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Effect of Cyclicity on Ovsynch Synchronization Treatment Efficiency in Dairy Cows

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Abstract: Administration of PGF-2α and GnRH is a widely used method for ovulation synchronization. The objective of this study was to assess whether regular cyclic activity prior to synchronization treatment increases conception rate. The experiment was performed on 326 multiparous Italian Friesian dairy cows synchronized with GnRH and PGF-2α 80 days at least, after calving. The treatment begins with an injection of GnRH, given at any stage of the estrous cycle. Seven days later, an injection of PGF-α is given followed two days later by a second injection of GnRH. Cows were inseminated 8 to 24 h after the second GnRH injection without regard to estrus behavior. Cyclic activity of animals before hormonal treatment was assessed by daily measurement of milk progesterone. Moreover progesterone concentrations were used to discriminate animals in two groups: HHL (H = high progesterone levels at the first GnRH injection; H = high progesterone levels at the PGF2α injection; L = low progesterone levels at insemination) and NNN (HLL, HHH or LHL). This experiment demonstrates that synchronisation treatment is more efficacious on cows showing regular cyclic activity (conception rate of 49.4% vs 19.4%; $p<0.001$). This difference between cyclic and non-cyclic cows is also observed in the HHL group (48% vs 31%, $p<0.01$) and in the NNN group (38% vs 7%, $p<0.01$). In conclusion, this study demonstrated that a regular cyclic activity improved synchronization treatment efficiency.

Key words: Progesterone/conception rates/GnRH/ PGF2α

INTRODUCTION

In the last decades, there has been a surge in large-scale dairy farms at the expense of small and medium producers. The larger number of cows on these farms hinders the monitoring of each individual animal and thus reproductive management becomes a critical issue[1]. In fact, estrus detection is the main factor limiting a herd's reproductive performanceand is below 50% in most farms[2], this deficient estrus detection increases the time elapsed between calving and conception. The optimal calving to conception interval is of 120 days, but according to a survey conducted by the Farmers Association of the Province of Vicenza, this interval lasted 127 days on average with the consequent reduction in profits. The use of a synchronization protocol makes reproduction management easier as it doesn't require heat detection which can reduces the time needed to fecund cows and cost associated. One of the most widely used ovulation synchronization method is described by Pursley *et al.*[3] and is based on the administration of PGF-2α and GnRH. Using this method, known as the ovsynch protocol, reproduction is controlled by performing insemination without heat detection and is thusx efficient in managing artificial insemination[4]. According to Cartmill *et al.*[5] and Momcilovic *et al.*[6], this treatment results in an increased number of pregnancies compared to other synchronization methods, while Schmitt *et al.*[7] and Stevenson *et al.*[8], reported no significant improvement of pregnancy rates. The efficiency of treatment reported in the literature is around 40%[3,6,8-11], this low efficiency of the treatment is probably due to the fact that the first treatment with GnRH does not always coincide with the presence of a follicle that is ready to ovulate[12]. The objective of this study was to assess whether regular cyclic activity prior to synchronization treatment can have an effect on the conception rate, considering that previous luteal activity is probably a determining factor for the development of a follicular wave able to respond correctly to the first GnRH dose.

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MATERIALS AND METHODS

Animals: The experiment was performed on 326 loosely-housed multiparous Italian Friesian dairy cows from the same farm. All cows partured at least 80 days before the beginning of the experiment. The conception rate of the herd before the experiment was 30.1%. Average milk yield was 9128±115 kg per lactation (mean±SE). The cows were fed once daily with a total mixed ration (primary components: corn silage, high moisture corn, alfalfa silage, dry hay, dehydrated beet pulp, soybean meal 44% crude protein solvent, dehydrated alfalfa meal 18% protein, sunflower meal). Sodium bicarbonate was added as mineral supply. Water was provided ad libitum.

Body condition score: The BCS was established for each animal every 15 days using a scale of 1 (very thin) to 5 (very fat), as described by Wright *et al.*[10]. The mean BCS at the beginning of the experiment was 3.5±0.04 (mean±SE). All animals included in the experiment did not loose more than 21% of their BCS between dry period and sone month postpartum, which is the maximum weight loss a cow can be submitted to without affecting her reproduction capacities[14].

Progesterone: Progesterone (P4) was determined in whey using the direct ELISA method described by Comin *et al.*[15]. Luteal activity of cows was monitored from 20 days after calving until the end of the experiment by determining progesterone concentrations in whey samples collected every Monday and Thursday. Estrus was considered regular if the animal showed progesterone levels below 300 pg mL^{-1} in 1 or 2 consecutive samples (3-7 days). Diestrus was considered regular if the animal showed progesterone levels above 300 pg mL^{-1} in 4 to 6 consecutive whey samples (14-21 days). Any cycle with luteal and estrous phases different from those described above during the 30 days before initial treatment was considered irregular.

Synchronization treatment: All animals were treated after more than 80 days post calving. The treatment began with an injection of GnRH (62.5 μg i.m.; Dalmarelin, Fatro, Bologne, Italy), given at any stage of the estrous cycle. Seven days later, an injection of PGF2α (0.15 mg i.m., Veteglan, Calier, Barcelona, Spain) was given followed two days later by a second injection of GnRH. Artificial insemination was performed 8 to 24 h after this last GnRH dose. Progesterone concentrations at the first GnRH injection at the PGF2α injection and at the insemination were use to discriminate animals in two groups. Animals in diestrous at the first GnRH injection and at the prostaglandin injection but were in estrus at the insemination were marked HHL (H = high progesterone at the first GnRH injection; H = high progesterone at the PGF2α injection; L = low progesterone at insemination), the other animals were marked NNN (HLL, HHH or LHL progesterone status). Forty days after insemination, pregnancy was determined by rectal palpation.

Statistical analysis: A shortlist of three qualifications was then attributed to each animal: Pregnancy (YES, NO); HHL (TRUE,FALSE); Cycle (regular, irregular). 326 shortlists were obtained of classified values for drawing the ramified map of the subdivisions, taking into account that the two HHL and CYCLE states were surveyed from the perspective of their influence on the end result of pregnancy. These being categorical data, two contingency tables were drawn up on the result in terms of number of pregnancies, taking the HHL state and cycle state as influencing factors, respectively Table 1 and 2.

Table 1: Contingency table of Pregnancy (No, Yes) by HHL (False, True) the meaning of the numbers in cells is listed in the corner upper left

Count total% col% row% expected cell chi^2	Preg No	Yes	
HHL			
False	126	36	162
	38.65	11.04	49.69
	58.33	32.73	
	77.78	22.22	
	107.337	54.6626	
	3.2448	6.3717	
True	90	74	164
	27.61	22.70	50.31
	41.67	67.27	
	54.88	45.12	
	108.663	55.3374	
	3.2053	6.2940	
	216	110	326
	66.26	33.74	

Table 2: Contingency table of Pregnancy (No, Yes) by Cicle (IRR, RE) The meaning of the numbers in cells is listed in the corner upper left

Count total% col% row% expected cell chi^2	Preg No	Yes	
Cicle			
IRR	140	36	176
	42.94	11.04	53.99
	64.81	32.73	
	79.55	20.45	
	116.613	59.3865	
	4.6901	9.2096	
RE	76	74	150
	23.31	22.70	46.01
	35.19	67.27	
	50.67	49.33	
	99.3865	50.6135	
	5.5030	10.8060	
	216	110	326
	66.26	33.74	

Figure 1 shoes graphically the partition of data. The test applied to tables 2x2 is usually not very probative because the value of the chi-square variable (standard deviation between observed and expected data) is calculated from very few numbers. Anyway, in our case, the almost nil probability of having a higher chi-square than that found contributes towards accepting the hypothesis of a strong positive influence of the HHL TRUE and CYCLE regular states on the induction of pregnancy.

RESULTS AND DISCUSSION

At the beginning of the experiment, about half of the herd (150 cows, 46%) showed regular cyclic activity as evaluated by milk progesterone. After synchronization treatment, the conception rate (CR) observed was of 33.7% whereas it was of 32.6% before. Prior cyclic activity have a major impact on conception rates observed, indeed, they were of 49.4 and 20.4% for cyclic and irregular cows, respectively. This difference between the CR of cyclic and non-cyclic cows was also observed in the groups HHL and NNN Fig. 1. In the HHL group, cyclic activity increased treatment efficiency of 21% and in the NNN group, of 32%. Progesterone levels in whey indicated that 87 of the 150 cyclic cows 56.6% had diestrous luteal activity at the first GnRH dose and at PGF2α injection, while this activity was absent (progesterone levels below 300 pg mL^{-1}) at the insemination (HHL) leading to a conception rate of 55.3% Fig. 2. The remaining cows showed different luteal activity during treatment (NNN) and their conception rate was 41.5%. The same analysis was carried out in animals with irregular cyclic activity. In this case, the pregnancy rate was 34.1% for HHL animals and 9.3% for the animals in group NNN Fig. 2.

This study showed that the PGF2α treatment does not improves conception rates as it was also observed by Cartmill *et al.*[9], instead Stevenson *et al.* [8] found that the Ovsynch treatment was efficacious in improving fertility. The mean conception rate recorded 33.7% was in the range of values mentioned in the litrature 27 to 44%[3,6,8-11].

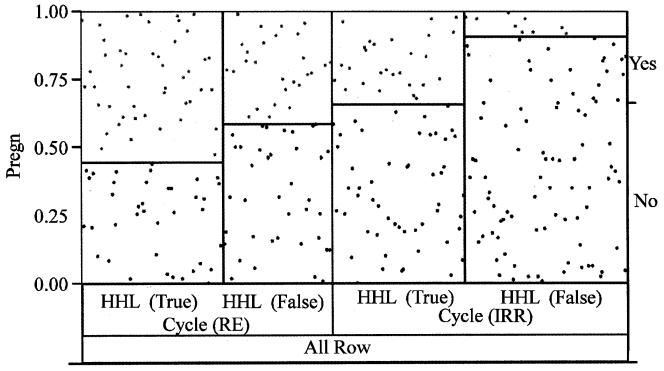

Fig. 1: Figure shows, in the form of mosaic plot, the superimposed frequencies of non-gravid (below, red dots) and gravid (above, green dots), divided according to the categories regular and irregular cycle, in their turn divided into true and false HHL levels. It can be seen that, to result in pregnancy, the conditions range from the most favourable (Cycle RE, HHL True) to the least favourable (Cycle IRR, HHL False)

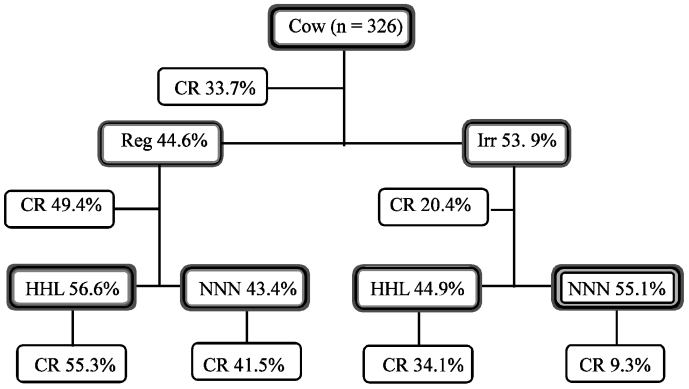

Fig. 2: Conception rates (CR) of cows with regards to their cyclic activity (Reg = regular, Irr = irregular) and HHL or NNN status

We observed an higher CR in HHL cows than in NNN cows which confirms previous studies[5,12] showing that the treatment efficiency depends on the HHL or NNN status of cows. Some authors[5,7,12] attribute this CR improvement to the fact that treatment is given at the initial stage of diestrus when there is a dominant follicle capable of ovulating in response to the first GnRH injection, such that many of the animals are in optimal condition for the subsequent stages of synchronzation[16]. We used progesterone profiling to assume cyclic activity before treatment[15]. Animals with regular cyclic activity showed a significantly higher CR than animals with irregular cyclic activity (49.4 % vs 20.4 %, $p<0.001$). This difference between cyclic and non-cyclic cows was also observed in the HHL group (55.3% vs 34.1%) and much more in the NNN group (41.7% vs 9.3%). In the previous studies, it was generally suppose that NNN cows won't respond to the synchronization treatment but we showed that only non-cyclic cows in this group won't respond and that cyclic cows would have a good conception rate. This important effect of cyclicity was also observed by Gumen *et al.*[17] as they observed a CR of 32% for cyclic and 9% for non cyclic cows. Yamada *et al.*[11] demonstrated that cows showing cyclic activity prior to 34 d post-partum better respond to the synchronization protocol than cows cyclic after 56 d post-partum in the same way, Pursley *et al.*[3] reported that when Ovsynch was conducted at 50-75d after calving CR were lower than if performed after 76 d. Even if these studies did not record cyclicity, these differences might be due to delayed resumption of cyclicity. in many cows was delayed at 50-75d postpartum. But the reports did not show the effects of postpartum ovarian cyclicity. The mechanisms underlying this process may be that progesterone is lower at first ovulation post partum[18]. The levels of progesterone are important for further embrio development[19] explaining that cycling cows have better CR. Cordoba and Fricke[10] did not find any significative difference in the pregnancy rates achieved by animals showing luteal activity and those showing anovulatory behavior during and after synchronization treatment. Correct luteal activity in terms of its duration and progesterone levels is essential for normal follicular development. Indeed, Savio *et al.*[20], Bigelow and Fortune[21] reported that if the period of luteal progesterone secretion extends beyond the normal interovulatory life of the corpus luteum, follicular waves continue to increase. On the contrary, low exogenous progesterone levels maintain plasma progesterone concentrations at subluteal levels supporting the growth of a single persistent or prolonged dominant follicle[22]; its premature luteinization compromising fertility in successive ovulations[23]. When progesterone levels are experimentally raised to those of normal luteal activity during a prolonged period of follicular dominance, the persistent follicle regresses and another follicle becomes dominant, such that normal fertility is recovered[23-24]. This would suggest the importance of only treating developed follicles with progesterone concentrations typical of diestrus. In effect, it was possible to notably improve the pregnancy rate in non cyclic animals using a modified version of the ovsynch program that contained a norgestomet implant between the GnRH and PGF2α treatment[25].The present findings indicate that normally cyclic animals (in terms of the duration of estrus/diestrus and their luteal activity) before and during synchronization treatment are in the best condition to give rise to a high conception rate. It is sufficient that one of these two conditions (regular cyclicity before treatment or HHL) be fulfilled to ensure an improved conception rate.

CONCLUSION

In conclusion, the synchronization treatment is a useful method to synchronize cows which allows a reduction of farmer work and cost as all insemination are done in the same time. The measurement of the cyclicity before treatment allows to discriminate the cows that would effectively respond to the treatment and doing so would reduce cost linked to treatment. The difference between conception rates of HHL and NNN (45% vs 22%) is similar to that of cyclic non cyclic but the HHL status can not be known before treatment and so is not useful.

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