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Plasma Progesterone Hormone Levels After Utilizing a Newly Developed Estrus Inducing Device [P-Sync™], as Well as Field Studies Comparing a Commercial Intravaginal Device for Timing of Insemination in Dairy Cattle

W Pattanawong[1]*, P Promtan[2], S Teepatimakorn[3] and W Thaworn[1]

[1]*Faculty of Animal Science and Technology, Maejo University, Thailand*

[2]*Namchuewongwai Company Limited, Thailand*

[3]*Lampang Provincial Livestock Office, Lampang Maejo University 63 No.4 Nongharn, Sansai District, Chiang Mai, 50290, Thailand*

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***Corresponding author**: W Pattanawong, Faculty of Animal Science and Technology, Maejo University, Thailand. Email: wpattanawong@gmail.com

Abstract

The study of plasma progesterone hormone level after synchronization with a newly produced estrus-inducing device in comparison with a commercial device and the field test by determined artificial insemination [AI] in dairy cattle. A total of 15 dairy cattle were studied, consisting of 3 treatments [5 heads. per treatment]: control group, with injection of 2 ml $PGF_{2\alpha}$ at days 0 and 6 [T1]; insertion of a commercial device [T2]; and newly self-produced device, P-sync™ [T3]. As such, T2 and T3 used devices to induce estrus for 7 days. On days 0 and 6, the cows were injected with 2 mL of $PGF_{2\alpha}$. On day 7, the device was withdrawn, and blood samples were collected from cows for 12 days. Plasma progesterone was later measured from the blood using the ELISA technique, which also included the measurement of ovulation by the LH Ovulation Test. The results showed that the use of P-sync™ device didn't show any difference in the concentration of plasma progesterone with commercial intravaginal device [$P<0.05$]. Analysis of ovulation indicated that cows using P-sync™ gave a similar response to cows using the commercial device. Field trial results of cows using the self-made device [n= 200] in comparison with the commercial device [n = 100] showed no difference in estrus induction. According to this study, the use of the P-sync™ device can be used to induce estrus in cows and has a much lower cost than the commercial device. Moreover, the project was able to register for two patents.

Keywords: Dairy cattle; P-syncTM; Device to Induce Estrus; Artificial Insemination

Abbreviations: FSH: follicle stimulating hormone; LH: luteinizing hormone

Introduction

At the moment, when farmers are able to determine the estrus and ovulation of cattle-buffaloes with accuracy and precision, this would mean that cattle cows and buffalo cows would be able to have artificial insemination and become pregnant at the right time to reduce damage from the day-open period and to increase the calf population by synchronizing the hormonal program to induce estrus and ovulation with artificial insemination to solve problems. Problems may include irregular estrus or silent heat, and as such, hormones are used to control or induce estrus, so cows can show signs of estrus uniformly or almost at the same time. At present, commercial devices that are used to induce estrus are already available on the market, but because these devices are imported from abroad, they are quite expensive. Therefore, the development of devices to control or induce estrus has been done locally to reduce importation expenses, although the technology is from outside the country and the devices are suitable for the anatomy of the reproductive systems of local cattle and buffaloes. The main goals of this research were to study how the devices that cause estrus affect the physical development of dairy cattle and to do field tests to see how well the devices work compared to commercial devices that help dairy cattle have their estrus at the same time.

Methods

In this study, the development of the estrus-inducing device was done in two [2] experiments, as

- Experiment 1 In vitro [laboratory]
- Experiment 2 In vivo [actual animals]
- Experiment 3 Use of self-made device of the project in farmers' farms raising dairy cattle.

Experiment 1 In vitro [laboratory]

Development of the design of an estrus-inducing device in the form of plaster attached to the skin

In this study, the conceptualization and design development of the estrus-inducing device in the form of a dermal plaster hormone, which has properties that expand on the skin surface of the cow, was considered satisfactory because it did not peel off during the test period. In addition, it could analyze the target area on the cow where it was pasted with a thin area around it, and most importantly, it was nearest to a vein. Thus, in summary, the area where the estrus-inducing device was pasted in the form of a hormone plaster was anywhere around the tail of the cow because in this area the skin was thinner than in other parts of the cow. Besides, the coccygeal vein was found to pass across this area.

Designing the estrus-inducing device in the form of skin hormonal plaster

The rectangular shape of the estrus-inducing device in the form of skin hormone plaster had a size in width x length equivalent to 30 x 5 cm. In the middle of the plaster was the progesterone hormone in various amounts. This estrus-inducing device, in plaster form with hormone concentration, was pasted on the skin surface as illustrated below.

Determining the quantity of the progesterone hormone suitable to be contained in the estrus- inducing device in the form of a skin hormone plaster.

In this study, there was a need to determine the amount of progesterone hormone suitable to be contained in the estrus-inducing skin hormone plaster device, to be able to know the minimum amount needed to enable the cow to show signs of being in heat, and at the same time, to reduce cost by producing the greatest number of devices. As such, experimental planning involved the packing of progesterone hormone into the device at various levels, as follows: 0.75 g, 1.0 g, and 1.5 g, respectively, were used with the experimental cows in comparison with the use of a commercial intravaginal estrus-inducing device [Progesterone device]. Blood samples were then collected to measure the amount of progesterone hormone in the plasma of the experimental cow to determine the amount that is highly like commercial devices.

Analysis of progesterone hormone

Determining the suitable dilution rate of MAb P4 and P4-HRP by ELISA method

MAb P4 used 100uL per well and concentration ratios of 1:1, 1:2, 1:5, 1:10, and 1:50 to coat 96- well plates. The plates were then left at 4°C for 16 hours. The plates were later diluted, after which the wells were filled with 2% gelatin at 200 uL per well and then incubated at room temperature for a period of 60 min. Afterwards, the wells were diluted with P4-HRP at concentration rates of 1:10,000, 1:20,000, 1:30,000, and 1:40,000 at a volume of 10 uL per well and then incubated at room temperature for 90 minutes. After washing the plates, the diluting substance was added to cause color at a volume of 100uL per well, then incubated at room temperature in a dark place for 20 minutes. Using $4NH_2SO_4$ at a volume of 100uL per well stopped the reaction. Light wave absorbance was then measured at 420 nm using the microplate reader.

Determining standard graph

Mab P4 was used to coat 96-well plates with a concentration of 1:10 at 100 uL per well. The plates were kept at 4°C for 16 hours and then washed with a solution that made the concentration lower. Afterwards, gelatin solution at 2% concentration was poured into each well at a volume of 200 uL and then incubated at room temperature for 60 minutes. The same plates were then washed with standard progesterone at concentrations of 0, 1, 2.5, 5, 10, 25, 50, 100, 250, 500, and 1,000 pg/50 uL per well. After 90 minutes at room temperature, 10 uL of P4-HRP 1:40,000 was added to each well. This was again incubated at room temperature for 90 minutes. The plates were then washed with a diluting solution that caused coloration at 100 uL per well. Again, it was incubated at room temperature in a dark place for 20 minutes, and the reaction was ceased using 4NH2SO4 at 100 uL per well. Light absorbance was read at 492 nm using a microplate reader.

Analysis of P4 amount in samples using the Competitive ELISA method

The 96-well plate coated with Mab P4, which has a concentration ratio of 1:10 at 100 uL per well, was incubated at 4 oC for 16 hours. Afterwards, the plates were washed with diluting solution and then filled with 2% gelatin diluting solution at 200 uL per well, and then incubated at room temperature for 60 minutes.

Experiment 2 In vivo [actual animals]

Experimental design

A total of 15 Friesian crossbred dairy calves were divided into 3 treatment groups, as follows:

- Group 1 Control group with animals not using the device [5 calves]

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➢ Group 2 Intravaginal estrus-inducing device inserted to the uterus of the cow [Progesterone device] that contains the progesterone hormone at 1.9 g [5 calves]

➢ Group 3 Estrus-inducing hormonal skin plaster device [5 calves]

Experimental method

Before the experiment, each animal received 2 ml of the prostaglandin F2 alpha hormone by intramuscular injection at the rear leg to dilute the corpus luteum and disable its functioning.

➢ The estrus-inducing intravaginal device [Progesterone device] was inserted into the uterus of the Group 2 experimental animals. For Group 3 calves, the estrus-inducing device in the form of a hormonal skin plaster was pasted around the area of the tail for 7 days.

- The steps in inserting the intravaginal device in Group 2 [Progesterone device] consisted of cleaning the area around the mouth of the vagina before inserting the device and then applying gel for lubrication of the device and the vagina. Then the device was slowly inserted into the uterus. The stability of the device could be observed from the cord hanging outside of the uterus.

- The steps involved in the use of the estrus-inducing device in the form of a hormonal skin plaster started with the cleaning of the area around the tail by 70% alcohol using cotton after which the plaster was pasted to the skin. When pasting, the plaster must stick to the skin surface of the rear end around the tail.

➢ Collection of 5 ml blood samples from the jugular vein from each of the experimental animals. The samples were then contained in the pipette with an anticoagulant EDTA at 500 uL. Afterwards, the blood samples were taken for centrifuge to separate the plasma component at 2000 rpm for 10 minutes. After separation of the plasma from the surface of the microcentrifuge tube [1.9 ml], it was then stored in - 20oC until taken for analysis to determine the amount of progesterone [P4], estrogen [E2], follicle stimulating hormone [FSH] and luteinizing hormone [LH] at the first collection of blood samples during the first day of the experiment [Day 0]. After that, collecting blood samples was done every day throughout the 15-day duration of the study for a total of 16 days. Collection was done at a uniform time each day, at 7.30 in the morning.

➢ Scanning of the body to measure the size of the follicles, dominant follicles, and corpus luteum of each of the experimental animals throughout the entire period of the experiment, to study the effect of using the estrus-inducing device on the physiology of the reproductive system of the experimental animal.

➢ Measuring the amount of P4 in plasma by using the ELISA technique.

➢ Observing the behavior of the animal in showing signs of estrus after withdrawal of the estrus- inducing device at the last 3 days [Day 8, 9 and 10] for 2x/day at 2 hours each during 07: 00-09: 00 hours and 15:00-17:00 hours.

➢ Measuring ovulation using the commercial LH Ovulation Test, with the following steps:

- Preparing the animals to start the test.
- Collecting blood sample in a vial.
- Opening the envelope and pulling out the test strip from the envelope
- Dipping the test strip into the blood sample for about 10-20 seconds but carefully making sure the blood sample did not go beyond the maximum line on the strip or it would be inaccurate.
- Withdrawing the strip from the blood sample and then lying it down on a smooth
- surface that was clean, dry, and non-water absorbent. For the most appropriate reading of the test result, it must be done 5 minutes after withdrawal.
- Waiting for the color to appear on the line depending on the LH concentration in the plasma. Normal results show a positive reading for about 40 seconds. However, the result would be confirmed as negative if the reading allowed a complete reaction in 10 minutes. In this case, the reading should not be done for 30 minutes after testing.

Experiment 3 Use of the project-made device in the farmers' dairy farm

Experiment among dairy cows

A total of 300 cows was used in this study, divided into two groups consisting of:

Group 1. Use of intravaginal device to induce estrus [Progesterone device] [100 heads] Group 2. Use of device to induce estrus in the form of a skin plaster [200 heads]

Experimental procedure

➢ The insertion and pasting of the device to induce estrus to the experimental cows in Groups 1 and 2 took place for 7 days based on the program on estrus induction for artificial insemination. The procedure included the insertion and pasting of the device for 7 days and then blood sample collection throughout the time the device was used. The device remained inside the uterus for 10 days after insertion at Day 0 [D0] together with an injection of 100 mg P4 + 2 mg E2. On Day 6 [D6], the cows were injected with $PGF_{2\alpha}$ and then the estrus-inducing device was removed at Day 7 [D7]. After withdrawing the device, 24 hours a Day 8 [Day 8], the cows were injected with estradiol benzoate [EB] hormone and were then observed for signs of being in estrus

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within 24-36 hours. Observation was done twice per 2 hours and on Day 9 [D9], breeding was done when the cow showed signs of being in estrus. (Figure 2).

- Examination of estrus and breeding. After inducing estrus to all cows and then observing for
- signs of estrus in cows and buffaloes done at 2 days after withdrawal of the device at twice per 2 hours during 07:00-09:00 hours and 15:00-17:00 hours.
- When examination found behavioral signs of estrus, breeding was done twice with intervals of 12 hours in between.
- Examination of return to estrus after breeding at 21- 24 days and pregnancy test at 55-50 days after breeding.

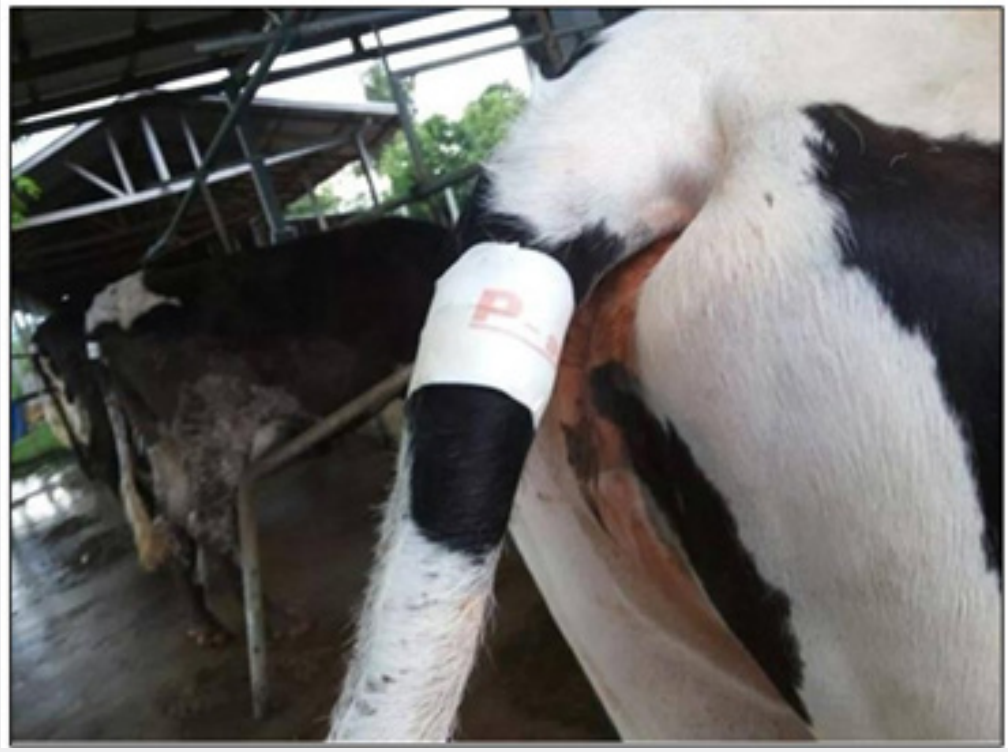

Figure1: Shows the estrus-inducing device in hormone plaster form.

Source: Purchased from Namchuewongwai Company Limited.

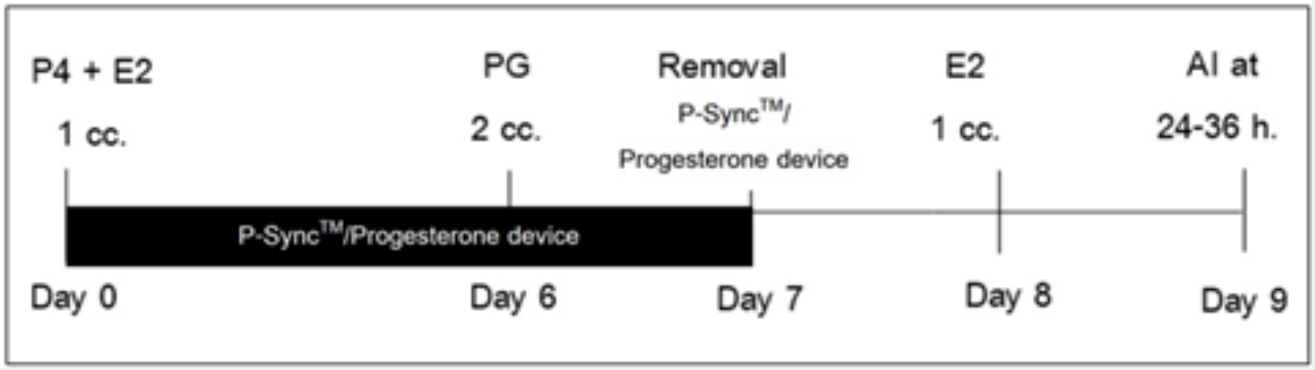

Figure2: Shows the steps in the breeding experiment in cows after estrus by using estrus-inducing device.

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Results

Experiment 1. In vitro [Laboratory]

Development of the model of the estrus-inducing device in plaster form [P-sync™]

In this study, the conceptualization and development of the model of the estrus-inducing device in plaster form [P- sync™] included excellent properties to allow it to adhere to the skin surface of the experimental cow without dripping out until the experimental period was finished. In addition to that, analysis of the target area on the body of the experimental cow where the plaster was to be pasted [P- sync™], on a skin area with a thin surface and importantly, that area was nearest to a vein. Therefore, it was summarized that the best area would be around the rear end at the tail because this area has the thinnest skin layer. Moreover, this area contains the coccygeal vein that passes through it.

Designing the plaster form estrus-inducing device [P-sync™]

The design of the plaster form estrus-inducing device [P-sync™] was characteristically of a rectangular shape with area of 30 x 5 cm [width x length]. Directly in the middle of the plaster was a case containing the progesterone hormone in various amounts. This design of the plaster form of the estrus- inducing device [P-sync™] was conceptualized by the researcher, is shown in Figure 1.

Examination of microbial contamination and infection around the uterine region

The examination of any microbial contamination and infection around the uterus of the experimental animal which were estrus-induced using the intravaginal device and the hormonal plaster form device, was conducted by dividing the experimental animals into 3 groups: Group 1 was the control group which did not use any device at all [2 heads]; Group 2 used the intravaginal estrus-inducing device [2 heads]; and, Group 3 used the estrus-inducing device in plaster form pasted to the skin [P- sync™] [2 heads]. Before the experiment on estrus, each animal was injected with 2 ml prostaglandin F2 alpha hormone intramuscularly at the rear leg to dilute the corpus luteum to block them from functioning. After that, the intravaginal estrus-inducing device was inserted and the P- sync™ was passed around the area of the tail for 7 days. After completing the 7 days, the uterus was felt and grasped to feel it together with observation of any mucus and pus coming out from the vagina. Results of the study showed that experimental cows in the group that used the intravaginal device were observed to have mucus mixed with pus that appeared in yellow color mixed with blood and with rotten smell that came out of the uterus in the 2 cows. Meanwhile, in the group that used the plaster device and the control group, no mucus mixed with pus was observed in both animals, as shown in Table 1.

Table 1: Shows the results of the experiment on microbial contamination and infection around the area of the cervix and of the 2 groups.

Treatment	Cow 1	Cow 2
Group 1 Control	-	-
Group 2 Intravaginal device	+	+
Group 3 P-sync™	-	-

+ means there is infection of uterus; - means no infection of the uterus.

Experiment 2 In vivo [actual animals]

Determination of the quantity of progesterone hormone suitable for containing in the estrus- inducing device in the form of plaster pasted on the skin [P-sync™]

In this experiment, the appropriate quantity of the progesterone hormone to be packed or contained in the estrus-inducing device in the form of a plaster pasted on the skin of the animal [P-sync™], must first be determined to identify the least or minimum amount needed to allow the animal to show signs of being in estrus. This would maximize the reduction of cost investment for the device production, as indicated in the research plan of containing the progesterone hormone in the P-sync™ device during the actual experiment in comparison with the containment of the progesterone hormone in the commercial intravaginal estrus-inducing device [Progesterone device]. Before conducting the estrus experiment, each animal was injected intramuscularly with 2 ml prostaglandin F2 alpha in the rear leg to dilute the corpus luteum to disable them from The rate of release of progesterone hormone [P4] among animals in the Control, Progesterone device and Plaster groups for a period of 16 days [using the device in days and collecting blood for testing in 9 days for a total of 16 days], was measured. In computing for progesterone hormone in the estrus-inducing device of the 2 groups released into the blood of the experimental animal, the average amount of the experimental animals in Groups 2 and 3 was subtracted by the average of the animals in Group 1 because the average amount in Group 1 was the amount of the hormone naturally built by the body. It was found that the amount of progesterone in the estrus-inducing P- sync™ device showed a similar trend to the commercial intravaginal estrus-inducing device. But the average amount of progesterone hormone circulated in the blood was higher than the commercial intravaginal estrus-inducing, as shown in Table 2 and Figure 3.

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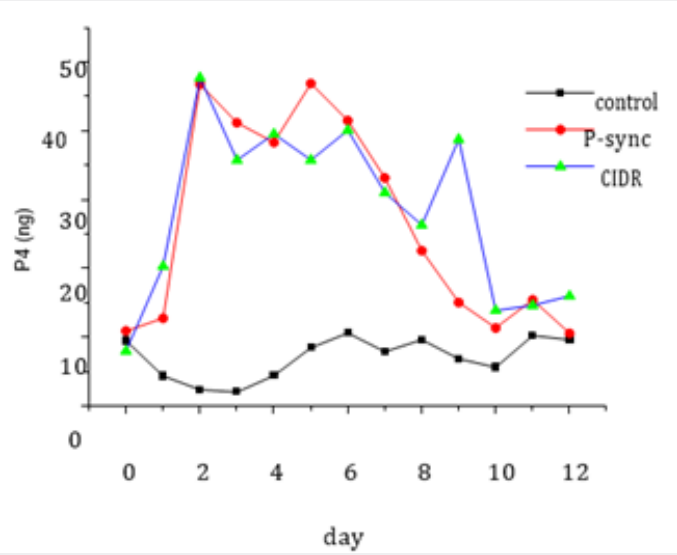

Figure3: Shows the average amount of progesterone hormone circulated in the blood of experimental cows in the 3 groups and indicates the use of progesterone hormone of the P- syncTM group.

circulated in the blood had similar trend as in the commercial intravaginal device.

Table 2: Shows the level of progesterone hormone in the blood of cows in the control group, intravaginal device group and P-sync™ group.

	Average P4 [µg/ml]		
Day	Control [n=5]	Commercial Intravaginal Device [n=5]	P-sync™ [n=5]
0	9.52 ± 3.04[a]	10.92 ± 5.04[a]	8.02 ± 4.04[a]
1	4.39 ± 4.70[a]	12.79 ± 4.70[b]	20.35 ± 3.70[c]
2	2.39 ± 4.35[a]	46.82 ± 7.35 [b]	47.76 ± 3.75[b]
3	2.04 ± 6.27[b]	41.22 ± 1.77[b]	35.78 ± 4.77[b]
4	4.44 ± 0.86[a]	38.35 ± 3.86[b]	39.57 ± 3.26[b]
5	8.49 ± 2.55[a]	46.93 ± 2.48[c]	35.79 ± 4.42[b]
6	10.63 ± 0.78[a]	41.52 ± 1.78[b]	40.12 ± 5.17[b]
7	7.93 ± 5.47[a]	33.18 ± 6.47[b]	31.04 ± 3.47[b]
8	9.67 ± 6.10[a]	22.59 ± 7.10[b]	26.33 ± 4.15[b]
9	6.89 ± 2.41[a]	15.09 ± 4.41[b]	38.81 ± 4.41[b]
10	5.66 ± 7.61[a]	11.37 ± 5.62[b]	13.91 ± 4.60[b]
11	10.27 ± 1.22 [a]	15.46 ± 3.21[b]	14.63 ± 2.87[b]
12	9.67 ± 3.45 [a]	10.58 ± 4.17[b]	16.01 ± 2.01[b]

[a,b,c] Values within a column have significant difference [P<0.05].

In testing the amount of progesterone hormone in the 3 treatment groups from Day 0 to Day 12, results showed that at Day 0, or the starting day of the experiment, the 3 groups were not different. If compared during the days after Day 0 of the experiment, results showed that from Day 1–12, the cows in the groups of commercial intravaginal devices and P-sync™ were not different. It can be clearly seen from the values that the amount of hormone that permeated into the blood showed no significant difference in statistics. And in addition, several values were similar, but the two groups were different from the control group. Thus, the resulting value could be confirmed to be applicably similar and not different from the two treatment groups of the commercial intravaginal device and the P-sync™ device.

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LH Ovulation Test

The examination of ovulation among the experimental cows from the 3 groups, after testing their plasma on Day 8, 9, 10 and 11, was done by LH Ovulation Test. It was found that cows in the Progesterone device and P-sync™ groups gave positive results during the 4 testing days. Meanwhile, cows in the Control group gave negative test results for the 4 days. Table 3 showed that the results of this study indicated the high level of the progesterone hormone circulating in the blood. This might be due to the stress exerted to the pituitary gland allowing the secretion of the follicular stimulating hormone [FSH] to disable the development of the follicles until ovulation. After the progesterone hormone ceased to be given [after the device was withdrawn], the body of the animal started to secrete LSH from the pituitary gland and follicles began to fully develop and were ready for ovulation. Afterwards, the body began to secrete high amounts of LH until it was able to stimulate ovulation and the animal showed behavioral signs of being in-heat, which were observed in animals in Progesterone device and P-sync™ groups.

Table 3: Shows test results of ovulation by LH Ovulation Test among cows in the 3 groups.

Treatment	Replication	Day 8	Day9	Day 10	Day11
Group 1 [Control]	1	-	-	-	-
	2	-	-	-	-
	3	-	-	-	-
	4	-	-	-	-
	5	-	-	-	-
Group 2 [Progesterone device]	1	-	+	+	+
	2	-	+	+	+
	3	-	+	+	+
	4	-	+	+	+
	5	-	+	+	+
Group 3 [P-sync™]	1	+	+	+	+
	2	+	+	+	-
	3	+	+	+	+
	4	-	+	+	+
	5	-	+	+	-

+ means there is ovulation,
- means, no ovulation.

Table 4: Shows results on the use of estrus-inducing device to determine breeding dates among cows.

Treatment group [n]	Progesterone device [100]	Psync™ [200]
Loosening of device [%]	1 [1%]	7/200 [3.5%]
Uterine infection [%]	21/99 [22.2%]	0
Estrus induction [%]	99/99 [100%]	193/193 [96.5%]
Pregnancy from 1st A.I. [%]	55/99 [55%]	113/193 [58.5%]
Pregnancy from 2nd A.I. [%] *	39/44 [88.6%]	73/80 [91.3%]

Experiment 3 Use of project made device for dairy farmers.

Estrus response when using a self-made estrus-inducing device in comparison with commercial device among dairy cows

The Table showing the results of the experiment using the device and indicating several factors involving the induction of estrus among cows such as loosening of device [%], uterine infection [%], estrus behavior [%], pregnancy rate after 1st breeding, and pregnancy rate after 2nd breeding. It was found that most of the recurring problems related to estrus induction among cows were uterine infection. In using the P-sync™ device, no uterine infection was detected [0%], which was different from cows using the commercial intravaginal device [Progesterone device] which showed 22% uterine infection. On animals showing signs of being in estrus, results showed that cows using the P-sync™ device displayed more signs than those in Progesterone device group [100% and 96.5%, respectively], and pregnancy

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rates from the 1st and 2nd breeding were shown higher by cows in the P-sync™ group than those in the Progesterone device group. But in cases where the device got loosened, cows in the P-sync™ group showed higher rates of loosening than in cows using the Progesterone device, at 3.5 and 1.0%, respectively.

Discussion and Conclusions

In this study, an estrus-inducing device had been designed differently from the characteristically T-shaped commercial device. This device was self-designed in the form of a plaster to be pasted to the skin. When this device was used to be tested in cows, it was pasted firmly to the skin near the tail of the 8 cows for a period of 7 days. The trial was done twice. Results of the test showed that the device remained attached firmly during the entire duration of the trial. With a 100% firm attachment, the device proved suitable to the body of the cow and did not cause any stress to the animal prior to the containment of the progesterone hormone in the device in the laboratory.

From the study on the level of progesterone hormone in the blood after the device was pasted into the skin to control estrus in the cows, results showed that the average amount of the progesterone hormone in the blood was equivalent to 4.21 nanogram/ml which could be due to the progesterone hormone being circulated through the cell membrane and which from there, self-functioned directly in the nucleus by attaching to the plasma membrane receptor leading it into the cell. The synthesis of progesterone hormone in the ovary, placenta and adrenal glands using the same method of having a cholesterol add a precursor to the change in pregnenolone to become progesterone hormone [1], which showed that the amount of progesterone hormone synthesized in each part of the organ was different among non-pregnant animals. The progesterone hormone is mostly created from corpus luteum such as in small luteal cells [SLC, about 19%] and large luteal cells [LLC, about 4%. The remainder of about 7% is comprised of other cells [Chainarong, 2008]. Much of the amount of secreted progesterone hormone during the luteal phase of the estrus cycle, are usually excreted at about 20-40 mg/ml. Secretion starts after the ovulation phase and slightly increase continuously until reaching the optimum level in day 16 of the estrus cycle. From there, it starts to decrease when ovulation starts at about 3-4 days. The progesterone hormone is then absorbed at a fast rate. After about 90% has entered the body by blood circulation into the target organ, it is metabolized in the first liver together with other un-used progesterone hormone which are then converted by the liver into inactive form as pregnenolone which are conjugated and excreted through feces. About 20% of the progesterone hormone found in the blood is excreted through urine in the form of sodium pregnanediol glucuronide.

Since progesterone hormone is important in fertility, the implantation and survival in the young animal, through the development of follicle, ovulation and estrus cycle, the circulation of the blood to remove the progesterone hormone in the liver was related to the level of progesterone hormone circulated by the blood. If given to be digested, the effect is very minimal as compared to when inserted into the uterus [2]. Applying the progesterone hormone directly through the skin caused changes in the mucus membrane in the uterus, like the one created in the luteal phase of the estrus cycle. This showed that in giving the progesterone hormone through the skin to permeate to the veins in the area around the tail of the cow, helped in the changes in the mucus membrane of the ovarian wall thus making the development of the follicles to be like when they were created during the normal estrus cycle.

In the measurement of the amount of progesterone hormone in the plasma, the level of progesterone hormone can determine the number of days of the estrus cycle by using the level of progesterone hormone as an index [3]. Normally, the level of progesterone hormone during the estrus period [Day 0] and Day 1-2, is very low, with values so small it could not be measured, until it reaches about 1 ng/ ml. That value decreases for 2-3 days. After that, the value increases until it reaches the highest value. First, the maximum value of the progesterone hormone is found at the 6th day. But sometimes, it can be seen even on the 4th day to the 8th day. The amount of the progesterone hormone that can be measured from the highest level during the first cycle, changes based on the form of the level of progesterone hormone of each cycle with amounts ranging from higher than 1 ng/ml to more than 10 ng/ml. Afterwards, the amount of progesterone hormone that can be measured from the highest level remains at high level such as in day 7-10 which is during the luteal phase. At a 2-5 cycle in each cycle, the amounts reach higher than 2 ng/ml until more than 10 ng/ml. After that, the level of progesterone hormone decreases at a very fast rate until it has the lowest amount at day 17 to day 19 depending on the estrus cycle, with the level of progesterone hormone at day 19 to day 22 of the estrus cycle having the lowest amount of less than 1 ng/ ml [4]. But if estrus-induction is done by using the intravaginal device, the amount of the progesterone hormone level would not be like the normal amount of progesterone hormone. When the self-made device was used to induce the estrus, it was found that the concentration of progesterone hormone of the cow increased very rapidly with the concentration level of progesterone hormone in the blood of the cow increasing to the highest level after using the device for only 24 hours and remaining at the original level at about 7 days until the device was withdrawn when the amount of concentration of the progesterone hormone in the blood decreased rapidly.

In this study, it is shown that a self-made estrus-inducing device could be used to induce estrus with effect on the measurement of the progesterone hormone in the plasma. When the estrus-inducing device was compared with the commercial device, the self-made device was shown to contain a higher amount of progesterone hormone than the commercial device. And when the self-made estrus- inducing device was used in combination with estradiol benzoate and prostaglandin F2 alpha hormones to

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regulate the estrus and ovulation as in this study, it enabled the heifer to ovulate at an acceptable rate and the duration of ovulation became precise thus allowing the effective synchronization of time for artificial insemination. When comparing the percentage estrus of the animals between the group using the self-made device with the group using the commercial device, it gave a 100% rate for the two groups. This was also likewise reported by Siwat [5] who found that the use of progesterone hormone [1 mg/ml] at 24 hours after withdrawal of the estrus-inducing device led to the induction of the growth of follicles and caused ovulation at almost the same time thus increasing the rate of conception. The application of estradiol benzoate hormone at 24 hours after withdrawal of the estrus-inducing device also caused faster ovulation and becoming in heat than cows not using estradiol benzoate hormone. Cows injected with estradiol benzoate hormone were found to show signs of estrus as in being still between 48-60 hours after being injected with estradiol benzoate. In a fixed time, artificial insemination, breeding time must be done at 48 hours after removal of the estrus-inducing device to cause a higher rate of conception. As for prostaglandin F2 alpha hormone, it was applied to dilute the corpus luteum which might have settled during the time when the estrus-inducing device was inserted, as similarly reported by Cavalicri et al [6], who found that application of estrogen hormone together with the intravaginal insertion of the estrus-inducing device caused the animal to show signs of estrus and followed by ovulation. By applying the estrogen hormone, it caused the uniform time for building up of follicles. The application of estrogen may cause it to be similar with the insertion of the estrus-inducing device or injection with estrogen hormone before the start of the stimulation using progesterone hormone in the form of intravaginal device which showed that when administered at day 1 or 3 and before day 6 during the time the follicles were developing. This led to an increase in ovulation because the use of estrogen hormone caused the ovary to become full. This effect allowed the level of luteinizing hormone to increase to the highest thus causing ovulation within 24 hours and causing the cows to display signs of being in-heat.

In using the hormonal program of inducing estrus based on the model recommended by this study, heifers attained 100% estrus and a 67% ovulation thus enabling the fixation of time for effective artificial insemination. Aside from this, this would also reduce investment cost related to control of ovulation, as reported by Bo et al. [7], who found that injecting progesterone hormone at 100 mg/ml could induce dominant follicles to be diluted and to make certain that the cow was really in the period of luteal phase. This was in conformity with [6] who found that injecting estrogen hormone could stimulate ovulation of dominant follicles and could result to the development of the new set of follicles, which could then be used together with the program of estrus-induction with progesterone hormone in the form of intravaginal insertion. This agreed with the report of Xu and Burton [8] who found that injection of estradiol benzoate hormone at 1 mg/ml at 24 or 48 hours after the cessation of progesterone hormone by intravaginal insertion could induce estrus in cows which displayed clear signs of estrus. More than 90% of the cows showed signs of being in estrus within 24 hours after injection of estradiol benzoate hormone, which agreed to the findings of Day et al. [9] who found that 24 hours after the injection of estradiol benzoate hormone, ovulation period ranged from 60-80 hours after the withdrawal of progesterone hormone and led to a 40-40% rate. This was also like the report of O'Rourke et al. [10] and Siwat [5] who found that progesterone hormone through intravaginal insertion could be used together with prostaglandin F2 alpha and estradiol benzoate, resulting to the induction of estrus and ovulation with higher efficiency. This also agreed to the report of Martinez et al. [11] who found that the use of the intravaginal device together with the injection of estrogen hormone became a widely accepted method in a fixed time artificial insemination with much effectivity among dairy and beef cows. This was like the report of Nusara [12], who found that the use of progesterone hormone inserted intravaginally to induce estrus, served as a new guideline to solve the problem of mating system efficiency and assisted in the management of artificial insemination and having this hormone used to cows with or without estrus cycle. But the use of progesterone hormone inserted intravaginally [Progesterone device] and in combination with other substances such as prostaglandin F2 alpha and estradiol benzoate hormones, could lead to estrus induction and greater ovulation. Similarly, the report of Martinez et al. [13], stated that commercial intravaginal devices could be used to induce estrus together with progesterone hormone [50 mg/ml] and estradiol benzoate hormone [1 mg/ml]. It was found that estrus in animals injected with estradiol hormone reached a rate of 92.3%. This was also in conformity with Colazo et al. [14] who reported that using intravaginal device could induce estrus together with prostaglandin F2 alpha hormone and estradiol benzoate [1 mg] plus progesterone hormone [100 mg]. Results showed that rate of estrus was high at 88%, which was like the report of Pursley et al. [15]. The use of commercial intravaginal device to induce estrus combined with estradiol benzoate hormone [1 mg] and progesterone hormone [100 mg], showed a 100% estrus rate, 76% conception rate and as high as 100% pregnancy rate, as likewise reported by Martinez et al. [13], who stated that when using estradiol benzoate hormone at 1 mg after insertion of estrus-inducing device, 90% of the cows in the herd attained estrus after 24 hours Similarly, Cavalicri et al. [6] , found that the use of estradiol benzoate hormone combined with commercial intravaginal estrus-inducing device, could be used to regulate the occurrence of follicular wave, control estrus and ovulation after giving estradiol benzoate hormone during estrus-induction thus causing ovulation in heifer cows and mother cows. Likewise, the report of O' Rourke et al. [10] showed that applying estradiol benzoate before estrus after using the commercial intravaginal device to induce estrus and prostaglandin F2 alpha, helped in reducing the time gap between estrus and ovulation thus enabling effective calculation of the best time for easier breeding.

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The use of hormone to induce estrus resulted to a greater rate of induced estrus leading to an acceptable rate of ovulation with duration of ovulation much more precise thus enabling the time for an effective artificial insemination, in agreement with Tenhagen et al. [16] who reported that applying progesterone hormone through intravaginal insertion in combination with the ovulation program and a good response in fixed time artificial insemination. The synchronization program for estrus-induction and ovulation by progesterone hormone in the form of intravaginal insertion, agreed with the report by Bo et al. [7], who found that the use of progesterone inserted intravaginally to the uterus to induce.

estrus, gave a higher rate of conception in cows with problem of not having estrus and no conception. The advantage of using the intravaginal device to induce estrus together with the program for hormonal induction of ovulation, was its ability to rehabilitate the reproductive organ of cows with problem of returning to normal estrus. Similarly, Chenault et al. [17] reported that about 3.1% of cows showed no response to the use of the intravaginal device to induce estrus in combination with the program to induce ovulation. In addition, a report mentioned that the use of commercial estrus-inducing device in combination with injection of estrogen hormone after withdrawal of the device, was able to effectively induce estrus and ovulation in cows. This report agreed with Whittier et al. [18] who used a commercial intravaginal estrus-inducing device in combination with an injection of estrogen thud resulting to an increased percentage rate of pregnancy among cows leading to cost-effective economics most particularly during the hot season when cows were usually under stress.

The main objective of this research was to develop and test a self-made device that can induce estrus and be used to regulate the estrus among dairy and beef cattle while being capable of controlling the ovulation effectively among cows to a precise period of ovulation, to create a good appropriate model of program for estrus induction intended for cows raised in Thailand. This research was also aimed to reduce investment cost for the use of hormones by designing a device that can be applicable in local conditions while at the same time, being able to regulate ovulation with greater efficiency and with an acceptable breeding rate in comparison with commercial device. Aside from these, it was hypothesized in this study that the precision and capability of detecting low estrus which is the main reason for the lower rate of pregnancy among cows as expected in beef cattle farms in the country. Therefore, the use of a self- made estrus-inducing device in regulating ovulation and fixed time artificial insemination will help in increasing the breeding efficiency among cows on the farm. Moreover, it reduces the cost of the technology that was imported from abroad and allows farmers to have the opportunity to access the technique of inducing estrus. In increasing the number of pregnant cows by this method of inducing estrus, knowledge was used to develop the method in using the estrus-inducing device and fixed time artificial insemination even without observing the signs of estrus, inducing ovulation for fixed time artificial insemination.

For future studies, the interesting issues may include the development of the design of the device to be more suitable and capable of being commercially produced since estrus-inducing device that is being produced now can use the locally available and cheaper raw materials particularly in field trials in comparison with the commercial device. For results to be more credible, future research should focus on the use of the device in the farm to examine the function of the body, expression of estrus behavior, rates of conception and pregnancy after using the device. Aside from these, if this technology is used together with the rapid pregnancy test technology such as pregnancy test by ultra-sound [30 days after breeding] or determining the level of progesterone after breeding, will increase the efficiency of reproduction of cows in the farm by increasing the number of pregnant cows. Finally, this research project would successfully apply for 2 patent rights, the production of the estrus-inducing device and the design of the estrus-inducing device for commercial prospect of producing the device and transfer of technology in the future.

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