauck, M.D. Holland and K.G. Odde, 1995. Synchronization of estrus in suckled postpartum beef cows with melengestrol acetate and PGF_{2α}. *Theriogenology*, 43: 389-400.