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Application of GnRH Administration at Post Artificial Insemination in Synchronized Estrus Heifer and Dairy Cows by $PGF_{2\alpha}$ Induction on Conception Rate in Phupan Dairy Co-operative, Sakol-Nakon Province, Thailand

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Abstract: This experiment was conducted to study the effects of GnRH on conception rate of 32 crossbred Holstein-Friesian dairy cows. All cows were uniform of body condition score and were taken care by the management of small holders in Phupan dairy co-operative, Sakol-Nakon. They were randomly placed in 2 x 2 factorial in randomized complete block design. Factor 1 was for dairy heifers and lactating cows comparison and factor 2 was assigned to compare between distilled water and GnRH injection on day 5 post insemination under the treatments. Blood samples were collected and analyzed for progesterone concentration examination by RIA technique. The environment effects, general physiology and hematology changes were recorded in the entire period of the experiment. The result showed that, the Temperature Humidity Index (THI) was over than 72, which affected the increase of general physiology changes, co-efficient of heat tolerance, sweating rate, hematology and cortisol level concentration that were over standard value. However, there were no significant differences ($p>0.05$). The examination of serum progesterone concentration on day 11, 17 and 24 post artificial insemination including the conception rate were significantly different ($p<0.01$).

Key words: GnRH, $PGF_{2\alpha}$, synchronized estrus, AI, conception rate, phupan dairy co-operative

INTRODUCTION

The project of dairy cow promotion has been one approach of the government that expects to improve the national dairy cow production by establishing a good systemic plan for dairy cow production and management. However, there were still many problems for dairy cow raising in some area such as the Project of dairy cow promotion in Pattaloong province. Within 4 years of the said project operation the farmers had to cancel the dairy cow raising due to losses as much as 37 per cent of business cost (Thamawasorn and Luengwatanawilai, 1992). Another is the poor fertility of dairy cows that were raised in tropical zone (Jantalakana, 1994; Umpapol, 2002), anoestrus; silent heat and infertility affected the decrease of milk yield and calf population (Janpongsaeng, 1996). The imbalance nutrients related to gonadotropin secretion, estrous cycle or ovulation, breeding management, heat detection, breeding techniques and semen quality could affect the absolute fertility (Janpratheep, 1996).

The efficient feed value improvement (Janpongsaeng, 1996) which related with prostaglandins application for estrous cycle control or for indicating the suitable breeding time could be one means to reduce the problems of poor fertility on cows due to corpus luteum regressed in luteal phase and followed by follicular phase that caused the cows become in heat in about 45 day post-parturition. The $PGF_{2\alpha}$ (cloprostenol) administration by intramuscular injection for dairy cows in luteal phase could induce normally estrus of the cows (Iamlamai, 2003; Patarajinda, 2003).

The integration of $PGF_{2\alpha}$ and GnRH application at post artificial insemination would be one of the methods to solve the problems of poor fertility of cows during summer because of low level of progesterone. Both hormones could induce the production of accessory corpus luteum and could increase the tissue of corpus luteum and the progesterone level that caused effectively on endometrium preparation for the implantation of zygote in blastocyst phase; reducing the contraction of uterus and stimulating the uterine milk secretion for nourishing zygotes; but the early regression of corpus luteum could cause the embryonic mortality to lower the level of progesterone (Shelton *et al*., 1990). If the

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function of corpus luteum failed it caused the reduction of progesterone level that affected on the conception and gestation (Wiebold, 1998). If the level of progesterone was low after mating it could negatively interfere on zygote implantation and acceptable response of cows for zygote implantation (Inskeep, 2004).

This research aimed to study the conception rate of dairy heifers and lactating cows of the farmers in Phupan Dairy Co-operative (PDC) which raised under small holder management and the animals were induced for synchronized estrus by application of $PGF_{2\alpha}$ (Dinoprost trometamol) and GnRH injection on day 5 post artificial insemination for investigation the effects for improving and increasing the conception rate of dairy cattle under heat stress condition.

MATERIALS AND METHODS

Holstein-Friesian (HF) crossbred dairy heifers and milking cows (82.5% or over of HF blood with uniform body score), under the similar management condition of small holders who were the members of PDC, by randomization the problematic dairy cattle in conception of the voluntary small holders in the number of 32 heads (16 dairy heifers and 16 lactating cows). The experiment was conducted by 2 x 2 factorial in RCBD, factor 1 aimed to heifer and lactating cow comparison (2-4 lactation cows were used) and factor 2 was assigned to compare between 2 ml of distilled water and GnRH (Receptal ®) injection on day 5 post artificial insemination by intramuscular injection.

The synchronized estrus induction was conducted by $PGF_{2\alpha}$ intramuscular injection of 2.5 ml/head at the rump region because they were in estrus on different days but it was very close period therefore the injection was done 2 times. The first injection was conducted on day 9 post-estrus and the second injection followed 11 days thereafter. The cattle would respond to the second injection and artificial insemination could be done during 72-96 h after application. Generally, the cattle would respond to $PGF_{2\alpha}$ injection around 4-5 days before estrus and until 4-5 days after estrus. The estrus would occur 2-5 days after the injection (Willard *et al*., 2003), by observing the estrus symptom and followed by the artificial insemination services (Iamlamai, 1999).

The data collection was conducted with concerns on ambient environment and environmental effects, Temperature-Humidity Index (THI), general physiological changes, haematology, co-efficient of heat tolerance, sweating rate (Umpapol, 2002), pedigree, milk yield, ovary examination by rectal palpation, blood sampling for progesterone examination and pregnancy test by collecting blood on day 0, 5, 11, 17, 24 and 42 post artificial insemination (day 0 meaned a proestrus day before estrus and day 5 indicated a GnRH injection day) (Shelton and David, 2002) and confirmed the certain pregnancy by the results of rectal palpation on day 60 post artificial insemination.

RESULTS

The general condition of the ambient environment: The results of the study on the general ambient environment in the entire duration of the experiment found that maximum, optimum and minimum temperatures, and the different temperature between maximum and minimum temperatures were 34.80±0.40, 29.40±0.60, 24.20±0.80 and 11.20±0.70°C, respectively. The relative humidity was 84.40±4.40 (%) and THI was 86.40±5.02 (Table 1).

Table 1: Effect of the ambient environment on temperature, relative humidity and THI in experiment

Environmental	Mean±STD
Maximum temperature (°C)	34.80±0.40
Optimum temperature (°C)	29.40±0.60
Minimum temperature (°C)	24.20±0.80
Different temperature (Max. - Min.) (°C)	11.20±0.70
Relative humidity (%)	80.40±4.40
Temperature Humidity Index (THI)	86.40±5.02

Table 2: Effect of $PGF_{2\alpha}$ injection on the synchronized estrus induction in heifers and lactating cows

Item	Heifers	Cows
Number of animals (head)	16 (100.00)	1616 (100.00)
Response on estrus induction (head)	16 (100.00)	16 (100.00)
• Standing heat (head)	16 (100.00)	16 (87.50)
• Silent heat (head)	-	2 (12.50)
Anoestrus (head)	-	-

Effect of $PGF_{2\alpha}$ injection on the synchronized estrus induction: The results of the synchronized estrus induction by $PGF_{2\alpha}$ injection found that all of the dairy heifers and lactating cows showed estrus symptom and two of the lactating cows were in silent heat (12.50%) (Table 2).

Effect of the ambient environment on the general physiological changes: The study on the effect of the ambient environment on the general physiological changes among Group 1 (distilled water injected heifers), Group 2 (GnRH treated heifers), Group 3 (distilled water injected lactating cows) and Group 4 (GnRH treated lactating cows), were; the rectal temperatures were 39.38±0.20, 39.40±0.12, 39.42±0.40 and 39.42±0.20°C, skin temperatures were 36.80±0.80, 36.80±0.84, 36.70±0.74 and 36.48±0.92°C, pulse rates were 72.60±2.60, 72.42±1.48, 72.80±2.80 and 73.64±2.02 times/minute, respiration rates were 71.20±2.76, 70.60±1.48, 70.60±3.02 and 72.40±3.46 breathing/minute, sweating rates were 920.20±30.20, 916.20±31.48, 904.40±33.70 and 922.80±40.20 ml/square metre/hour, respectively with non-significant differences ($p>0.05$) (Table 3).

Table 3: Effects of environment factors on general physiology

	Heifers		Lactating cows	
General physiology	Distilled water	GnRH	Distilled water	GnRH
Rectal temperature (°C)	39.38±0.20	39.40±0.12	39.42±0.40	39.42±0.20
Skin temperature (°C)	36.80±0.80	36.80±0.84	36.70±0.74	36.48±0.92
Pulse rate (times/min)	72.60±2.60	72.42±1.48	72.80±2.80	73.64±2.02
Respiration rate (breaths/min)	71.20±2.76	70.60±1.48	70.60±3.02	72.40±3.46
Sweating rate (ml./m^2/h)	920.20±30.20	916.20±31.48	904.40±33.70	922.80±40.20

Means within row with different superscript differ significantly ($p<0.05$)

Table 4: The values of hematology and cortisol level

	Heifers		Cows	
Hematology	Distilled water	GnRH	Distilled water	GnRH
Hematocrit (%)				
• Before experiment	32.20±0.80	32.10±0.34	34.02±0.20	31.80±0.40
• During experiment	33.40±0.64	34.24±0.42	34.68±0.60	32.80±0.46
Hemoglobin (g./100 ml. blood)				
• Before experiment	9.60±0.50	10.64±0.62	9.48±0.42	11.04±0.40
• During experiment	11.20±0.34	11.96±0.74	11.28±0.50	13.04±0.62
Cortisol (ηg/mg)				
• Before experiment	12.14±0.40	12.68±0.64	12.60±0.60	13.04±0.42
• During experiment	12.82±0.26	13.26±0.34	13.68±0.76	13.40±0.62

Means within row with different superscript differ significantly ($p<0.05$)

Effect of the ambient environment on the haematological changes: The haematological values of the experimental cattle in Group 1 (distilled water injected heifers), Group 2 (GnRH treated heifers), Group 3 (distilled water injected lactating cows) and Group 4 (GnRH treated lactating cows) were studied on haematocrit percentages. In the preliminary period of the experiment (before experiment value) the percentages were 32.20±0.80, 32.10±0.34, 34.02±0.20 and 31.80±0.40% but during the experiment (during experiment value) the percentages were 33.40±0.64, 34.24±0.42, 34.68±0.60 and 32.80±0.46. The hemoglobin concentrates in the preliminary period of the experiment had the levels of 9.60±0.50, 10.64±0.62, 9.48±0.42 and 11.04±0.40 gm/100 ml of blood but during the experiment, it had the levels of 11.20±0.34, 11.96±0.74, 11.28±0.50 and 13.04±0.62 gm/100 ml of blood. The cortisol concentration in the preliminary period had the levels of 12.14±0.40, 12.68±0.64, 12.60±0.60 and 13.04±0.42 ηg/ml but during the experiment it had the levels of 12.82±0.26, 13.26±0.34, 13.68±0.76 and 13.40±0.62 ηg/ml, respectively with no significant differences ($p>0.05$) (Table 4).

The comparison of progesterone levels (ηg/mg) at post artificial insemination: The comparative results of progesterone levels of the experimental cattle in Group 1 (distilled water injected heifers), Group 2 (GnRH treated heifers), Group 3 ((distilled water injected lactating cows) and Group 4 (GnRH treated lactating cows) showed that the progesterone levels on day 0 post artificial insemination were 0.12±0. 04, 0.14±0.02, 0.10±0.20 and 0.18±0.03 ηg/mg respectively and on day 5 post artificial insemination were 1.28±0.12, 1.06±0.20, 1.12±0.14 and 1.24±0.32 ηg/mg respectively with no significant difference.

But the comparison of progesterone levels on day 11 post artificial insemination were 2.90±0.20, 4.62±0.16, 2.80±0.24 and 4.20±0.42 ηg/mg respectively, on day 17 post artificial insemination were 3.70±0.26, 6.70±0.28, 3.40±0.30 and 6.60±0.39 respectively and on day 24 post artificial insemination were 2.20±0.32, 5.80±0.24, 2.60±0.24 and 5.78±0.46 ηg/mg respectively with significant difference ($p>0.05$) (Table 5).

Pregnancy examination: The results of pregnancy examination of the experimental cattle in Group 1 (distilled water injected heifers), Group 2 (GnRH treated heifers), Group 3 ((distilled water injected lactating cows) and Group 4 (GnRH treated lactating cows) by rectal palpation on day 60 post artificial insemination found that the numbers of examined pregnant cattle were 8, 8, 8 and 8 heads respectively, but the real pregnant cattle were 3, 7, 2 and 6 heads respectively and the conception rate were 37.5, 87.5, 25.0 and 75.0% respectively with significant differences ($p<0.05$) (Table 6).

DISCUSSION

The influences of ambient environment: The results of this study indicated that the climate was tropical zone (Vajrabukka, 1996). The influence of temperature and relative humidity were correlated in term of Temperature-Humidity Index (THI) (Johnson, 1985) and when THI mean was raised up to 72 it would cause the heat stress to the dairy cows (McDowell, 1972).

Table 5: The comparison of progesterone level (ηg/mg) at post artificial insemination

Day on test (day) (Post artificial insemination)	Heifers		Cows	
	Distilled water	GnRH	Distilled water	GnRH
0	0.12±0.04	0.14±0.02	0.10±0.20	0.18±0.03
5	1.28±0.12	1.06±0.20	1.12±0.14	1.24±0.32
11	2.90±0.20	4.62±0.16[a]	2.80±0.24	4.20±0.42[a]
17	3.70±0.26	6.70±0.28[a]	3.40±0.30	6.60±0.39[a]
24	2.20±0.32	5.80±0.24[a]	2.60±0.24	5.78±0.46[a]

Means within row with different superscript differ significantly (p<0.05)

Table 6: The results of pregnancy examination and conception rate at day 60 pot artificial insemination

Item	Heifers		Cows	
	Distilled water	GnRH	Distilled water	GnRH
Number of cattle (head)	8	8	8	8
Checked pregnant cattle (head)	8	8	8	8
Actual pregnant cattle (head)	3	7	2	6
Conception rate (%)	37.5[b]	87.5[a]	25.0[b]	75.0[a]

Means within row with different superscript differ significantly (p<0.05)

Particularly, the climate in PDC areas and the adjacent areas that raised the dairy cows in Sakol-Nakorn province had THI value of 82.20±4.50 which were affected on the heat stress to the dairy cows (Wiersma *et al*., 1984). The heat stress affected the general physiological changes, haematological values, hormones, fertility and milk production performance (Yousef, 1985).

The impact of the ambient environment on general physiological changes: The responses of the dairy cows under the condition of high THI, would adjust to the mechanism of heat expulsion and heat regulation for static body temperature control by which the changes of general physiology of the body such as rectal temperature, pulse rate, respiration rate and sweating rate increased so the cows reduced the voluntary feed intake (Stermer *et al*., 1986). Similarly, their related to Smith (1984) reported that feed intake reduction (NRC, 2001) was the response of body to the high temperature environment (Maust *et al*., 1972). For decreasing heat combustion from metabolism and for increasing respiration rate and water consumption, and changing the behavior such as movement reduction which related to Thermo-regulatory system under the control of hypothalamus (Ruckebusch *et al*., 1991). The changes of haematology includes such as the values of haemoglobin and heamatocrit were reduced but cortisol level was increased (Abilay *et al*., 1975) that affected the feed intake for regulating the body temperature balance (Umpapol, 2002).

The impact of the ambient environment on endocrine system: The heat stress affected on endocrine gland system, interfered negatively to the mechanism of gonadotropin secretion due to the responses through the hypothalamo-pituitary-gonad axis that were less effective (Iamlamai, 1999). It caused the reduction of estrogen secretion and directly affected to the condition of fallopian tube and uterus (Pilachai, 2005). Luteinizing Hormone (LH) level was lower than normal level, glucorticoid and serum progesterone were also low (Wolfenson *et al*., 2000).

Effect of $PGF_{2\alpha}$ application on the estrus induction: The application of $PGF_{2\alpha}$ could effect on the decrease of LH secretion from anterior pituitary gland and corpus luteal capillaries. They were slightly contracted during the day 6-18 of estrous cycle that caused the progesterone level to rapidly reduce within 12 h and at the least level within 24 h. The estradiol level would elevate highly during 48-72 h and the cows became in estrus around 72 h (+ 24 h) after the $PGF_{2\alpha}$ injection. However, $PGF_{2\alpha}$ application had the limited usage in each case, considering the cow condition of $PGF_{2\alpha}$ could influence the corpus luteum regression. Furthermore, the progesterone level was reduced but estrogen level was elevated and the cows would turn back to their natural behavior and condition (Iamlamai, 1999; 2003).

The responses of GnRH application at post artificial insemination: This experiment was conducted while THI value was raised up over 72 so it caused the heat stress to the dairy cattle during the experiment. The plasma progesterone was decreased due to the functions of corpus luteum which was also decreased as well as the follicle size was smaller including the decrease of granular cell number and androstinidiol secretion from thaca cell. These were the causes of progesterone secretion reduction (Howell *et al*., 1994; Wolfenson *et al*., 2002). These causes affected the reproductive system viz. shortening the estrus period, low conception rate, reducing the development and growth of follicle, extension of corpus luteum regression, high embryonic mortality and abnormal functions of gonadotropin occurred (Jorden, 2003). Furthermore, it affected the ovarian functions of the cows because when the cows

were under the ambient temperature of 29°C and relative humidity of 60% that which interfered negatively to lengthen the duration of the system of corpus luteum regression, because when estrogen concentration was reduced. It could stimulate corpus luteum regression because the endometrium epithelial decreased the building of enzyme that synthesis of prostaglandins (Pilachai, 2005).

However, when ovulation or scar follicle regression was reduced and progesterone level was also decreased (Wilson *et al*., 1998), it affected to the GnRH and LH secretion. In addition, the decrease of estrogen secretion during estrous period because the follicle became smaller than normal and the ovulation system was delayed, silent heat or undetected heat, prolonged luteal phase. This is due to the low level of prostaglandin secretion from endometrium epithelial because of the increase of high temperature would cause the endometrium epithelial secreted less level of prostaglandins (Fabio and Scaramuzz, 2003).

The synthesis of progesterone by motivating in corpus luteum functions and the developing the new corpus luteum by GnRH application in early luteal phase would effect on LH function in inducing the ovulation of the first wave of follicle. Ovulation could occur by the present and influence of LH and later effected on new corpus luteum forming (Techakampu, 2000), progesterone level on day 11 and 17 post artificial insemination of the GnRH treated cattle were highly and significantly different ($p<0.01$) and they were pregnant (Schmitt *et al*., 1996).

The application of GnRH effected on LH secretion and the mechanism of LH function would begin by LH united with LH receptors on SLC wall and became the second messenger that stimulated the function of adnylate cyclase to synthesize increasingly the cyclic AMP from ATP. Later on, the cyclic AMP would stimulate the function of PKA which was the enzyme for catalyzing in adding the phosphate group with some protein that caused the increase of cholesterol transferring into the mitochondria. So cholesterol changed into progesterone more than the direct synthesis from SLC with similar results as Schmitt *et al*. (1996). It concluded that the responses of hCG or GnRH by intramuscular injection of GnRH (Burserelin) 8 μg or hCH (Chorulon®) 3,000 IU compared with normal saline on day 5 post artificial in dairy heifers and lactating cows found that the hormone treated groups formed accessory corpus luteum 93% of the cattle and could elevate progesterone level in the blood but there was no significant difference in pregnancy result of both dairy heifers and lactating cows in summer (Stevenson and Mee, 1991; Thatcher *et al*., 1993).

Conclusion: The ambient environment that caused THI value over than 72 could effect the heat stress to dairy cattle by resulting in the changes of general physiology, haematology and cortisol level. In addition, the application of $PGF_{2\alpha}$ effected to stimulate the dairy heifers and lactating cows under small holder management in Phupan Dairy Co-operative areas and Sakol-Nakorn province region found that they became in clear about estrus symptom. The dairy heifers and lactating cows under the ambient environment of THI value over than 72, were treated with $PGF_{2\alpha}$ for estrus induction and further treated with GnRH on day 5 post artificial insemination could effect to elevate the plasma progesterone that enhanced well to conception rate.

Recommendations:

- A study on conception rate in dairy cattle which was low in heritability characteristics but the influence of environment was higher therefore the consideration for control of many factors that could affect the study should be recognized such as feed and feeding management, house and equipments, climate and the sufficient number of experimental cattle for accurate results of the experiment.
- The health management of dairy cattle must be complete for the efficiency of hormone effects, particularly the lactating cows should be perfect in nutritional condition when application of hormone were being done for stimulating the body.
- The improvement of environment, feed quality and management would effect to the efficiency of hormone when the hormone was applied.

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