Article - Human and Animal Health

Comparison of the Results Synchronization with Progesterone Implants and Fixed Time Artificial Insemination in Dairy Cows

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HIGHLIGHTS

- A field synchronization study.

- Solution suggestions for the dairy industry.

- Progesterone different uses.

- High pregnancy result was obtained.

Abstract: Dairy cows that are being cared for in family businesses were used to compare the pregnancy rates by applying different time periods, using progesterone devices, and using fixed-time artificial inseminations. The cows were randomly divided into 3 groups. Group 1(n = 56); On day 0, GnRH (IM) (10mg Buserelin acetate, Ovarelin, CEVA DIF) + Progesterone implants (PRID®) (1,55 g progesterone CEVA), Day 8 PGF2α (Dinoprost tromethamine, 25 mg, Dinolytic, Pfizer), Day 9 Progesterone implants removal + PGF2α, insemination was performed at 56th hours insemination after the second PGF2α injection. Group 2 (n = 50); on day 0, GnRH (IM) + Progesterone implants, PGF2α (IM) on the 8th day, Progesterone implants removal + PGF2α on the 9th day, insemination was performed at 48th and 72th hour after the second PGF2α injection. Group 3 (n = 53); on day 0 GnRH (IM) + Progesterone implants, on the 5th day PGF2α injection, day 6 PGF2α (IM) and remove progesterone implant and insemination at 56 hours after the second PGF2α. The average milk yield was recorded Group 1 16.66 ± 5.28 kg, Group 2 13.46 ± 3.05 kg. and Group 3 was recorded as 15.35 ± 4.60 kg. Pregnancy diagnoses were performed 35 days after the artificial insemination. The pregnancy rate was 41.07% in Group 1, 42% in Group 2, and 52.83% in Group 3. As a result; pregnancy rates were similar in all three groups. It has been concluded that synchronization with progesterone can solve many reproductive problems.

Keywords: cow; progesterone; family business; synchronization; artificial insemination.

INTRODUCTION

Increasing the success of artificial insemination in cows and various applications to obtain a calf per year are performed regularly. Systematic synchronization programs are needed for a sustainable farm due to the constantly increasing herd sizes [1]

CIDR® (Controlled Internal Drug Releasing) and PRID® (Progesterone Releasing Intravaginal Device) are inserted intravaginally for 7-10 days, both mimicking luteal phase progesterone secretion. One day before CIDR or PRID is removed from cows, prostaglandin F2 alpha (PGF2α) is administered to eliminate the potential source of endogenous progesterone. This provides the proper endocrinological order for the development and maturation of the dominant follicle. It is observed that most of the animals show estrus and insemination can be made within the next 3 - 5 days. In addition, fixed-time artificial insemination can be performed 48-64 hours after the removal of these implants. Progesterone therapy using implants can be used for hormonal manipulation of the estrus cycle and resynchronization of animals [2].

The success of synchronization is attributed to the absence of corpus luteum (CL) at the end of the application and the presence of a new dominant follicle selected [3]. The duration of progesterone therapy depends on the time interval for the development of the new dominant follicle and the hormone used. If the hormone selected for the beginning of the application is Gonadotrophin releasing hormone (GnRH), the duration of the PRID is 5-7 days, however; If the selected hormone is estradiol benzoate, it has been reported that the application time of PRID should be 7-12 days [3].

In recent years, many studies have investigated the addition of progesterone to Ovsynch or modified Ovsynch protocols. In studies using progesterone (PRID, CIDR) with the Ovysnch protocol, an increase in the number of pregnancies in cows was observed [4-6]. Pregnancy rates were 59% (Progesterone + Ovsynch) and 36% (Ovsynch), respectively [6].

In this study, it was aimed to apply intravaginal progesterone-secreting preparations at different times and to determine the effect of one and two inseminations on fertilization in a certain period of time in multipourus cows raised in small family farms in Amasya.

MATERIAL AND METHODS

Animals

This study; was carried out in family farms (with a maximum of 20 cattle) located in Merzifon District of Amasya Province. Working time is from March to December 2018.

The animals used in the study gave at least one birth and formed cows between the ages of 36-72 months (Total, 159) (in Holstein, Brown Swiss, and Simmental breeds), dairy cows. When animals were examined, it was observed that at least 90 days postpartum, no true anestrus, PGF2α, and Ovsynch were applied, but estrus did not occur and problems were not resolved.

The animals were thoroughly examined for genital organs, and care was taken to ensure that there was no cystic structure and abnormal vaginal discharge. It was determined by the anamnesis finding that the last births of the animals to be included in the study were problem-free, and the phenomenon of retention secundinarum was not experienced.

Since they were animals in the same region, the conditions of care and feeding were the same. They were fed with high quality hay consisting of dry clover grass and sainfoin, corn silage and concentrated feed (3-4 kg/day) daily. Water ad libitium was given.

Age, Body Condition Scores (BCS), and milk yields of cows

The number of animals used in Group 1 was 56. 31 of these animals were Simmental, 19 were Holstein, and 6 were Brown Swisses. The average age was 57.05 ± 27.40 months, the BCS average was recorded as 2.75 ± 0.43, and the average milk yield was recorded as 16.66 ± 5.28 kg.

The number of animals used in Group 2 was 50. 31 of these animals are Simmental, 14 were Holstein, and 5 were Brown Swiss. The average age was 45.48 ± 26.81 months, the mean BCS was 2.74 ± 0.37, and the average milk yield was 13.46 ± 3.05 kg.

The number of animals used in Group 3 was 53. 26 of these animals were Simmental, 19 were Holstein, and 8 were Brown Swisses. The average age was 44.73 ± 20.25 months, the BCS average was recorded as 2.70 ± 0.34, and the average milk yield was recorded as 15.35 ± 4.60 kg.

Determination of animal groups

Milk yields of the cows included in the study were recorded and for the BCS of the animals, the scoring determined by Çitil and Uzlu [7] was used.

BCS; 1.0 Extremely bad, 1.5 Very bad, 2.0 Bad, 2.5 Medium, 3.0 Good, 3.5 Very good, 4.0 Fat, 4.5 Very fat, and 5.0 Too much fat was scored as Çitil and Uzlu [7].

Group 1 (n = 56): In the cows included in this group, Day 0 GnRH (IM) (10 mg Buserelin acetate, Ovarelin, CEVA DİF) + Progesterone implants PRID® (1.55 g progesterone CEVA), Day 8 PGF2α (Dinoprost tromethamine, 25 mg, Dinolytic, Pfizer), Day 9 Progesterone implants removal + PGF2α, insemination was performed at 56th hours insemination after the second PGF2α injection (Figure 1).
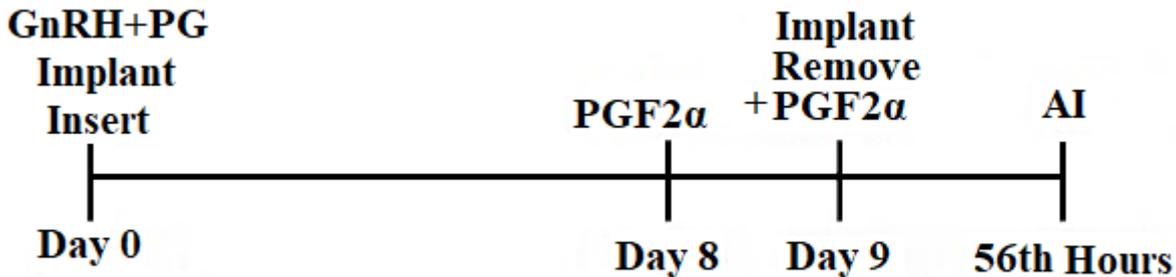

Figure 1. Synchronization application to animals in Group 1.

Group 2 (n = 50): on day 0, GnRH (IM) + Progesterone implants, PGF2α (IM) on the 8th day, Progesterone implants removal + PGF2α on the 9th day, insemination was performed at 48th and 72th hour after the second PGF2α injection.
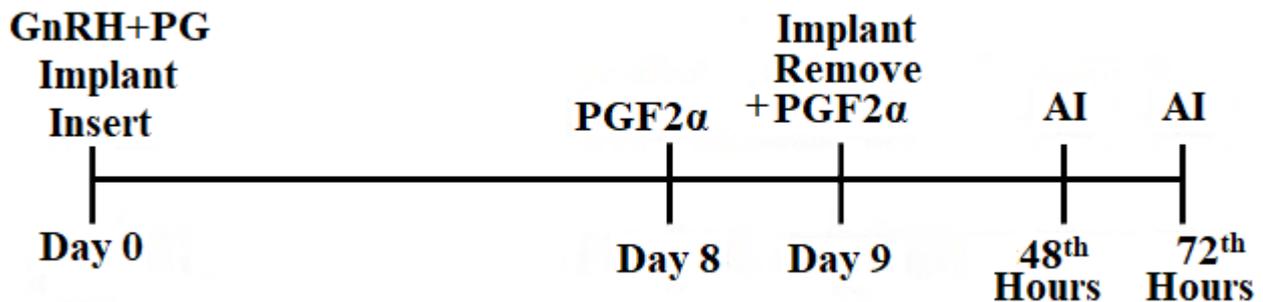

Figure 2. Synchronization application to animals in Group 2.

Group 3 (n=53): on day 0 GnRH (IM) + Progesterone implants, PGF2α (IM) on the 5th-day insert of Progesterone implants, day 6 Progesterone implants removal + PGF2α (IM), and insemination at 56th hour after the second PGF2α injection (Figure 3).

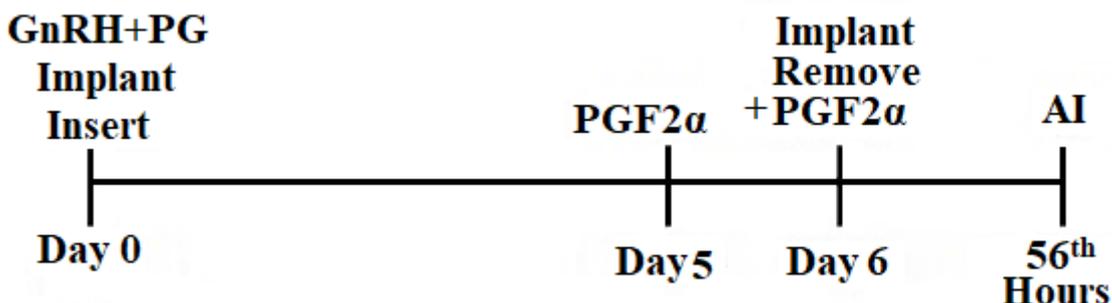
Figure 3. Synchronization application to animals in Group 3.

Pregnancy diagnoses were made with an ultrasonography device (Welld® WED-3000 Shenzhen WELLD Medical Electronics Co.) 35 days after the artificial insemination of all cows.

Oestrus monitoring and artificial insemination

Table 1. Oestrus symptoms and grading at the time of insemination.

Oestrus Criteria	Rating	
	NO	YES
Mooing	0	1
Vaginal discharge	0	1
Do let it jump on it	0	3
Uterine tone	0	3

The most important symptoms of estrus are do let it jump on it and uterine tone is graded with 3 points and it was decided that cows with at least 4 points were successfully synchronized.

Statistical analysis

Before performing the statistical analysis, data were examined with the Kolmogorov-Smirnov test for normality and the Levene test for homogeneity of variances as parametric test assumptions. The relationship between groups, race, animal owners' complaints, BCS, and pregnancy rates were determined using the Chi-square test. Relationship coefficients were calculated with Cramer's V coefficient. The differences between age, estrus signs, and milk yields in pregnant-no-pregnant groups were determined using the Student t-test. $P < 0.05$ was considered significant in all analyses. All statistical analysis was performed by using SPSS 14.01 package program for Windows.

The data obtained were analyzed in the SPSS package program according to their variability and continuity with different statistical methods. Pregnancy rates by groups, Chi-square test was used to determine the differences between pregnancies and races, the complaints of animal owners and pregnancies, BCS, and pregnancies. Independent Sample T-Test was used to determine the difference between age and pregnancy, to determine the difference between estrus findings and pregnancies, and to determine the relationship between milk yields and pregnancy.

Ethics committee permission

Ethics Committee approval was not obtained because our study was evaluated within the scope of "Good Clinical Practices".

RESULTS

Table 2: Complaints determined as a result of anamnesis received from breeders in the animals used in the study (for 12 months).

	Doesn't Show Oestrus	Ovsynch Applied Unsuccessful	No response to PG
Group 1. n=56	24	12	20
Group 2. n=50	17	6	27
Group 3. n=53	19	6	28

In Table 2; It is shown which applications the animals in the groups have been exposed to before.

Table 3. Mean scores and pregnancy rates of the oestrus of the animals used in the study at the time of insemination.

	Oestrus (point)	Pregnancy
Group 1. n=56	5.39±1.56	56/23 41.07%
Group 2. n=50	5.6±1.38	50/21 42%
Group 3. n=53	5.49±1.52	53/28 52.83%

In Table 3; The scores given according to the estrus scoring and whether they were pregnant at the end of the study were shown.

The mean score of the animals used in group 1 (n = 56) was recorded as 5.39 ± 1.56 according to the scoring system prepared. The pregnancy rate was 41.07%. (Table 3).

The average score of the animals used in group 2 (n = 50) was recorded as 5.6 ± 1.38 according to the scoring system prepared. The pregnancy rate was 42%. (Table 3).

The mean score of the animals used in group 3 (n = 53) was recorded as 5.49 ± 1.52 according to the scoring system prepared. The pregnancy rate was 52.83%. (Table 3).

Table 4. Evaluation of pregnancies by groups.

		Pregnancy		Total
		No Pregnant	Pregnant	
Groups	Group 1	33(20.8%)	23(14.5%)	56(35.2%)
	Group 2	29(18.2%)	21(13.2%)	50(31.4%)
	Group 3	25(15.7%)	28(17.6%)	53(33.3%)
Total		87(54.7%)	72(45.3%)	159(100.0%)

X^2:1.837, sd:2, p:0.399>0.05; Cramer's V=0.107
No difference was found between the groups in terms of pregnancy rates (P > 0.05).

Table 5. Determination of the difference between race and pregnancy.

		Pregnancy		Total
		No Pregnant	Pregnant	
Race	Simmental	48(30.2%)	30(18.9%)	78(49.1%)
	Holstein	29(18.2%)	21(13.2%)	50(31.4%)
	Brown Swiss	6(3.8%)	13(8.2%)	19(11.9%)
	Simmental x Brown Swiss	3(1.9%)	7(4.4%)	10(6.3%)
	Brown Swiss x Holstein	1(0.6%)	1(0.6%)	2(1.3%)
Total		87(54.7%)	72(45.3%)	159(100.0%)

X^2:8.271, sd:4, p: 0.082>0.05; Cramer's V=0.228
There is no difference in whether or not pregnancies differ by race (P > 0.05).

Table 6. Determination of the difference between animal owners' complaints and pregnancies.

		Pregnancy		Total
		No Pregnant	Pregnant	
Complaints	Doesn't Show Oestrus	43(27.0%)*	17(10.7%)*	60(37.7%)*
	Ovsynch Applied Unsuccessful	6(3.8%)	18(11.3%)	24(15.1%)
	No Response to PG	38(23.9%)	37(23.3%)	75(47.2%)
Total		87(54.7%)	72(45.3%)	159(100.0)

X^2:16.007, sd:2, p:0.000<0.05; Cramer's V=0.317, *= P<0.05

There was a statistically low relationship between the complaints of the cattle participating in the study and their pregnancies (P = 0.000, Cramer's V = 0.317). It was also determined that there was a difference between their anger complaints and their pregnancy.

Table 7. Determination of the difference between BCS and pregnancies.

		Pregnancy		Total
		No Pregnant	Pregnant	
BCS	2.00	12(7.5%)	4(2.5%)	16(10.1%)
	2.50	35(22.0%)	29(18.2%)	64(40.3%)
	3.00	38(23.9%)	29(18.2%)	67(42.1%)
	3.50	2(1.3%)*	10(6.3%)*	12(47.2%)
Total		87(54.7%)	72(45.3%)	159(100.0%)

X^2:9.777, sd:3, p:0.021<0.05; Cramer's V=0.248, *= P<0.05

In the examination on whether there is a relationship between BCS and pregnancies, it was determined that there was a significant (P < 0.05) pregnancy in the group above BCS 3.5 (P < 0.05).

DISCUSSION

It has been reported that in cows and heifers, vaginal hyperemia, uterine tone, and vaginal discharge increase during the estrus period, which is the appropriate insemination time, and with these findings, the cow that is in heat when jumped on does not escape, and that it can become pregnant when artificial insemination is performed [8]. In the presented study, the above estrus findings during artificial insemination were scored and followed with the estrus scoring system, but all cows were applied fixed-time artificial insemination regardless of whether there were any estrus findings after the progesterone implant synchronization protocol.

Additional applications are made to increase the synchronization rate in estrus synchronizations with CIDR. In a study on 2077 heifers, Mihm [3] divided heifers into 4 groups, applied CIDR to the heifers (n = 517) in Group 1 for 7 days, and injected 25 mg PGF2α on the day of removal of the CIDR, followed by the estrus for 84 hours. They were inseminated. The heifers that did not show estrus for eighty-four hours were inseminated by applying 100 µg of GnRH. The heifers in Group 2 (n = 504) were also applied to those in Group 1, but the only difference was the application of 100 µg of GnRH on the day the CIDR application started. The heifers in group 3 (n = 531) were treated with GnRH on the day of CIDR application, and PGF2α on the day of removal, followed by the second GnRH application at 60th hour, and artificial insemination was performed without looking at the signs of estrus. The heifers in group 4 (n = 525), on the day of removal of CIDR, were treated with PGF2α, followed by GnRH at 60th hour, and artificial insemination was performed regardless of estrus symptoms. As a result, there was no difference in pregnancy rates between Group 1 (54.5%) and Group 2 (57.3%), but a higher pregnancy rate was obtained than those inseminated without estrus [3]. In this study, artificial insemination was performed in three different groups without synchronization, and the pregnancy rate of animals used in group 1 (n = 56) was determined as 41.07%, and the pregnancy rate of animals used in group 2 (n = 50) was 42%. The pregnancy rate of the animals used in the third group (n = 53) was determined as 52.83% and was found consistent with the mentioned study.

The researchers (9) report that 66.7-90% of estrus can be seen as a result of PRID applications for 6-12 days. Xue et al. [10] reported that the rates of estrus obtained with PRID applied in different periods are different, accordingly, 80-100% of cyclic cows and 75% of animals in anestrus are observed. Grunert [11] reports that 74% of estrus was seen with 12 days of PRID applications in postpartum 40 and 80 days to induce ovaries in cows with symptoms of subestrus. In this study, according to Table 1, the most important symptoms of estrus are grading permitting, and uterine tone is graded with 3 points and it is decided that the cows with at least 4 points are successfully synchronized [10]. The average score of the animals used in group 1 (n = 56) was recorded as 5.39 according to the scoring system prepared. The average score of the animals used in group 2 (n = 50) was recorded as 5.6 according to the scoring system prepared. The average score of the animals used in group 3 (n = 53) was recorded as 5.49 according to the scoring system prepared.

It has been reported that a single PGF2α application is not very effective in regressing the newly shaped CL using the fixed-time artificial insemination protocol [12]. Various investigators have been reported on which day and interval of synchronization of the second PGF2α injection [13, 14]. In another study, no difference was reported between the pregnancy rates in the fattening cows subjected to the 5-day Cosynch + CIDR program with the removal of the CIDR applicator 2 doses at 8-hour intervals or 2 doses in a single treatment [15].

CL's early regression deficiency is an interesting biological condition that is not yet fully understood [16]. However, the inability to fully activate intracellular signal transduction pathways has been suggested and supported by various researchers [17,18].

Some studies have reported statistical differences between pregnancy and in multiparous cows as a result of fixed-time artificial insemination. Others did not report any significant differences in conception rates between primiparous and multiparous cows [19-21]. In the study presented as a result of the aforementioned studies, a PGF2α injection was made in each of the three groups that were synchronized with an interval of 24 hours.

The initiation of new ovulation at the beginning of the synchronization protocol has been reported to increase plasma Progesterone concentrations during PGF2α injection [22]. In the early stage induced by exogenous GnRH application, it is noted that the ovulation of a dominant follicle will be ovulated as a result of the second follicular wave and decrease the luteal function and conception in beef breed cattle [23]. However, in a previous study, it was reported that there was no significant relationship between the diameter of follicles to be ovulated in dairy cows and pregnancy rates [24].

It is reported that in animals with inactive and active ovarium, estrus mostly occurs within 2-3 days with PRID application for 7-12 days for the treatment of estrus synchronization or functional disorders [25]. The

GnRH hormone used in the treatment of inactive ovaries causes the release of LH, thus contributing to the maturation and ovulation of the dominant follicle [26]. In some studies, it has been suggested that a high progesterone environment with long-term progesterone administration creates follicular dominance, and as a result, an aged oocyte may be produced [27,28]. In this study, which was presented by making use of the researches reported above, GnRH was applied together with short- time PRID.

CONCLUSIONS

As a result; as the yield of cows increases, it becomes harder for them to become pregnant. For sustainable livestock, it is necessary to obtain one cub per year from a cow. Many hormonal applications and many formulas have been developed to achieve this. In various studies conducted in the world and our country and the field study presented, it is tried to ensure that the cows are conceived at any time with the tools containing progesterone and fixed-time artificial insemination protocols. As it is known, it is very difficult to carry out a study with hundreds of business owners in field conditions. In this study, which was carried out in animals fed the same ration in a certain area and kept under the same care conditions, it was seen that the highest pregnancy rate with 52.83% (statistical analysis did not show the superiority) was in the 3rd group implanted with Progesterone for 6 days and artificially inseminated at 56 hours by injecting PGF2 alpha on the 5th and 6th days. In this group, a better pregnancy result was obtained than the group applied for 9 days (Group 2). It is thought that oocyte aging that may occur with the prolonged application of progesterone implants leads to such a result. Besides, it is more economical because of single insemination in terms of cost. The study should be done in more detail in more animals in the future and question marks should be removed.

Conflict of interest: The authors declare that they have no conflict of interest. Statement of Animal Rights all applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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