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FERTILITY IMPROVEMENT IN LACTATING DAIRY COWS USING A PRESYNCHRONIZATION PROTOCOL BEFORE OVSYNCH

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Abstract

Pregnancy rates obtained after artificial insemination following Ovsynch in lactating dairy cattle are currently around 40-45%. Therefore, various fertility programs have been established in an attempt to increase these percentages. The aim of the present research was to use a simple and cost-effective presynchronization protocol, that would allow maximization of ovulatory response after the first GnRH of Ovsynch, and therefore improve fertility of treated animals, in comparison to those in which Ovsynch was used alone. A total of 240 non-pregnant and lactating dairy cows were divided in two groups (n = 120). Group 1 was synchronized with Ovsynch alone, while in group 2, PGF2α and GnRH were administered almost simultaneously, in different injection sites, 7 days before the initiation of Ovsynch. Ovarian structures were observed by ultrasonography on the day when the first GnRH injection of Ovsynch was made and 4 days later. Results showed a 42.5% pregnancy rate in group 1 and a 57.5% pregnancy rate in group 2. Therefore, presynchronization treatments allowed an improved pregnancy rate of 15%, which totally covers the supplemental costs implied by such measures.

Key words*: dairy cows, fertility, presynchronization, Ovsynch.*

INTRODUCTION

Estrus synchronization protocols are nowadays considered to be almost mandatory in dairy cattle farms, for the enhancement of reproductive function and optimal organization of artificial insemination activity. One of the most common methods is currently Ovsynch (Pursley et al., 1995), which involves synchronization of follicular wave development and ovulation using GnRH and PGF2α. On the other hand, the classical techniques tend to be replaced by more complex fertility programs, which yield better results, and therefore allow for superior pregnancy rates as they involve a pre-synchronization, before the classical Ovsynch is performed. There are currently several fertility programs which are generally accepted to be the most efficient: Presynch-10, Presynch-11, Presynch-12, Presynch-14, Double Ovsynch, G6G etc. (Bello et al., 2006; Astiz et al., 2013; Dirandeh et al., 2015; Souza et al., 2008; Ayres et al., 2013; Herlihy et al., 2012).

Presynchronization brings significant advantages, as it was shown that fertility of cows is improved if the first GnRH of Ovsynch induces ovulation of a pre-existing dominant follicle. This leads to a new corpus luteum formation and initiation of a new follicular wave, that produces a new dominant follicle. Administration of Ovsynch's PGF2α induces luteolysis of an active corpus luteum and subsequent estrus due to the dominant follicle reaching the mature stage.

Beside the advantages that such fertility programs bring, there are also several drawbacks, that result from their complexity and necessity of multiple hormonal treatments, and therefore multiple farm visits.

It was shown by several authors (Stevens et al., 1993; Peters et al., 2003) that if PGF2α and GnRH are administered together, they do not adversely influence each other in what induction of luteolysis (by PGF2α) or ovulation (by GnRH) are concerned.

Therefore, presynchronization before Ovsynch, using a simultaneous administration (in two

different injection sites) of PGF2α and GnRH was attempted in lactating dairy cattle (Yousouf et al., 2016; Martins et al., 2017) with good results.
Thus, the aim of the present research was to use a simple and cost-effective presynchronization protocol, that would allow maximization of ovulatory response after the first GnRH of Ovsynch, and therefore improve fertility of treated animals, in comparison to those in which Ovsynch was used alone.

MATERIALS AND METHODS

Our research was carried out in a private dairy farm from Cluj County, Romania.
All animals included in the experiments were multiparous Holstein cows, 3.5-6 years of age, housed in free stalls and fed a combined fixed ration, made up of corn and alfalfa silage as well as concentrates. Salt and water were offered ad-libitum.
Production of these cows averaged approximately 30 liters of milk/day/cow.
Females were randomly chosen, without establishing the phase of the estrous cycle that they were into.
Farm records were consulted and they confirmed that all cows that were included in the experiment were either between 55 and 65 days post-partum, waiting for their 1st insemination or were confirmed non-pregnant by ultrasounds 40 days after the previous AI.
All hormonal products that were used were licensed for cattle, while the producer's recommended dose was respected, as follows: PGF2α - 25 mg dinoprost tromethamine (Dinolytic, Zoetis) and GnRH - 50 µg D-Phe 6-gonadorelin (Gonavet 50, Veyx Pharma).
All treatments were performed as intramuscular injections, using sterile single use syringes and needles.
A total of 240 non-pregnant and lactating dairy cows were divided in two equal groups (n = 120). Group 1 was synchronized with Ovsynch alone, while in group 2, PGF2α and GnRH were administered almost simultaneously, in different injection sites, 7 days before the initiation of Ovsynch (Figure 1).
In both groups Ovsynch was performed as follows:

- Day 0 - GnRH,
- Day 7 - PGF2α,
- Day 8 - PGF2α,
- 32 hours later - GnRH,
- 16 hours later - artificial insemination.

Ovarian structures were observed by ultrasonography on the day when the first GnRH injection of Ovsynch was made, as well as 4 days later, in order to confirm any modifications that occurred.
All ultrasound examinations were performed using a Mindray DP-10 Vet ultrasound scanner and a 4-6 MHz linear transducer, by the usual trans-rectal approach.

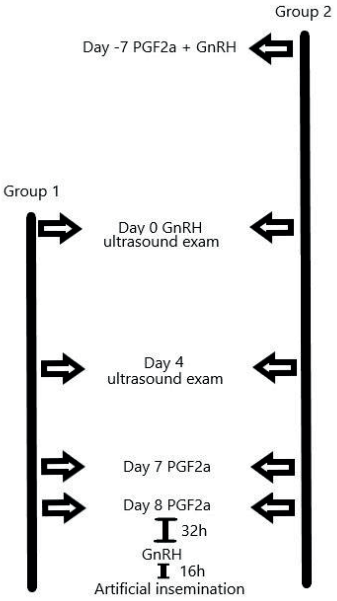

Figure 1. Graphical representation of the two estrus synchronization protocols

Artificial insemination was performed 16 hours after the second GnRH administration of Ovsynch, using frozen/thawed bull semen, using the classical transcervical technique.
Pregnancy diagnosis was performed 35 days after artificial insemination by ultrasonography. Re-confirmation of pregnancy, as well as fetal sexing was also performed by ultrasonography 55 days after insemination.
Cows were closely monitored throughout the entire experiment, regarding their health status, behavioral estrus-associated modifications as well as general welfare.

RESULTS AND DISCUSSIONS

One of the first differences that was observed between the two groups of cows was related to the percentage of females that had a functional corpus luteum at the time of the first GnRH injection of Ovsynch, and therefore did not ovulate following this treatment.

In group 1, which had no presynchronization treatment, only 73 out of 120 cows (60.83%) had a large follicle (Figure 3) on their ovary when the first GnRH administration of Ovsynch was performed, and were confirmed to have ovulated 4 days later, by ultrasounds. The other 47 cows (39.17%) had an active corpus luteum on their ovary on day 0 (Figure 4), when the first GnRH was administered, and only small follicles (below 0.8 cm in diameter), which became luteinized after the treatment (Table 1, Figure 2).

In group 2, in which PGF2α and GnRH were administered 7 days before the beginning of Ovsynch, 98 out of 120 cows (81.66%) had a dominant follicle at the time of the first GnRH injection of Ovsynch, as confirmed by ultrasounds and ovulated following this treatment, as the follicle had disappeared and a new active corpus luteum was identified by ultrasounds 4 days later.

Table 1. Results of ovarian ultrasound examination on the day of first GnRH administration of Ovsynch

	Group 1 No. (%)	Group 2 No. (%)
Cows with large follicle at the first GnRH of Ovsynch	73 (60.83%)	98 (81.66%)
Cows with active corpus luteum at the first GnRH of Ovsynch	47 (39.17%)	22 (18.33%)

Table 2. Results of pregnancy ultrasound examination and fetal sexing

	Group 1 No. (%)	Group 2 No. (%)
Pregnant cows	51 (42.5%)	69 (57.5%)
Identified sex of fetuses	22 males 29 females	32 males 37 females

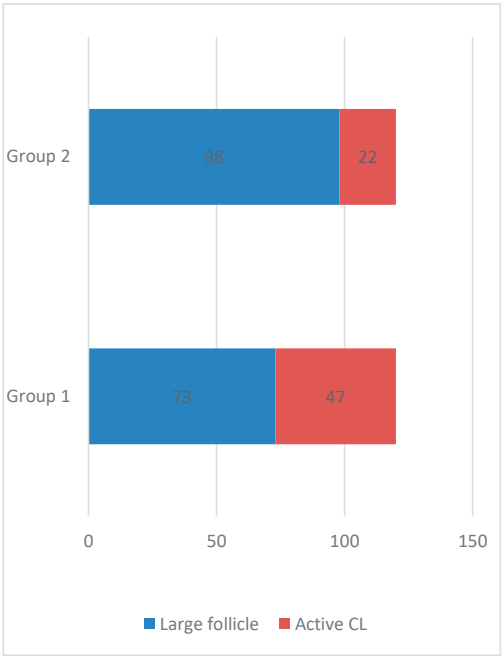

Figure 2. Graphical representation of the results obtained on ultrasound examination on the day of first GnRH administration of Ovsynch

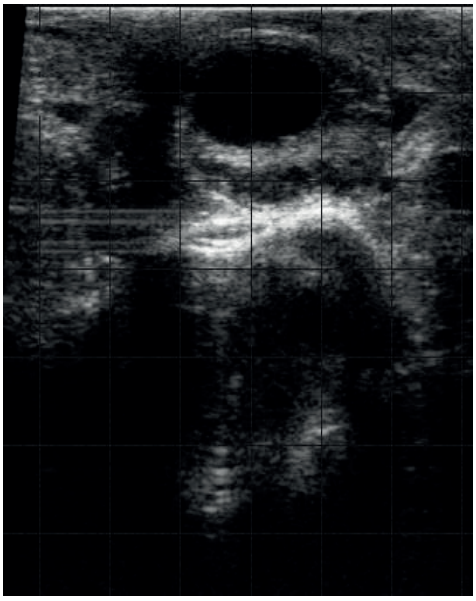

Figure 3. Ultrasound image of a large follicle

The other 22 cows (18.33%) had an active corpus luteum on their ovary and small follicles that became luteinized after the first GnRH administration of Ovsynch (Table 1, Figure 2). Therefore, the presynchronization treatment significantly increased the number of cows that ovulated after the first GnRH of Ovsynch.
After the second PGF2α administration of Ovsynch, estrus behavior was monitored and only 53 out of 240 cows were shown to display behavioral estrus, without significant differences between the two groups (27 cows from group 1 and 26 cows from group 2).
Nevertheless, timed artificial insemination (TAI) was performed in all cows, 16 hours after the second GnRH of Ovsynch in all females.

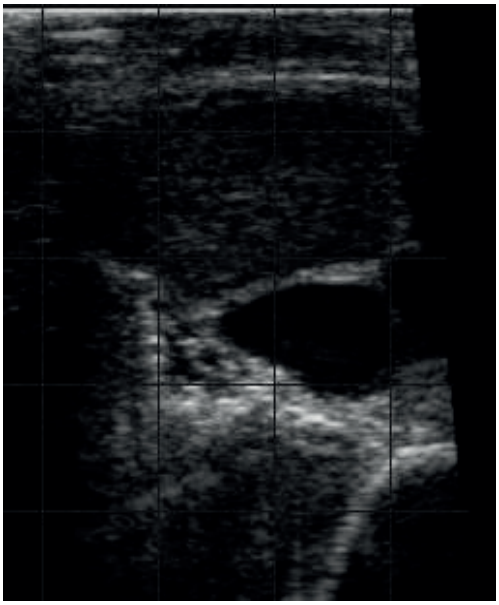

Figure 4. Ultrasound image of an ovary with a corpus luteum

Out of the 120 cows from group 1, 51 (42.5%) were diagnosed pregnant by ultrasounds 35 days after artificial insemination and all of them were reconfirmed pregnant at 55 days after insemination. Upon fetal sexing, 22 males and 29 females were found (Table 2, Figures 5, 6).
Out of the 120 cows from group 2, 69 (57.5%) were diagnosed pregnant by ultrasounds 35 days after artificial insemination and all of them were reconfirmed pregnant at 55 days after insemination. Upon fetal sexing, 32 males and 37 females were found (Table 2, Figures 5, 6).

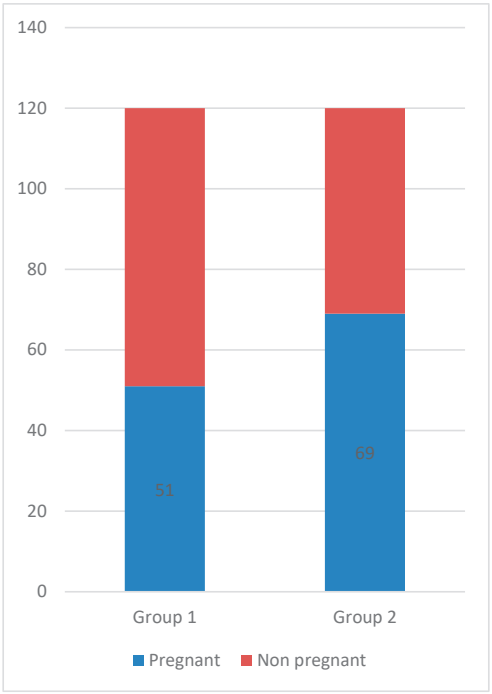

Figure 5. Graphical representation of the results obtained on pregnant diagnosis

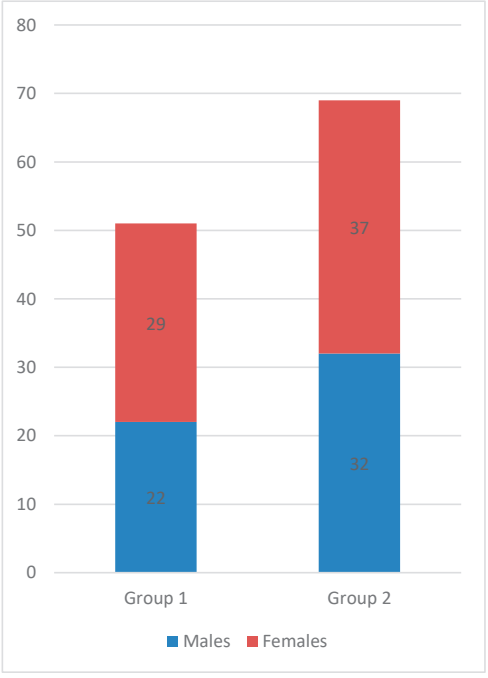

Figure 6. Graphical representation of the results obtained on fetal sexing

As shown above, the results obtained were significantly better in group 2, where a superior pregnancy rate following estrus synchronization was obtained, due to the presynchronization treatment, which allowed a better response to Ovsynch.

This improved response was mainly due to an increased number of ovulations that occurred after the first GnRH injection of Ovsynch.

The fact that a significantly number of cows presented a large follicle after pre-synchronization was due to the positive effect of the combined administration of PGF2α and GnRH.

The former induced luteolysis of any luteal structure that was present on the ovary while the latter produced luteinization of any follicular structure. Therefore, a new follicular wave was initiated, which selected a dominant follicle, that was ready to ovulate when the first GnRH of Ovsynch was administered. This also led to the presence of an active corpus luteum, which was lysed under the influence of the two PGF2α injections of Ovsynch, and this allowed growth and ovulation of the new dominant follicle.

In group 1, where no presynchronization protocole was performed, the cows presented various ovarian structures and therefore not all responded to the first GnRH injection of Ovsynch. As the percentage of ovulations was lower, the amount of luteal tissue on the occasion of Ovsynch's PGF2α administration was diminished, as was luteolysis induced by it, and therefore ovulation rate before artificial insemination was also low. Thus, the pregnancy rate was obviously lower too.

These observations are supported by the research of Vasconcelos et al., 1999, who showed that the ovulation rate after the first GnRH injection of Ovsynch is much higher, if this administration is performed on days 6-7 of the estrous cycle, as compared to a random moment.

This presynchronization protocol involves less work and also less costs than other such methods, as it only needs one single extra farm visit, before the onset of Ovsynch. As the pregnancy rate is significantly higher (15%), the costs of labor and hormonal products are easily covered by the extra profit obtained.

This technique allows an adequate management of reproductive activity in dairy farms, allows timed artificial insemination to be performed, without the need of estrus detection, and also involves no risks for the cows and neither for the consumer of dairy products obtained from treated animals.

CONCLUSIONS

Results showed a 42.5% pregnancy rate in group 1 and a 57.5% pregnancy rate in group 2. Therefore, presynchronization treatments allowed an improved pregnancy rate of 15%, which totally covers the supplemental costs implied by such measures. Also, the presynchronization protocol was easy to apply, as only one supplemental farm visit was needed as compared to the classical Ovsynch.

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