CHAPTER V

5.0 DISCUSSION

5.1 Fertility responses of synchronized cows

Generally, the present study was conducted to observe the effect of farming system, body condition score (BCS), breeds, and days of postpartum interval on estrus and pregnancy of synchronized cows. The discussions for the fertility responses of synchronized cows were described in the following sub-sections.

5.1.1 Effect of farming system on body condition score (BCS) and fertility responses of synchronized cows

The present study showed that integrated farm had cows with the lowest BCS compared with the cows in the intensive and semi-intensive farms. Probably, this was due to the small grazing area, that was 500 hectares with large number of cows, 600 cows. According to Zainur and Wan Zahari (2005), one hectare of oil palm could only support one head of cow. In intensive and semi-intensive farms, cows were supplied with cut grass (Napier) or cut oil palm leaves (fronds) and palm kernel cake (PKC) or other concentrates. Probably, grazing area and feed supplement are some of the factors that affect BCS in cows. Ferguson and Chalupa (1989) reported that, deficiency in nutrient intake was a common problem in grazing herd, where supplement was frequently absent. Improper feed ratio or handling might also lead to insufficient nutrient intake. On the other hand, excessive amount of feed or certain feed component, such as protein, might also reduce fertility in cows (Ferguson and Chalupa, 1989).

The present study showed that cows with high body condition score (BCS) had better response to estrus and percentage of pregnancy. Ambrose *et al.* (1999), reported that as BCS increased, sign of behavioral estrus was stronger and fertility was improved. Low BCS had been associated with increased risk for cystic ovarian disease and an increase in the percentage of anovular cows (Crane *et al*. 2006). The present result observed lower percentage of cows that showed behavioral estrus as to that reported by Wahid *et al*. (2001). This probably due to short observation time of estrus that was only 24 hours prior to artificial insemination (AI). Wahid *et al*. (2001) reported that 91.7% of CIDR treated Kedah-Kelantan cows showed estrus after 96 hours observation.

In the present result, cows bred in the integrated farm had the lowest percentage of pregnancy when compared to the intensive and semi-intensive farms. In the integrated farm, cows grazed intensively on pastures available in the oil palm plantation and increased number of cows could cause over grazing. Insufficient food intake could reduce fertility and low percentage of pregnancy. Wild and aggressiveness of cows in the integrated farm caused difficulty in handling the cows during the treatment. This probably could be one of the factors that can affect and lower the percentage of pregnancy in the integrated farm. According to Mann (2001), stressed cows during experiment would give poor pregnancy percentage. Beever *et al*. (2001) reported that the nutritional status of the cows had a significant effect on fertility which influenced both the interval from calving to first ovulation and conception. Poor nutritional status of cows after calving was the most important reason for the delay of first ovulation and prolonged the commencement of luteal activity (Shresta *et al*., 2005).

Jusoh and Mohd Noor (2002) reported that the 40-60% sunlight intensity passing through oil palm fronds was necessary for a reasonable amount of grass and other cover to grow under oil palm trees. Cows grazing were sufficient even if the area had only 60-70% grass cover (Jusoh and Mohd Noor, 2002). However, Wong and Chin (1998) indicated no negative effect of shading on the intake and digestibility of tropical forages.

The present result indicated that BCS and fertility responses of synchronized cows in intensive and semi-intensive farms were higher than integrated farm. Probably, this was due to extra feeding of the cows in both the intensive and semi-intensive farms that was cut grass (Napier) or cut oil palm fronds in the morning and palm kernel cake (PKC) or other concentrates in the afternoon.

5.1.2 Effect of breeds on body condition score (BCS) and fertility responses of synchronized cows

In the present study, Charolais crossbreds had better BCS when compared to Brahman crossbreds and Kedah-Kelantan (KK) cows. Probably this Charolais crossbreds was imported breed and were very big in size and has good growth performance. Charolais crossbreds were also reported to adapt well to the Malaysian environment, and is comparable to KK (Zainur and Wan Zahari, 2005). Suriyasathaporn *et al*. (1998) reported that cows with low BCS at parturition had poor reproductive performance.

The current result indicated no significant difference ($p<0.05$) in the number of cows that showed estrus among the Charolais crossbreds, Kedah-Kelantan, and Brahman crossbreds cows. A total of 67.3% of cows showed behavioral estrus, which was lower than that reported by Wahid *et al*. (2001) in Kedah-Kelantan cows that showed 91.7% estrus when treated with CIDR for 12 days and injected with prostaglandin after 24 hours of CIDR removal for 96 hours observation. Probably in this study, low number of cows that shows behavioural estrus was caused by the limited time to observe the estrus sign.

The result of the current study also indicated no significant difference in the percentage of pregnancy ($p<0.05$) in Charolais crossbreds, Brahman crossbreds, and Kedah-Kelantan. However, previous study showed 82.8% of dairy cows showed estrus and 57.7% of 146 lactating dairy cows were pregnant after synchronization of estrus with PGF2 alpha injection, 18 days after progesterone treatment in lactating dairy cows (Xu and Burton, 1998).

This result was in agreement with Ryan *et al*. (1999) who reported 57.9% cows pregnant in lactating dairy cows after 8 days of CIDR treatment with 1 mg of estradiol injection after 10 hours CIDR removal the result was also similar to a report by Rekwot *et al*. (1999) in zebu cows (*Bos indicus*) when treated with 25 mg of prostaglandin as the dinoprost tromethamine salt (Lutalyse, Upjonh), 60.6% showed estrus and 53.3 percentage of pregnancy.

5.1.3 Effect of body condition score (BCS) on fertility responses of synchronized cows

The present result showed that as BCS increase, percentage of pregnancy also increased. This result was in agreement with Rae *et al*. (1993), who observed lower percentage of pregnancy (59%) for BCS ≤ 4 when compared to BCS ≥ 5 (90%). Ambrose *et al*. (1999) indicated that an increase in 1 unit of BCS would increase 13% of pregnancy.

Body condition score (BCS) was a reliable method to assess the nutritional status of cows (Richards *et al*., 1986; John and Lamb, 2008). Insufficient intake of energy, protein, vitamins, and micro- and macro-minerals had been associated with suboptimal reproductive performance (John and Lamb, 2008). Of these, energy balance was probably a single most important nutritional factor related to poor reproductive function in cows. Linear relationship exists between body weight change and BCS, where an approximate 40 kg weight change was associated with each unit change in BCS (using the 1–9 scale).

Lamb *et al*. (2001) found that BCS affect the percentage of pregnancy. Cows with greater BCS at the onset of the breeding season experienced an overall improvement in the percentage of pregnancy. For every unit increase in BCS (range 3.8

to 6.2) on day 0, conception rate would increase. One unit increased in BCS would result in 16.3% increase in the proportion of cycling cows at the onset of the breeding season and 22.9% increase in the proportion of pregnant cows after synchronized ovulation (Lamb *et al*., 2001). Houghton *et al*. (1990) reported that calving cows with poor BCS had longer intervals (89, 70, and 60 days for BCS of 3, 4, and 5, respectively) before resuming their estrus cycles compared with calving cows with good condition (52 and 31 days for BCS of 6 and 7, respectively).

Herd and Sprott (1986) observed 62% of beef cows with BCS 4 or lower at calving were on heat at 80 days after calving as compared to 88% to 98% for cows with BCS 5, 6 or higher. Other studies found that cows with BCS below 5 required more services per conception. This situation indicated depression in fertility of cycling beef cows (William and Robert, 2001). According to Lamb *et al*. (2001), beef cows should conceive by 80 days after calving to maintain 365 days calving interval which was desired in most breeding programs.

Cows with low BCS were less likely to have a corpora lutea on day 21 as compared to cows with higher BCS (Crane *et al*., 2006). Low BCS, or a change in BCS over time, had been associated with an increase risk for cystic ovarian disease (Lopez *et al*., 2000) and increase in the percentage of anovular in cows (Gumen *et al*., 2003). Previous study indicated that embryonic lost due to low BCS was associated with the failure to sustain an antiluteolytic mechanism (Thatcher *et al.*, 1997).

5.1.4 Effect of days of postpartum interval on estrus and percentage of pregnancy

The present result showed that percentage of pregnancy for cows with days of postpartum < 55 was lower than cows with > 55 days of postpartum, which was in agreement to that reported by Ryan *et al*. (1999). Cows with < 55 days of postpartum had a lower percentage of pregnancy (48.9%) when compared to cows with > 55 days of postpartum (56.0%) (Ryan *et al*., 1999).

For the normal cycle, ovulation occurred not more than 45 days after calving, followed by regular ovarian cycle with about 2 weeks of luteal phase and 1 week of follicular phase (Shresta *et al*., 2005). A delay after calving of at least 40 days before rebreeding was essential for the health of cows, enhanced the chance of conception, and reduced the probabilities of breeding difficulties as compared to earlier rebreeding (Salisbury *et al*., 1978).

According to Lamb (2005), for the best conceivement, synchronization of estrus should not occur before 45 days of postpartum. Producers who aim to shorten or shift the calving season should consider a fixed time AI in cows less than 45 days of postpartum. A fixed-time AI should not be considered in cows less than 21 days of postpartum (Lamb, 2005).

Time is an essential component of the postpartum period. Stevenson *et al*. (2003) demonstrated that the proportion of cows cycling on the first day of the breeding season was influenced by calving date. The proportion of cows initiating estrus cycles increased by approximately 7% for every 10-day interval from calving. Thus, more cows had initiated estrous cycles when they had longer periods of time between calving and the breeding season (Anderson, 2010).

A low level of nutrition, either during the gestation or after parturition, would delay the process of return to estrus (Bearden and Fuquay, 2000). Silent ovulations were also more frequent in cows with low plane of nutrition. Other factors that might extend postpartum anestrus included infectious diseases, metabolic disorder, uterine infection, and other health problems (Bearden and Fuquay, 2000).

5.2 Progesterone and estradiol profiles of synchronized cows

5.2.1 Progesterone profile of synchronized cows

The present result found an increase concentration of progesterone when CIDR was inserted on day 0 and started to decrease after day 3. This was due to the presence of natural hormone, progesterone in the CIDR which was released once it was implanted into the vagina. Progesterone has a dominant role in regulating the estrus cycle. During diestrus with the functional corpus luteum, high concentration of progesterone inhibits the release of FSH and LH through its negative feedback control of the hypothalamus and anterior pituitary. High concentration of progesterone also inhibits behavioral estrus (Bearden and Faquay, 2000). High progesterone level acted as if the corpus luteum was still active thus, preventing the release of FSH from the pituitary gland. Low level of FSH in the blood would inhibit the growth of ovarian follicle thus, preventing estrus and ovulation in the treated animals. Estrus cycle activity would not occur until growing and maturing follicles appeared in the ovaries (Asdell, 1946; Hammond, 1957).

The present study showed that all cows exhibited behavioral estrus, irrespective of the estrus cycle stage at the beginning of the treatment. In cows, one of the key requirements for estrus synchronization is to know the precise onset of estrus, independent on the endocrine status or follicular ovarian stage of each animal at the beginning of treatment (Taniguchi *et al*. 2007). Moreover, distribution of estrus or degree of synchrony is important for timed breeding.

In the present result, the progesterone level started to decline after day 3. Low level of progesterone spark off new wave of follicular development. It stimulates the pituitary to release FSH which stimulates the growth of ovarian follicle until it becomes a matured follicle. At this stage, ovum is then released from the ovary. It was reported that there was different in the time estrus and ovulation` for the same breed depending on the dosage of progesterone in CIDR sponges (Bearden and Fuquay, 2000).

In the present study, cows were treated with CIDR for 7 days and injected with estradiol benzoate 24 hours after CIDR removal. Fike *et al*. (1997) observed more cows and heifers exhibited estrus after 7 days treated with intravaginal progesterone-releasing device (PRID) insert followed by an injection of estradiol benzoate 24 to 30 hours after PRID removal compared with females that were not treated with estradiol benzoate. Subsequent work by Lammoglia *et al*. (1998) with heifers and cows treated with 7 days of prostaglandin insertion on day 6 of treatment followed by estradiol benzoate treatment with various doses, 24 to 30 hours after removal resulted in a greater number of estradiol benzoate treated females in estrus compared with no estradiol benzoate treatment.

Bearden and Fuquay (2000) stated that the exogenous progestins worked by preventing the released of GnRH, which reduced the release of gonadotropins so that estrus and ovulation were prevented until withdrawal of progestin. Upon withdrawal, progestin level decreased which allowed pulsatile release of GnRH. This would result in the release of gonadotropins and estrus would occur in 2 to 6 days later.

5.2.2 Estradiol profile of synchronized cows

The insertion of the CIDR implant caused a slight drop in estradiol level and later increased after estradiol benzoate injection on day 8. In the present result, 70% cows treated with estradiol benzoate, exhibited estrus after 7 days of CIDR treatment. According to Brown *et al*. (1972) administration of estradiol benzoate after progesterone treatment would enhance ovulation in cows.

Peters (1986) showed that when estradiol benzoate was injected after CIDR removal, onset of estrus following 12 days of progesterone treatment was more synchronized than without injection of estradiol benzoate. Sreenan *et al*. (1977) found that progesterone treatment alone did not affect the corpus luteum function at either early (day 2) or midluteal (day 12) phase. However, when progesterone and estradiol benzoate were administered intramuscularly on day of device insertion, the development of corpora lutea was inhibited at day 2, and functional corpora lutea was regressed in cows at day 12 (Tjondronegoro *et al*., 1986).

Ayres *et al*. (2008) proposed that the estradiol benzoate treatment would induce an earlier increase in circulating estrogen, which in turn would induce the LH surge and, consequently, interrupted the follicular growth and promoted ovulation. Lammoglia *et al*. (1998) found that estradiol benzoate administration amplified the circulation of 17 estradiol concentrations during the interval from 4 to 28 hours post-treatment, with a peak occurring after 11 hours. In addition, this previous study also reported that LH surge occurred on average of 21.5 hours after estradiol benzoate administration. Ross *et al*. (2004) found no differences in the time to ovulation among animals treated with estradiol benzoate on day 8 or 9 (median interval of 3 days in both cases).

Bridges *et al*. (1999) compared the effect of three short-term treatments with progesterone and estradiol benzoate on follicular growth, synchronization of estrus, and percentage of pregnancy after fixed-time insemination in lactating postpartum beef cows. The authors found that the injection of 2 mg of estradiol benzoate at time of CIDR insertion would terminate the growth of the largest ovarian follicle in 16/16 (cows received a CIDR for 7 days with injection of EB (2 mg, im) at the time of device insertion) and 14/15 (cows received a CIDR for only 5 days with injection of EB (2 mg, im) at the time of device insertion). Estrus was also detected within 8 hours and no difference in the percentage of pregnancy was observed between the two groups. It was concluded that treatment with CIDR for 5-7 days followed with 1 mg of estradiol benzoate 30 hours later would induce high degree of estrus synchronization and ovulation which was necessary for fixed-time insemination.

In the present study, insemination was carried out 24 hours after estradiol benzoate injection. The estradiol benzoate was administered at CIDR insertion would resynchronize follicle growth and development which resulted in ovulation of a new and fertile follicle after CIDR removal. Ross *et al*. (2004) detected no difference in percentage of pregnancy when cows were inseminated 48 hours after CIDR withdrawal, regardless of whether time of ovulation was synchronized using estradiol benzoate on day 8 or 9. Artificial insemination should occur near the time of ovulation to maximize sperm accessed to the ovum, but not too late because awaits sperm arrival (Ayres *et al*., 2008).

In the current study, probably estradiol benzoate injection triggers the release of estradiol from growing follicle which drastically increases LH secretion and within 24 hours, ovulation occurs. This was in agreement with Roelofs *et al*. (2006) that the optimal AI time was between 12 and 24 hours before ovulation for the most desirable rate of fertilization and 12–16 hours for greatest percentage of greater quality of recovered embryos (89%). More precisely, Maatje *et al*. (1997) obtained an optimal percentage of pregnancy when AI was performed 16.2 hours before ovulation.

Intramuscular administration of 0.5 mg estradiol benzoate, 24 hours after removal of CIDR significantly increased the number of cows exhibiting estrus. The administration of estradiol benzoate also altered the time to estrus so that significantly more cows were in estrus and conceived by 24 hours after CIDR removal.

The present result showed that estradiol reached the peak nearest to time of estrus. High level of estradiol in the blood before the onset of estrus would prevent release of FSH and promoted the release of LH. Presence of LH peak in circulation was the signal to the highly-developed follicle to complete the process of ovulation (Lindsay *et al*., 1982). At this stage, the animal is said to be in estrus phase.

CHAPTER VI

6.0 SUMMARY AND CONCLUSION

All experiments were completed successfully and the main objectives of the studies were achieved. This study showed the effect of farming systems and body condition score and fertility responses of three different breeds of cows, Charolais crossbreds, Brahman crossbreds and Kedah-Kelantan. Intensive farm had better estrus response and percentage of pregnancy when compared to the cows in semi-intensive and integrated farms.

Synchronization of estrus in cattle not only allow one to predict the time of estrus with reasonable accuracy but also help the management by which a proper breeding programme may be planned and organized. Synchronized cows with a high body condition score (BCS) had better response to estrus after CIDR treatment and more likely to become pregnant than the cows with a lower BCS. To achieve optimal pregnancy rates with CIDR based estrus synchronization protocol, cows should be in a good body condition (BCS $\geq$4) and treatments should be initiated only when cows were at least 55 days of postpartum.

Charolais crossbreed had better response to estrus and higher percentage of pregnancy when compared to Brahman crossbreds and indigenous Malaysian cattle, Kedah-Kelantan. The used of CIDR for 7 days insertion that contains progesterone for 7 days has important roles in estrus synchronization. Intramuscular injection of 0.5 mg estradiol benzoate 24 hours after removal of CIDR implant significantly increased the number of cows exhibiting estrus within the observation period.

Progesterone profile of synchronized cows increased when CIDR was inserted on day 0 and reached the peak on day 3. After CIDR removal on day 7, level of progesterone started to decline. The insertion of CIDR caused a slight drop in estradiol and later increased after estradiol benzoate injection on day 8.

There are many factors that influence estrus synchronization in cows. This includes body condition score, parity, days of postpartum, facilities, labor, and methods for estrus synchronization. To improve percentage of pregnancy, all the above factors must be considered by the farmers.

To produce large number of cattle in a very short time, it needs advance reproductive biotechnology techniques such as artificial insemination (AI), in vitro fertilization, embryo transfer and cloning. Also need to consider by farmer to improve reproductive efficiency in cows.

It is hoped that the result obtained from this study will enlighten the understanding of farm management and hormonal control of reproduction in synchronized cows. Thus, it would somewhat overcome many problems related to reproduction in farm animals. Therefore, this would be tandem with the nation aspiration to increase cattle population in Malaysia to meet self-sufficiency and possibility for exportation.