Reproductive Responses of Lactating Dairy Cows According to Estrus Behavior and CIDR Uses in a Heatsynch Protocol

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ABSTRACT

Background: The timed artificial insemination (TAI) protocols have been used worldwide as a first service artificial insemination (AI) breed system as well as to resynchronize cows diagnosed as not pregnant. The benefits associated to Heatsynch are the lower cost of the protocol and the increase in the number of cows detected in estrus. Furthermore, it has been suggested that protocols combining AI after estrus detection followed by TAI could increase overall conception rates, as well as increased AI submission rates. The aim of this study was evaluate reproductive responses of dairy cows submitted to a Heatsynch protocol according the estrus behavior and controlled internal device release (CIDR) uses.

Material, Methods & Results: Holstein lactating cows (n = 562) were enrolled in protocol using (CIDR) (1.9 g of progesterone) insertion at (day 0) D0 either a new one, a previous used once (7 days - second use) or twice used (14 days - third use). Based on the detection of estrus and the moment of AI after the CIDR removal, cows were divided into 3 groups: cows showing signs of estrus and therefore AI 36 to 60 h after CIDR removal (EPTAI group; n = 241); cows showing estrus and AI 72 h after CIDR removal (ETAI group; n = 207); and cows that did not show any sign of estrus after CIDR removal and were TAI 72 h later (NoETAI group; n = 335). Previous to the first postpartum insemination (47.01 ± 5.43 days in milk) cows were pre-synchronized using one dose of PGF2α (Lutalyse, Pfizer Saúde Animal, Brazil). At 60 ± 3.52 days in milk (DIM), day 0 of the Heatsynch protocol, cows were subjected to gynecological examination by a Veterinarian, where only those animals considered as healthy cows with no signs of uterine disease were kept in the study. In a subset of animals (105/783; 13.4%) an ovary evaluation was performed by ultrasonography (WED-3000, B-mode, linear array, ultrasound scanner equipped with a 5.0-MHz transducer, Welld, Shenzhen, China) at the beginning of synchronization protocol aiming to check the presence of a corpus luteum (CL) and classify the cows as cycling or not cycling. Ovarian structures were examined aiming to check the CL presence on day zero of the program in a subset of the cows and pregnancy status was also determined by ultrasonography. Higher conception rates were found in EPTAI and ETAI groups than NoETAI, respectively 44.8%, 38.2% and 24.8%. Pregnancy losses were calculating considering cows that were not pregnant at 60-65 days re-check and those that showed signs of estrus before the first pregnancy determination. The CIDR reutilization did not affect the estrus behavior, conception rate or pregnancy losses. In conclusion, reproductive responses in dairy cows during a Heatsynch protocol are not affected by CIDR reutilization and are improved in cows showing estrus signs.

Discussion: Cows submitted to Heatsynch protocols that demonstrate estrus signs have increased pregnancy rates and lower pregnancy losses. In addition to that, cows exposed to low estradiol concentrations were more likely to experience subsequent premature luteolysis. Thus, coupled with the estradiol cypionate (ECP) action, it can be postulated that cows showing estrus signs during a timed AI protocol could have a higher ovulation rate. This can assume great importance in high producing dairy herds, since in response to ECP becomes possible to overcome the lower concentrations of estradiol due to high hepatic clearance and catabolism of esteroid hormones.

Keywords: dairy cows, estrus, pregnancy, timed artificial insemination.
INTRODUCTION

Estrus synchronization as well as timed artificial insemination protocols provides an important contribution to reproduction efficiency in dairy herds. The main benefit of TAI is the reduction in the calving to first service interval as a result of the increased AI submission rate [2]. The Heatsynch protocol was developed using ECP as a substitute to gonadotropin releasing hormone (GnRH) aiming to induce ovulation. This ECP injection is able to induce LH surge, ovulation and CL formation as well as estrus behavior between PGF<sub>2α</sub> injection and TAI moment [17]. Conception rates in dairy cows submitted to Heatsynch have been described as similar to Ovsynch outcomes [17]. Some studies have demonstrated a 50% increase in cows showing estrus in comparison to Ovsynch [10], which could be related to an enhancement in fertility.

The use of controlled internal drug release devices (CIDR) containing progesterone increase the synchronization efficiency in TAI programs [11] and can have positive effects on conception rates in dairy cows [7]. The advantages of CIDR utilization are the reduction in the LH pulsatility and the blockade in the pre-ovulatory LH surge [21]. It has been demonstrated similar fertility results when CIDR used up to 36 days in Bos indicus beef cows [14]. Moreover, serum progesterone levels were kept above 1 ng/mL up to 24 days of treatment with CIDR in crossbred cows [22]. Despite that, only one published study has attempted to test CIDR reutilization in lactating dairy cows [6].

Therefore, the aim of this study was to evaluate the reproductive responses of lactating dairy cows according to estrus behavior and CIDR uses in a Heatsynch protocol.

MATERIALS AND METHODS

Experimental cows enrolled in this study (n = 562) were a subset from a grazing-based herd of 1067 Holstein cows in Southern Brazil (39°36′S 174°18′E). A total of 252 primiparous and 310 multiparous cows were selected and subjected to 783 insemination procedures with the Heatsynch protocol. The period of the study was from June 2011 to April 2012. The average DIM of the cows in this study was 113 ± 34.1 days and the annual rolling herd average was 6,653.1 kg of milk (milked twice daily). All cows were fed to meet or exceed NRC [16] recommendations for lactating cows. A total mixed diet consisting of sorghum silage, and a concentrate-trace-mineral mix was offered twice daily. Besides, cows were kept on winter pastures (raygrasses and clovers) and summer pastures (sorghum) and had ad libitum access to fresh water. Previous to the first postpartum insemination (47.01 ± 5.43 DIM) cows were pre-synchronized using one dose of PGF<sub>2α</sub> (Dinoprost trometamin, Lutalyse, 25 mg, im). At 60 ± 3.52 DIM (day 0 of the Heatsynch protocol) cows were subjected to gynecological examination by a Veterinarian, where only those animals considered as healthy with no signs of uterine disease were kept in the study. The Heatsynch consisted of one injection of GnRH (100 μg - Gonadorelin diacetate tetrahydrate, Cystorelin<sup>2</sup>) and CIDR (Controlled intravaginal drug-releasing device)<sup>3</sup> insertion at D0, either a new one, a previous used once (7 days- second use) or twice used (14 days- third use). Seven days later, cows received an injection of PGF<sub>2α</sub> (25 mg, im) and the CIDR insert was removed. To induce ovulation, 24 h after CIDR removal (day 8), cows received ECP (Estradiol cypionate, 1.0 mg, im)<sup>1</sup>. Subsequently all cows were observed for visual signs of estrus (twice daily at 7:00 and 18:00) for 3 days following CIDR removal and those detected in estrus were then inseminated 8-12 h after the detection. Cows that were not detected in estrus were timed artificially inseminated 72 h after CIDR removal (Figure 1). The estrus detection and insemination were performed by a single technician and the semen used was from different sires selected according genealogy and productive criteria adopted by the farm. Used CIDRs had been thoroughly rinsed with a mild disinfectant solution, air-dried, and stored in a dry, enclosed container after first or second use.

Based on the detection of estrus and the moment of AI after the CIDR removal, cows were divided into 3 groups: cows showing signs of estrus and therefore AI between 36 to 60 h after CIDR removal (EPTAI group; n = 241); cows showing estrus and AI 72 h after CIDR removal (ETAI group; n = 207); and cows that did not show any sign of estrus after CIDR removal and were TAI 72 h after CIDR removal (No ETAI group; n = 335). In a subset of animals (105/783; 13.4%) anovary evaluation was performed by ultrasonography (5.0-MHz, WED-3000, B-mode)<sup>3</sup> at the beginning of synchronization protocol aiming to check the presence of a CL and classify the cows as cycling or not cycling. Ovarian structures were examine aiming to check the CL presence on day zero of the program in a subset of
the cows and pregnancy status was also determined by ultrasonography, at 30 to 35 days and 60 to 65 days after AI. Cows that were pregnant at 30 to 35 days but were diagnosed as not pregnant at 60 to 65 days or showed signs of estrus after first pregnancy confirmation were considered as having pregnancy loss. The cows diagnosed as not pregnant, were re-synchronized using the same Heatsynch protocol.

Statistical Analysis

Binomially distributed data, such as, estrous response, conception rate, pregnancy rate, and pregnancy losses, were analyzed by logistic regression using the logistic procedure of SAS [16]. Pregnancy rates at 30-35 days and 60-65 days as well as pregnancy losses were considered in the model for the effect of parity (primiparous vs. multiparous), the number CIDR uses (first, second and third time) and the group of estrus behavior during the Heatsynch protocol (EPTAI, ETAI, and NoETAI). The bull effect was initially considered in the model, however there was no effect (P > 0.05), therefore, it was excluded from the final model.

RESULTS

The reproductive responses to the Heatsynch protocol were affected (P < 0.01) by estrus behavior, where higher conception rates at 30 and 60 days were found in EPTAI and ETAI groups than NoETAI group (Table 1). Also, pregnancy losses were lower in EPTAI and ETAI groups than NoETAI group (Table 1). The overall conception rates in the Heatsynch program at 30 days was 34.5% and the pregnancy rate at 60 days was 25.4%. General pregnancy losses rate (the difference of results between the 30 days and 60 days examination) was 26.3%. In the current study, the pregnancy losses were affected by the estrus behavior, where higher rate of losses were observed in the No ETAI group (Table 1). Estrus behavior and AI until 60 h after CIDR removal was detected in 30.8% of the cows (EPTAI group), and 26.4% of the cows showed signs of estrus 60 h after CIDR removal and were AI 72 h after CIDR removal together with TAI cows (ETAI group, Figure 2). Therefore, 42.8% of cows in the Heatsynch protocol did not show any signs of estrus, and were TAI 72 h after the CIDR removal (No EPTAI, Figure 2). The CIDR reutilization did not affect the estrus behavior, conception rate or pregnancy losses (Table 3). These results are useful to dairy herd reproduction management, especially in countries that cost effectiveness is important for the adoption of some practices like timed AI. In the current study, it was not possible to measure the serum progesterone levels during the protocols.

Thus, we could verify in a small sample (n = 105) that 74% of the cows already had ovulated by 60 DIM at the first Heatsynch protocol submission. Therefore, it can be speculate that the majority of cows enrolled in this study were cyclic at the beginning of timed AI protocol, once the average of DIM was 113 days.

Figure 1. Diagram of activities during the study. Based on the detection of estrus and the moment of AI after the CIDR insert removal (day 7), cows were divided into 3 groups: cows showing signs of estrus and therefore AI 36 to 60 h after CIDR removal (EPTAI group, n = 241); cows showing estrus and AI 72 h after CIDR removal (ETAI group, n = 207); and cows that did not show any sign of estrus and then TAI 72 h after CIDR removal (No EPTAI, n = 335). GnRH = injection of 100 μg of GnRH; PGF = injection of 25 mg of PGF2 ; ECP = injection of 1 mg of estradiol cypionate.

Acta Scientiae Veterinariae. 43: 1256.

Figure 2. AI moment according with estrus behavior groups in a Heatsynch protocol.

Table 1. Reproductive responses of different estrus behavior groups during a Heatsynch protocol in dairy cows.

<table>
<thead>
<tr>
<th>Item</th>
<th>Conception rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EPTAI&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>30 d, % (n/n)</td>
<td>44.8 (108/241)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>60 d, % (n/n)</td>
<td>35.7 (86/241)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pregnancy losses, % (n/n)</td>
<td>20.4 (22/108)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-b</sup>Different superscripts within column indicate significance (P < 0.01). EPTAI - estrus and therefore AI 36 to 60 h after CIDR removal; ETAI = estrus and AI at timed AI; NoETAI = no estrus and TAI.

Table 2. Parity effect on estrus behavior in a Heatsynch protocol in dairy cows.

<table>
<thead>
<tr>
<th>Item</th>
<th>Primiparous</th>
<th>Multiparous</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrus group, % (n/n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPTAI&lt;sup&gt;1&lt;/sup&gt;</td>
<td>33.3 (112/336)</td>
<td>28.9 (129/447)</td>
<td>0.63</td>
</tr>
<tr>
<td>ETAI&lt;sup&gt;1&lt;/sup&gt;</td>
<td>21.1 (71/336)</td>
<td>30.4 (136/447)</td>
<td>0.03</td>
</tr>
<tr>
<td>NoETAI&lt;sup&gt;1&lt;/sup&gt;</td>
<td>45.5 (153/336)</td>
<td>40.7 (182/447)</td>
<td>0.23</td>
</tr>
<tr>
<td>Conception rate, % (n/n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 d</td>
<td>39.9 (134/336)</td>
<td>30.4 (136/447)</td>
<td>0.005</td>
</tr>
<tr>
<td>60 d</td>
<td>30.1 (101/336)</td>
<td>21.9 (98/447)</td>
<td>0.01</td>
</tr>
<tr>
<td>Pregnancy losses, % (n/n) d 30 to60</td>
<td>24.6 (33/134)</td>
<td>27.9 (38/136)</td>
<td>0.38</td>
</tr>
</tbody>
</table>

<sup>1</sup>EPTAI - estrus and therefore AI 36 to 60 h after CIDR removal; ETAI = estrus and AI at timed AI; NoETAI = no estrus and TAI.
DISCUSSION

Estrus behavior has been reported as a positive effect on conception rates and the reduction of pregnancy losses during timed AI protocols [5]. This effect is possibly related with the better synchrony of the follicular wave at the beginning of the protocol, coupled with the higher dominant follicle development at CIDR removal and prostaglandin injection. As a result, there is an increase in the estradiol concentrations resulting in an increase of estrus signs manifestation. Dairy cattle revealed an improved synchrony of estrus when treated with estradiol benzoate (EB) within 24 h (100%) rather than 48 h (87.5%) of PGF2α administration and this was attributed to the timing of treatment in relation to the stage of follicle growth [4]. The CL produced after ovulation would have higher volume and more progesterone would be produced [12]. Therefore, cows submitted to Heatsynch protocols that demonstrate estrus signs have increased pregnancy rates and lower pregnancy losses [8]. In addition to that [13] also demonstrated that cows exposed to low estradiol concentrations were more likely to experience subsequent premature luteolysis. Thus, coupled with the ECP action, it can be postulated that cows showing estrus signs during a timed AI protocol could have a higher ovulation rate [9]. This can have great importance in high producing dairy herds, since in response to ECP becomes possible to overcome the lower concentrations of estradiol due to high hepatic clearance and catabolism of steroid hormones [24].

In previous studies, the rate of estrus detection reported in Heatsynch program varied from 60% [10] to 75% of cows [17,8]. In this study, 57.2% of cows subjected to the Heatsynch protocol were detected in estrus. Differences in estrous behavior can be explained by different factors, as the utilization of visual detection of estrus signs similar to what was used in a semi-extensive system where the cows were allowed to graze the majority of the time, which become one of potential limitations. The distribution of animals in EPTAI and No ETAI were similar for multiparous and primiparous cows (Table 2). However, it was observed a large number of multiparous cows showing estrus signs near to TAI (ETAI group) in comparison to the primiparous cows (Table 2). These results are in accordance with another study [7] and can be explained by the fact of multiparous cows have more demand for estradiol once they have greater levels of milk production and hepatic metabolism [24], therefore the dominant follicle takes longer to reach ovulatory size. As stated before, cows subjected to TAI protocols can experience higher pregnancy losses due to the presence of a less competent CL and as lower rise in progesterone concentrations [3].

Perry et al., [18] working with beef cows, observed that late embryonic losses occurred when small follicles (≤11 mm) were induced to ovulate with GnRH at the moment of timed AI. Therefore, the pregnancy losses found in the No ETAI group could be related to smaller ovulatory follicles, as well as to lower progesterone levels after insemination, both of which have a negative impact on the uterine environment and embryo development [12]. In addition, it was previously demonstrated that cows did not expressing signs of estrus after ECP injection have lower ovulation rate and progesterone concentrations seven days after AI, as well as higher incidence of short estrous cycles [9].

The results of our study are in agreement with the suggested by [23] where a reduction in the ovulatory follicle diameter had a negative effect on fertility, due to the development of small CL with lower capacity to synthesize progesterone. It has been well documented that higher concentrations of progesterone during early embryonic development increase the size of the embryo and positively influencing embryonic survival [15].

CONCLUSIONS

Cows showing signs of estrus during a Heatsynch protocol have increased conception rates and decreased pregnancy losses. The CIDR reutilization up to three times or 21 days of use does not have any effect on reproductive responses in a Heatsynch program. Therefore, CIDR reuse coupled with estrus detection can optimize reproductive performance in dairy cows subjected to TAI programs using estradiol cypionate and reduce the costs of the protocol.

MANUFACTURERS

1. Pfizer Saúde Animal. São Paulo, SP, Brazil.
2. Merial Limited. Duluth, GA, USA.
3. Well.d Medical Electronics Company Ltd. Shenzhen, China.

Acknowledgements. The authors would like to thank Granja 4 Irmãos S.A. for providing the cows and farm facilities for the experiment.

Ethical approval. The study was approved by the Committee for Ethics in Animal Experiments from UFPel, under protocol number 2827 on May 7, 2012, in accordance with Brazilian laws and ethical principles published by the Brazilian College of Animal Experimentation.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.
REFERENCES


