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Effect of GnRH or Estradiol Benzoate on Reproductive Traits during a Heatsynch Protocol in Dairy Cows

Laís Mielke[1], Mityelle da Costa Chaves Rodrigues[2], Márcio Érpen Lima[3], Diego Andres Velasco Acosta[3], Francisco Augusto Burkert Del Pino[1], Marcio Nunes Corrêa[3], Jéssica Halfen[1], Eduardo Gularte Xavier[4] & Cássio Cassal Brauner[1]

ABSTRACT

***Background*:** Milk production of cows is closely correlated with its reproductive efficiency. One of the several factors influencing the dairy efficiency is the decline in fertility of the cows/heifers. Use of hormonal protocol are often used to improve the fertility of the recipients. Several programs are available to inseminate dairy cows, with variation in the use of different types of hormones, time of use and range of duration period, as well as the time of AI. The objective of this study was to evaluate the efficiency of follicular wave emergence induction of estradiol benzoate in comparison to GnRH in a Heatsynch protocol and its influence on reproductive parameters.

***Materials, Methods & Results*:** In this study were used primiparous and multiparous Holstein cows (n = 325). Animals were divided randomly into two different treatments according with the hormone used to induce follicular wave emergence (D0). The GnRH group (n = 167), was given 25 mg, while in the EB group (n = 158) was used 2 mg. At same day, were inserted, a controlled internal drug-releasing containing 1.9 g of progesterone CIDR®. On day 7, CIDR was removed and cows were given 25 mg luteinizing hormone followed by an injection of 1 mg Inducer Ovulation at day 8. Cows were then observed for signs of estrus for at least two hours (twice daily at 7 am and 6 pm) for three days following CIDR® removal. Cows detected in estrus were inseminated 12 h later the estrus detection. Pregnancy status was determined by ultrasonography at 30 ± 5 d and 60 ± 5 d after AI. A single technician was responsible for the estrus detection and inseminations. A single technician was responsible for the estrus detection and inseminations. Ultrassonographic examinations with ultrasound, transrectal linear probe of 5 MHz, were performed for the diagnosis of pregnancy at 30 and 60 days after artificial insemination. Binomially distributed data, such as, estrous response, conception rate, pregnancy rate and pregnancy losses, were analyzed by Chi-square test. 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) and the group of estrus behavior during the Heatsynch protocol. Conception rate was similar between GnRH (36.1%, 57/158) and EB (37.7%, 63/167) treatments. Cows showing estrus signs after CIDR removed were 71.9% in GnRH and 62% in EB group ($P = 0.05$). Embryonic losses were different ($P = 0.03$) according to groups of cows showing estrus signals or not.

***Discussion*:** Our results demonstrate that conception rates are not affected by using either EB or GnRH at the beginning of the Heatsynch protocol. Therefore, the EB can be an alternative choice to GnRH at Heatsynch beginning. Similar results were found by autor in dairy cows, once it was also observed that there is no difference in conception rate in two TAI protocols using either GnRH or EB. In Heatsynch protocols the increase in estrus detection are a key factor to increase fertility in lactating dairy cows. It can be explained once animals in regular estrous cycle have a palpable corpus luteum and circulating progesterone concentration higher than 1 ng/mL which leads than to enhance pregnancy ratios. The use of BE at the time of CIDR insertion in TAI protocols for dairy cattle proved to be an alternative to GnRH considering conception rates. Therefore, the use of BE at the time of CIDR insertion in a Heatsynch protocol in dairy cattle proved to be an alternative to GnRH which can be an important tool to increase the TAI utilization by farmers. Furthermore, in Heatsynch protocol cows showing estrus have pregnancy rates improved and lower pregnancy losses.

Keywords: estrus, Timed AI, pregnancy, pregnancy losses.

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[1]Programa de Pós-graduação Zootecnia (PPGZ), Pelotas, RS, Brazil, [2]Programa de Pós-graduação Biotecnologia (PPGB) Pelotas. [3]Programa Pós-graduação em Veterinária (PPGV) Pelotas. [4]Granjas 4 Irmãos S.A., Rio Grande, RS. CORRESPONDENCE: C.C. Brauner [cassiocb@gmail.com - Fax: +55 (53) 3275-7136]. Programa de Pós-graduação em Zootecnia (PPGZ), Faculdade de Agronomia Eliseu Maciel, Campus Universitário s/n. CEP 96010-900 Capão do Leão, RS, Brazil.

INTRODUCTION

Milk production of cows is closely correlated with its reproductive efficiency. Use of hormonal protocols are often used to improve the fertility of the recipients [14]. Several programs are available to inseminate dairy cows, with variation in the use of different types of hormones, time of use and range of duration period, as well as the time of AI [17]. The controlled internal drug releasing (CIDR) is an intravaginal device that sustains adequate concentrations of progesterone in plasma to block an LH surge and prevent ovulation [20]. Hypothesized that incorporating a CIDR to a timed AI protocol using estradiol cypionate (ECP) to synchronize ovulation would positive affect protocols by improving detection of estrus and ovulation, resulting in greater pregnancy rates, which became well known as the Heatsynch plus CIDR protocol [7].

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 PGF2α injection and TAI moment [16]. Conception rates in dairy cows submitted to Heatsynch have been described as similar to Ovsynch outcomes [10].

When administered together with a progestin, estradiol causes atresia of the dominant follicle, initiating the emergence of a new follicular wave [15] since the GnRH promotes ovulation of the dominant follicle and the beginning of a new follicular wave [2].

Therefore, this study aimed to evaluate the efficiency of follicular wave emergence induction of estradiol benzoate in comparison to GnRH in a Heatsynch protocol and its influence on reproductive parameters.

MATERIALS AND METHODS

Study area

The study was conducted on a dairy farm located in southern Rio Grande do Sul (32°16' S, 52° 32' W) between the months of July 2012 to March 2013. With approval from the Federal University of Pelotas Ethics and Animal Experimentation under the number 2358.

Sampling

Three hundred and twenty five primiparous and multiparous Holstein cows pure, between three and six lactations, with average milk production of 28 ± 3.4 kg/day and 113.4 ± 3.5 days in milk were used. The animals were reared in a semi-extensive system with concentrate supplementation after each milking, based on milk production. The cows were milked twice a day at roughly 12 h interval.

The animals were randomly assigned to two different treatments for estrus synchronization (Heatsynch protocol) according to the hormone used at the beginning of the protocol (D0). The variation (between animals of the two groups) could be attributed to the differences in the protocol used to induce estrus: i.e GnRH or estradiol benzoate (EB) [Figure 1]. Cows injected In the GnRH (25 mg of lecirelin, Gestran Plus®, im)[1] group (n = 167), was injected at day zero, whereas in BE (2 mg, RIC-BE®, im),[1] group (n= 158). At same day, a controlled internal drug-releasing (1.9 g of progesterone, CIDR®)[2], either a new one or previously used once (7 d) or twice (14 d) were inserted. Seven days later (Day 7), CIDR was removed and cows were given PGF2α. (25 mg, Lutalyse®, im)[2], followed by an injection of estradiol cypionate at day 8 (1 mg, ECP®, im)[2]. Cows were then observed for signs of estrus for at least two hours (twice daily at 7am and 6pm) for three days following CIDR removal. Those cows detected in estrus were inseminated 12 h later the estrus detection. A single technician was responsible for the estrus detection and inseminations. Pregnancy status was determined by ultrasonography at 30 to 35 d and 60 to 65 d after AI.

Ultrassonographic examinations with ultrasound (5-0–MHz, Weld 3000, B-mode)[3], transrectal linear probe, were performed for the diagnosis of pregnancy at 30 and 60 days after artificial insemination. The result of the difference of these two measurements was considered as pregnancy loss in the period.

Statistical Analysis

Binomially distributed data, such as, estrous response, conception rate, pregnancy rate and pregnancy losses, were analyzed by Chi-square test at NCSS (2005). 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) and the group of estrus behavior during the Heatsynch protocol (estrus behavior at TAI and no estrus at TAI). The bull effect was initially considered in the model, however there was no effect ($P > 0.05$), as well as the number CIDR uses (first, second and third time) also initially was considered however it demonstrated no effect ($P > 0.05$). Therefore, both factors were excluded from the final model.

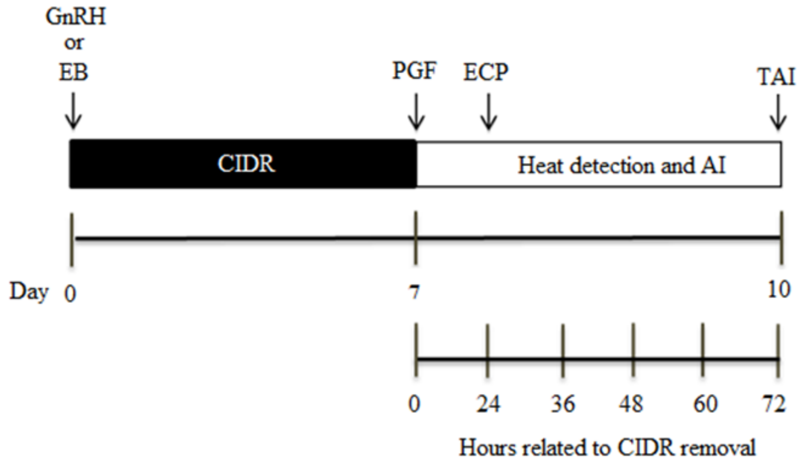

Figure 1. Diagram of activities during the study. CIDR® = intravaginal progesterone insert; GnRH = injection of gonadotropin releasing hormone; EB = estradiol benzoate; PGF2α = injection of prostaglandin F2α; ECP® = injection of estradiol cypionate; TAI = timed artificial insemination. Cows were then observed for signs of estrus for at least two h for three days following CIDR® removal. Those cows detected in estrus were inseminated 12 h later the estrus detection our TAI 72 h after CIDR® removal.

RESULTS

Among the 325 cows, conception rate was similar between GnRH (36.1%, 57/158) and EB (37.7%, 63/167) treatments, as shown in Table 1. But there was difference ($P < 0.0001$) compared the conception rates in TAI (15.8%) and TAI with heat detection (84.2%) among cows that did not show estrus and those who demonstrates estrus signs during the Heatsynch protocol. In Table 1 it is possible to observe the treatments, with 71.9% for the group receiving GnRH and 62% for the EB which received the beginning of the protocol ($P = 0.05$).

In table 2, is observed the hormones treatment, divided in groups primiparous and multiparous, showing results of estrus signs, timed AI, pregnancy rate.

Embryonic losses were different ($P = 0.03$) according groups of cows that showed or not estrus signs during Heatsynch protocol. These results can be analyzed in table 3.

Table 1. Reproductive characteristics of cows starting Heatsynch protocol either using GnRH or EB. Pelotas, Brazil.

	Hormones treatment		
Variable	GnRH (n = 167)	EB (n = 158)	*P*
Estrus signs	71.9 (120/167)[a]	62.0 (98/158)[b]	= 0.05
TAI	28.1 (47/167)[b]	38.0 (60/158)[a]	
Pregnancy rate. 30d	37.7 (63/167)[a]	36.1 (57/158)[a]	= 0.75
Pregnancy rate. 60d	33.5 (56/167)[a]	31.6 (50/158)[a]	= 0.71
Pregnancy losses	11.1 (7/63)[a]	12.3 (7/57)[a]	= 0.84

[ab]Different letters in the same row indicate significant difference ($P < 0.05$).

Table 2. Estrus behavior and conception rate (%) the hormone at beginning of Heatsynch. Pelotas, Brazil.

Variable	Hormones treatment					
	GnRH			EB		
	Primiparous (n = 82)	Multiparous (n = 85)	Overall (n = 167)	Primiparous (n = 77)	Multiparous (n = 81)	Overall (n= 158)
Estrus signs	70.7	67.7	71.9	67.3	55.2	62.0
Timed AI	29.3	32.3	28.1	32.7	44.8	38.0
Pregnancy rate 30 d (%)	36.2	27.4	37.7	42.3	24.1	36.1

Table 3. Reproductive characteristics of cows showing estrus signs or not (TAI) during the Heatsynch protocol. Pelotas, Brazil.

Variable	Hormones treatment		
	Estrus signs (n = 218)	TAI (n = 107)	*P*
Pregnancy rate. 30d	46.3(101/218)[a]	17.8(19/107)[b]	< 0.0001
Pregnancy rate. 60d	42.2 (92/218)[a]	13.1 (14/107)[b]	< 0.0001
Pregnancy losses	8.9 (9/92)[b]	26.3 (5/19)[a]	= 0.03

[ab]Different letters in the same row indicate significant difference ($P < 0.05$).

DISCUSSION

The reproductive management of dairy cattle is impaired due to failure of estrus detection. To minimize this negative effect protocols aiming to synchronize estrus and the use of TAI allow ovulation induction without the occurrence of estrus manifestation are important tools to increase reproductive performance [22]. The exogenous estradiol administered together with progesterone causes the dominant follicle atresia when administered before or during emergence of the wave suppressing FSH and LH [15]. According the results from the present experiment, conception rates are not affected by using either EB or GnRH at the beginning of the Heatsynch protocol. Therefore, the EB can be an alternative choice to GnRH at Heatsynch beginning. Similar results were found [25] in dairy cows, once it was also observed that there is no difference in conception rate in two TAI protocols using either GnRH or EB [21] comparing GnRH and EB treatments in beef cattle, did not found any effect of the two different hormones on conception rates. The largest number of cows showing estrus in GnRH group in this study may be could be explained due to the induction of an earlier follicular wave [1,23]. Thus, a greater size follicle has more time to grow and therefore it was able to produce more levels of estradiol at the time of CIDR removal.

In Heatsynch protocols the increase in estrus detection are a key factor to increase fertility in lactating dairy cows. 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% [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 [4]. In other studies, the rate of estrus detection reported in Heatsynch program varied from 60% [10] to 75% of cows [7]. Also, 57.2% of cows subjected to the Heatsynch protocol were detected in estrus [11]. 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. In this study, 57.2% of cows subjected to the Heatsynch protocol were detected in estrus. Therefore, hormones that are able to induce a higher estradiol production may cause higher estrus detection and then increasing fertility. In this present study cows that showed estrus during the Heatsynch (Table 3) protocol showed higher ($P < 0.0001$) pregnancy rates at 30 and 60 days as well as lower pregnancy losses ($P = 0.03$). According to the literature [11], 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 cows showing signs of estrus and therefore AI between 36 to 60 h after CIDR® removal and cows showing estrus at AI 72 h after CIDR® removal than cows that did not show any sign of estrus after CIDR® removal and were TAI 72 h after CIDR® removal. Also, pregnancy losses were lower in cows showing estrus signs than cows that did not show any sign of estrus. The increase in follicle size is followed by an increase in estradiol levels which facilitated estrus manifestation as well as ovulation. As a consequence of a greater size ovulatory follicle, a greater and more competent CL is produced, which is able to produce more progesterone and maintained pregnancy during the first ninety days of gestation.

Despite no differences ($P > 0.05$) in pregnancy rate in multiparous or primiparous, the Table 2 shows that could be an interaction among hormones at beginning of Heatsynch protocol. This fact is related with subfertility and could be due to higher hepatic steroids metabolism [18]. As a consequence, these animals have lower circulating estradiol and progesterone when compared to non-lactating animals [19] and animals with a low mil production [12]. There are evidences that indicate the lower circulating progesterone concentrations right after ovulation had a negative effect on pregnancy establishment [13], being one of the probably causes on reduced fertility on dairy cows [3]. Thus, animals with higher progesterone concentrations after insemination are highlighted as more fertile.

Embryonic losses were lower ($P = 0.03$) in animals that demonstrated estrus compared to the ones that had no signal (8.9% vs. 26.3% of losses) [Table 3]. It can be explained once animals in regular estrous cycle have a palpable corpus luteum and circulating progesterone concentration higher than 1 ng/mL which leads than to enhance pregnancy ratios. Indeed, it was already found a lower embryonic lost between days 29 and 57 in cows with high P4 concentrations (6.8%) compared to cows with low concentrations (14.3%), also the lower P4 concentrations at the device removal day resulted in lower conceptions rate (37.1%) compared to cows with higher P4 concentrations (51%), at day 29 [6]. Thus, there is a positive effect of high P4 concentrations before the AI on the following pregnancy maintenance, even 29 days after the AI. Also it is demonstrated the lower circulating progesterone concentrations after ovulation has a negative effect on pregnancy establishment [13].

Another fact to be considered about embryonic losses is the high sensibility of Holstein cows to elevated temperatures, which could mask all their genetic potential. Cows submitted to heat stress have the uterine blood flow reduced and also the heat exchange, consequently increasing the uterine temperature [9]. This alteration inhibits the embryonic development and reduces the AI success. A study demonstrated that Holstein cows which got pregnant during summer had 12.3% of embryonic losses, while during the winter the losses were only 2.1% [8]. The early stages of embryonic development are the me most sensible to temperature changes, higher embryonic losses happen before day 42 after AI in cows under heat stress [24].

CONCLUSION

In conclusion, the use of BE at the time of CIDR® insertion in a Heatsynch protocol in dairy cattle proved to be an alternative to GnRH which can be an important tool to increase the TAI utilization by farmers. Furthermore, in Heatsynch protocol cows showing estrus have pregnancy rates improved and lower pregnancy losses.

MANUFACTURERS

[1]Tecnopec. São Paulo, SP, Brazil.

[2]Zoetis Saúde Animal. São Paulo, SP, Brazil.

[3]Well.d Medical Electronics Company Ltd. Shenzhen, China.

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

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