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Early postpartum administration of equine chorionic gonadotropin to dairy cows calved during the hot season: effects on fertility after first artificial insemination

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Abstract

Heat stress reduces fertility of high-producing dairy cows, and early administration of equine chorionic gonadotropin (eCG) may improve it. Here, 401 heat-stressed, high-producing dairy cows on a single commercial farm were given eCG (500 UI, n = 214) or saline (n = 187) on days 11-17 after calving, and the effects on fertility after the first artificial insemination (AI) were assessed. On post-partum day 96.34±9.88, all cows were inseminated after a "double short Cosynch" synchronization protocol. Ovarian activity and uterine status were checked by ultrasound on the day of eCG administration and every 7 days thereafter for a total of 3 weeks; checks were also performed during synchronization, and 7 days after AI. On post-partum day 30, cytobrush uterine cytology was performed to check for subclinical endometritis. Pregnancy status was checked on days 30 and 60 after AI. The eCG and control groups did not differ significantly in terms of average lactations per cow (2.33±1.34), days in milk at first AI (96.33±9.88), average milk yield at AI (41.38±7.74 L), or the particular inseminator or bull used for AI. The eCG and control groups showed increasing ovarian activity with time, with approximately 75% of cows in both groups showing a corpus luteum at the beginning of the synchronization protocol. On post-partum day 30, 17.4% of eCG cows and 22.9% of control cows showed subclinical endometritis. Cows treated with eCG showed a tendency toward lower hyperecogenic intraluminal content (16.8 *vs.* 21.4%, $P = 0.15$), but ovarian activity during the synchronization protocol was similar between eCG and control groups, with 91% of animals in both groups showing luteolysis after prostaglandin application and 88% showing ovulation after the last administration of gonadotropin-releasing hormone. Fertility was similar between the two groups at both time points after AI (30 days, 34.9 *vs.* 31.8%; 60 days, 30.6 *vs.* 28.5%; $P >$ 0.2). These results suggest that early postpartum eCG administration does not improve fertility of heat-stressed dairy cows as long as 60 days after AI. Other strategies may be more effective at mitigating the ability of post-partum heat stress to reduce fertility of high-producing dairy cows.

Keywords: Heat stress, eCG, postpartum, cyclicity, timed AI, double Ovsynch

1. Introduction

High temperatures have been strongly linked to low fertility in dairy cattle [1-3]. Heat stress appears to reduce fertility by increasing the number of days open, reducing conception rate, and increasing the rates of anestrus, anovulatory or persistent follicles, and ovarian cysts [4-7]. In particular, heat stress during the post-partum period, which is a critical window for ensuring reproductive performance [8,9], can increase the risk that animals become anestrous or non-cycling [10]. The prevalence of such animals after the “voluntary waiting period” is a major problem among dairy herds, and it has grown more serious in recent decades [11 ,12].

Heat stress may reduce the fertility of cows through several mechanisms, including by reducing steroidogenesis, delaying follicle selection, and altering follicular waves, thereby harming oocyte quality [3, 13-18]. Heat stress also alters the uterine environment [2, 19-21], shortens corpus luteum life span and reduces progesterone production [22]. Heat stress may prolong the first post-partum ovulation by delaying follicular growth and estradiol production [1]. A prolonged luteal phase increases the risk of clinical and subclinical uterine infection as well as other uterine problems [23-24].

Cooling can diminish the deleterious effects of heat stress on the reproductive performance of dairy cows. Different strategies have been described for cases when cooling is not enough. These include fix timed artificial insemination (AI) [25] or administration of equine chorionic gonadotropin (eCG) in the early post-partum period [26].

The glycoprotein eCG is secreted by the endometrial cups of pregnant mares. It has a relatively long half-life and it sustainably exerts effects similar to those of follicle-stimulating hormone and luteinizing hormone in cattle [27]. Thus, eCG can be effective at improving post-partum fertility in anestrous cows, many of which are deficient in luteinizing hormone pulses [28-30] and possess a dominant follicle unable to ovulate [31]. Studies have shown that eCG administration on post-partum day 6 can enhance follicle growth and ovulation in the first-wave dominant follicle [32], and early eCG administration in the post-partum period can reduce the number of days until first service as well as the interval from calving to conception [26].

Based on these findings, we hypothesized that eCG administration soon after calving during the hot season could enhance fertility after the first artificial insemination (AI). We speculated that early post-partum administration would be effective because it would occur when a dominant follicle is likely to be present, thereby raising the likelihood that the animal would resume the estrous cycle. In addition, it would increase plasma estradiol concentration [33], improving the uterine environment. Therefore, we evaluated whether eCG administration on post-partum days 11-17 affected the conception rate after first timed AI of heat-stressed, high-producing dairy cows.

2. **Material and methods**

2.1. Animals

Experimental data were collected on the reproductive performance of 401 Holstein cows (132 primiparous, 269 multiparous) that calved during the hot season (July-September) on a commercial dairy farm in eastern Spain (SAT

More, Betere, Spain). Cows were housed in compost-bedded barns equipped with fans and sprinklers.

The cows in this study formed part of a herd of 1,216 cows, of which 37% were primiparous. All animals were managed in the same way. The replacement rate was 30%. Mean age at first calving was 24 months. Cows were milked three times daily; mean daily milk production was 35.6 kg per cow. The herd was fed twice daily with a total mixed ration (TMR) that was balanced according to recommendations for lactating dairy cows. The TMR consisted of brewer's grain, alfalfa or corn silage, orange, corn, cotton, soybean hulls, straw and soybean, as well as bicarbonate and corrector salts. All animals had *ad libitum* access to water.

The temperature-humidity index (THI) was monitored daily with a portable device (605-H1; Testo, Barcelona, Spain). THI was calculated by combining the maximum temperature (T) in Celsius and minimum relative humidity (h) using a published formula [34]. Mean daily THI over the study period was 78.18±2.40 (range, 73 to 86). Minimal daily THI was 67.48±2.34 (range, 63 to 75), and maximal daily THI was 88.87±4.13 (range, 79 to 102).

2.2. Experimental procedures

On post-calving days 11-17, cows were weekly randomized into two groups: one received 500 IU of eCG (n = 214) and controlled for parity, while the other received saline solution (n = 187). On post-partum day 96.34±9.88, all cows underwent AI performed by two experienced veterinarians, in which ovulation was synchronized based on the Double Ovsynch protocol [35] and the 5dCoS2 protocol [36] in a procedure that we called "double short Cosynch" (Figure 1). In this procedure, the gonadotropin-releasing hormone (GnRH) analogue was gonadorelin diacetate (100 µg; Cystoreline®, Ceva SA, Barcelona, Spain), and the prostaglandin $F_{2\alpha}$ (PGF) analogue was cloprostenol sodium (500 µg; Cyclix®; Virbac SA, Barcelona, Spain).

2.3. Outcome assessment

On post-partum days 21-25, uterine health was assessed in all cows using ultrasound. The presence of hyperechogenic content, likely corresponding to purulent intrauterine content, was determined, and the thickness (in mm) of the uterine wall was measured at the uterine body approximately 2 cm cranial from the cervix [37]. Vaginal discharge was also evaluated [38]. Endometritis was diagnosed based on the presence of hyperechogenic content and/or mucopurulent vaginal discharge comprising >50% pus [39]. On post-partum day 30, a randomly selected subset of cows (86 from the eCG group, 70 from the control group) was examined for subclinical endometritis using cytobrush uterine cytology as described [40]. The cut-off percentage of polymorphonuclear neutrophils was 20%.

Over the 3 weeks following eCG administration, all cows were examined by ultrasound every 7 days in order to evaluate ovarian activity (U1-U3 in Figure 1). During these exams, the presence of a corpus luteum, dominant follicle and follicular cysts were determined. A follicular cyst was defined as a follicle of diameter ≥2 cm without a corpus luteum in either ovary that was detected in two consecutive examinations 7 days apart.

During timed AI synchronization, several ultrasound examinations were performed in a subgroup of cows (89 from the eCG group, 75 from the control group) in order to evaluate the ovarian synchronization efficacy. This process was carried out as described [39]. Examination U4, conducted on the day of the first administration of GnRH in the second Ovsynch, was performed to check for the presence of corpus luteum (indicating that a cow was cycling) or of cystic structures (indicating that a cow was not cycling). A follicle ≥20 mm in diameter in the absence of luteal tissue was considered a cyst. Examination U5, conducted on the day of prostaglandin $F_{2\alpha}$ administration, was performed to detect the presence of corpus luteum. Examination U6, conducted on the day of treatment with GnRH as well as AI, served to determine the percentage of cows without a corpus luteum after prostaglandin treatment. Finally, examination U7, conducted one week after AI, was performed to check ovulatory response to the second administration of GnRH. Ovulation in response to this final hormone treatment was confirmed by the disappearance of the dominant follicle detected at examination U6 and by the presence of a corpus luteum in the same ovary.

On days 30 and 60 after AI, pregnancy was diagnosed in all cows using ultrasound. Additional data on each cow were also collected after AI, including the average number of lactations, days in milk (DIM) at first AI, average milk yield at AI, use of a particular inseminator (of 2 possible), and use of a particular bull (of 6 possible). A total of 13 cows were lost before pregnancy diagnosis (5 from the eCG group, 8 from the control group), so they were excluded from analysis of conception rate and pregnancy loss rate.

2.4. Statistical analyses

All data were analyzed using SPSS 22 (IBM, New York, USA). Probability values less than or equal to 0.05 were considered significant and those between 0.05 and 0.15 were considered trends. All data are reported as mean percentage or as mean ± SD.

Differences between eCG and control groups in percentages of frequencies of different ultrasound outcomes were assessed for significance using chi-squared tests. Differences in continuous variables (yield, lactation numbers, DIM) between the two groups were assessed using two-factor ANOVA to detect significant interactions.

Conception rate was analyzed using logistic regression and a stepwise forward method based on the Wald statistics criterion of $P > 0.10$. The statistical model included the effect of eCG administration and the following covariates for 389 cows that were diagnosed for pregnancy after AI: parity number, milk yield at insemination, DIM at insemination, inseminator, bull, presence or absence of endometritis on post-partum day 25 and hyperechogenic intrauterine content. The model included interactions between these effects as well as subclinical endometritis for the subset of 156 analyzed for this condition.

3. Results

THI during calving time was high, with maximal daily values ranging between 79 and 102. In fact, THI during calving of these cows was higher than the maximum THI values defined by Igono *et al.* (1992) [41] and West *et al.* (2003) [42] as critical for inducing heat stress. Thus, the cows in the present study were exposed to heat stress during calving. Parity did not differ between experimental groups (36,3 vs. 30,6% of primiparous in the control and eCG groups, respectively; $P>0.05$).

Uterine health soon after calving

On post-partum days 25-32, hyperechogenic intrauterine content tended to be less prevalent in the eCG group than in the control group (16.8 vs. 21.4%; $P = 0.15$). Other indices of uterine health in our study, i.e. uterine wall thickness and endometritis incidence, did not differ between groups (Table 1). Similarly, the incidence of subclinical endometritis was similar between the eCG group and control group (17.4 vs. 22.9%; $P = 0.826$).

Table 1. Uterine health of heat-stressed, high-yielding dairy cows on post-partum days 11-17.

	eCG group n = 214		Control group n = 187		Total	
	%	(n/N)	%	(n/N)	%	(n/N)
Endometritis	26.6	(57/214)	25.7	(48/187)	26.2	(105/401)
HIC	16.8	(36/214)	21.4	(40/187)	19.0	(76/401)
PMN ≥20%	17.4	(15/86)	22.9	(16/70)	19.9	(31/156)
Uterine thickness, mm	7.17 ± 1.68		7.34 ± 2.04		7.25 ± 1.85	

Values are shown as %, n/N or mean ± SD.

Abbreviations: HIC, cows with hyperechogenic intrauterine content; PMN, cows with a percentage of polymorphonuclear neutrophils ≥20%, based on cytobrush uterine cytology, indicating subclinical endometritis.

P values for inter-group differences in the same row were ≥0.2, except for HIC, for which $P = 0.15$.

Ovarian activity soon after calving

Ovarian activity in both the eCG and control groups increased with post-calving time: a corpus luteum was detected in 48% of cows at U3 but in only 3.7% at U1 ($P < 0.05$). The two groups showed similar ovarian activity at ultrasound examinations U1-U3 in terms of the presence of a corpus luteum, dominant follicle or follicular cysts ($P > 0.2$, Table 2). The presence of ovarian cysts at U1 is not known because our definition of cyst required detection on two consecutive examinations 7 days apart.

Table 2. Ovarian activity in heat-stressed, high-yielding dairy cows on post-partum days 11-17 based on three ultrasound examinations (U1-U3) at 7-day intervals.

	eCG group n = 214		Control group n = 187		Total	
	%	n/N	%	n/N	%	n/N
U1						
DF	73.4	(157/214)	77.0	(144/187)	75.1	(301/401)
CL	3.7	(8/214)	4.8	(9/187)	4.2	(17/401)
U2						
DF	70.1	(150/214)	71.7	(134/187)	70.8	(284/401)
CL	30.8	(66/214)	29.4	(55/187)	30.2	(121/401)
Cyst	18.2	(39/214)	13.9	(26/187)	16.2	(65/401)
U3						
DF	73.8	(158/214)	73.3	(137/187)	73.6	(295/401)
CL	48.6	(104/214)	48.1	(90/187)	48.4	(194/401)
Cyst	20.1	(43/214)	14.4	(27/187)	17.5	(70/401)

Abbreviations: CL, corpus luteum; DF, dominant follicle with a diameter of 8-20 mm; Cyst, a dominant follicle in either ovary with a diameter of at least 20 mm in the absence of a corpus luteum, detected in two consecutive examinations.

Ovarian activity during synchronization

At the beginning of the last Ovsynch in the synchronization protocol, 76% of all cows in our study had a corpus luteum, while fewer than 3% were non-cyclic. On the day of prostaglandin administration, more than 79% of cows showed a corpus luteum, compared to only 9% afterwards. The ovulation rate at 7 days after AI was 88.4%. All these rates were similar between the eCG and control groups (Table 3).

Table 3. Ovarian activity in heat-stressed, high-yielding dairy cows during the last Ovsynch after a Double Ovsynch-based synchronization protocol.

Parameter	eCG group n = 209 %	(n/N)	Control group n = 179 %	(n/N)	Total %	(n/N)	*P*
CL at first GnRH	71.9	(64/89)	80.0	(60/75)	75.6	(124/164)	*0.2294*
Cysts at first GnRH	2.2	(2/89)	2.7	(2/75)	2.4	(4/164)	*0.8214*
CL at prostaglandin administration	79.8	(71/89)	78.6	(59/75)	79.3	(130/164)	*0.2294*
no CL after prostaglandin	89.9	(80/89)	92.0	(69/75)	90.9	(149/164)	*0.8500*
CL: Ovulation rate after last GnRH+AI	88.5	(69/78)	88.2	(60/68)	88.4	(129/146)	*0.6401*
Conception rate (day 30)	34.9	(73/209)	31.8	(57/179)	33.5	(130/388)	*0.5211*
Conception rate (day 60)	30.6	(64/209)	28.5	(51/179)	29.6	(115/388)	*0.2075*

Abbreviations: AI, artificial insemination; CL, corpus luteum; DF, dominant follicle with a diameter between 8 and 20 mm; GnRH, gonadotropin-releasing hormone.

Conception rate after first timed AI

The conception rate at day 30 after AI was 33.5% among all cows in the study, and it was higher in primiparous cows (48.8%) than in multiparous cows (25.9%, *P* <0.0001). The conception rate was similar between the eCG and control groups (34.9 *vs.* 31.8%, *P* = 0.5211). Conception rates were similar, although slightly lower, at day 60 after AI (30.6 *vs.* 28.5%). The pregnancy loss rate was 11.54% across all cows in the study, and similar between the eCG and control groups (12.33 *vs.* 10.53%, *P* = 0.2075). Logistic regression analyses showed no evidence of joint influence of parity and eCG treatment on conception rate.

Cows that showed hyperechogenic intrauterine content at examination U2 were less likely to get pregnant after the first AI. The conception rate among these cows (19/75, 25.3%) tended to be lower than that among cows that did not show such content at U2 (111/313, 35.5%; P = 0.095). In contrast, we did not observe any difference in fertility between cows without endometritis (99/285, 34.7%) and cows with endometritis (31/103, 30.1%; P = 0.393), or between cows without subclinical endometritis (40/122, 32.8%) and with subclinical endometritis (7/31, 22.6%; P = 0.271).

Consistent with these results, logistic regression indicated that none of the following factors significantly influenced conception rate (all $P > 0.1$): endometritis, presence of hyperechogenic intrauterine content, subclinical endometritis, number of lactations per cow (eCG, 2.5±1.4; control, 2.1±1.2), liters of milk produced on the day of AI (eCG, 41.8±7.7; control, 40.9±7.8), and inseminator and bull used. The only factor that significantly affected conception rate when all cows were included in the model was DIM on the day of AI. DIM was 96.3±9.8 days across all cows in the study, 95.3±9.6 in the eCG group and 96.9±10.1 in the control group (OR 1.037, 95%CI 1.002 to 1.074; P = 0.040). Based on the model, eCG treatment did not influence fertility after the first timed AI (P = 0.213).

4. Discussion

Our results indicate that eCG administration on post-calving days 11-17 improved neither conception rate after first AI nor pregnancy loss rate by day 60 after AI in high-producing dairy cows that had calved during the hot season. This argues against our hypothesis that eCG injection soon after the calving period would improve uterine health and accelerate the resumption of ovarian activity, thereby mitigating the fertility reduction normally caused by heat stress.

We originally undertook this work because several studies provided indirect evidence that eCG administration might influence fertility in later breeding cycles. The glycoprotein benefits embryo development and survival, and eCG treatment on post-partum day 6 can facilitate early resumption of ovarian activity by enhancing ovarian follicle growth and early ovulation [32]. Early resumption of the ovarian cyclicity in dairy cows is associated with better reproductive efficiency [43, 44](Santos et al., 2016). On the contrary, a delay in this resumption is associated to a higher degree of inflammatory changes in the endometrium during the postpartum period [45] (Wathes et al., 2007). In addition, eCG administration improves development of the dominant and pre-ovulatory follicle [30], likely by binding to receptors for follicle-stimulating hormone and luteinizing hormone in the ovaries and acting as agonists of these hormones [46; Steward and Allen, 1981), thereby stimulating steroid secretion by granulosa and thecal cells. Administration of eCG during the post-partum period increases follicular development and folliculogenesis [33], inducing ovulation of the larger, more functional follicle with higher steroidogenic activity [33; 47; Ferrerira et al., 2013]. The earlier resumption of the cyclicity has been associated to a better reproductive efficienciy [43]. In contrast to these studies, our findings suggest that eCG treatment may not increase fertility of heat-stressed, high-producing dairy cows, highlighting the need for further studies to examine under what conditions eCG treatment may be effective.

Our results contrast with those of Vojgani *et al.* (2013) [26], who found that eCG injection into Holstein dairy cows on post-partum day 6 improved conception rate. Those authors proposed that eCG triggered earlier resumption of ovarian activity, but they did not measure that directly. In addition, they did not report on uterine health. We measured both ovarian activity and uterine health directly, allowing us to draw firm conclusions about any observed effects of eCG. The discrepancy between our study and theirs may reflect two key methodological differences. One is the timing of eCG dosing. Since the average interval from calving to first ovulation is 21-30 DIM [48,49], Vojgani *et al*. (2013) [26] may have added eCG before many cows had a dominant follicle, and so one could expect even better results than what those authors obtained if eCG were effective under our conditions. The second important difference is that our cows suffered intense heat stress during calving. Future studies should be performed in order to clarify the level of estradiol secretion during the early postpartum after eCG administration.

The level of clinical endometritis in our study population (26.2%) is similar to that in other studies [50-52], but lower than the 35-43% reported by others [53,54]. The greater prevalence in the latter studies may reflect that they diagnosed clinical endometritis using a vaginoscope, and they included cows that showed flecks of pus and mucopurulent discharge containing <50% pus. The incidence of subclinical endometritis in our study population was lower than in some studies [52, 55], but much higher than in others [54,56-57]. In fact, Gabler *et al.* (2010) [58] reported a decrease in polymorphonuclear neutrophils on post-partum days 30-31, which we did not observe. This discrepancy in rates of subclinical endometritis may reflect differences in threshold PMN percentages and the number of cells recovered for analysis. It may also reflect differences in temperature (heat stress exposure). Ambient temperature significantly influences animal body temperature [59], and higher temperature affects early postpartum uterine health [1].

Uterine wall thickness in our cows (7.25±1.85 mm), which was not influenced by eCG treatment, was slightly greater than the average of 5.9 mm reported on post-partum day 29 [60]. That study reported that the thickness reached a minimal size by post-partum days 22–36. We speculate that high THI may slightly delay uterine involution, as suggested by data published by Kornmatitsuk *et al.* (2008) [24] from heat-stressed dairy cows.

We relied on ultrasound to diagnose endometritis based on the presence of echogenic intrauterine fluid. We found that the group of cows with such fluid at examination U2 tended to have a lower conception rate than cows without such fluid (25.3 *vs.* 35.5%, $P = 0.095$). This is consistent with other studies [37,54]. These results imply that ultrasound is a valid and useful tool for diagnosing endometritis.

In contrast to endometritis at examination U2 (days 21-25), subclinical endometritis on post-partum day 30 did not significantly influence conception rate after first AI in our study. Similarly, Gobikrushanth *et al.* (2016) [54] observed no association between subclinical endometritis and the conception rate of non-heat stressed cows. Also consistent with our findings, those authors concluded that clinical endometritis was associated with a lower conception rate and lower pregnancy rate at post-partum days 150-250. It is difficult to compare the various published studies in detail because discrepancies can arise simply because of differences in how and when animals were diagnosed with endometritis during the post-partum period.

Our analysis of ovarian activity during post-partum weeks 3-5 indicates a slow resumption of cycling, similar to that reported by others [24]. In that study, 6.7% of cows had a corpus luteum at week 3 after calving, and 70.3% showed abnormal ovarian activity. Just under half of our cows (48.8%) had a corpus luteum at post-partum week 5, less than the ovulation rate of 60% reported for heat-stressed cows at post-partum week 5 [44] and less than the 61.5% of heat-stressed cows that were cycling at post-partum days 36-42 [61]. Our observation that eCG administration did not increase the rate of ovulation contrasts with the results of Rostami *et al.* (2011) [32]. However, the fact that none of the control cows in that study ovulated after the first post-partum wave suggests that their animals may have been under stresses that were absent from our study and that could be mitigated by eCG. For example, a report that eCG administration at 3 days before AI did not increase follicle size, follicle growth rate or serum estradiol concentration suggested that Body Condition Score (BCS) may be a more important factor in fertility after AI [62]. It is possible that the heat stress of the animals in our study was strong enough to cancel out any fertility gains induced by our eCG treatment protocol. The same may also be true of the prolonged interval between calving and first insemination in our study (96 days), which was longer than in some other studies. Further work is needed to verify our negative results with eCG and explore whether manipulating other farm or animal parameters may make early eCG administration effective at improving fertility after AI.

Just as it did not alter post-AI fertility in our study, early post-partum eCG administration did not alter ovarian response to the synchronization protocol. The rates of animals that had a corpus luteum or that ovulated in our study were broadly similar to those reported by Dirandeh *et al.* (2015) [63], who also analyzed heat-stressed cows. We observed 75.6% of cows to have a corpus luteum at the first administration of GnRH, and we observed 79.3% of cows to have a corpus luteum at the administration of prostaglandin; the corresponding rates in the study by Dirandeh *et al.* (2015) [63] were 88.5% and 96.2%. At the same time, we and they reported similarly high percentages of cows without a corpus luteum at the last administration of GnRH (90.9 *vs*. 93.5%), as well as similarly high percentages of ovulated cows after AI (88.4 *vs.* 87.2%).

Pregnancy loss was 11.5% across all cows in the present study. This rate is consistent with the 11.95% reported based on analysis of 24,391 pregnancies [10]. This was not affected by eCG administration in our study.

The average conception rate across all cows in our study (33.5%) is similar to rates reported in other studies from this geographical area [64] (Astiz y Fargas 2013-59), but higher than the rate reported for cows in Iran [63]. One reason for this difference may be differences in DIM at the time of insemination: animals in our study were inseminated on post-partum day 96, 30 days later than the animals in the study by Dirandeh *et al.* (2015) [63]. Indeed, analysis of data in the present study indicated a significant effect of DIM on conception after AI (OR 1.037, 95%CI 1.002 to 1.074, P = 0.040), whereas none of the other milk production-related parameters appeared to influence conception rate. Previous work on high-yielding cows has also suggested that DIM can significantly influence post-AI conception, independently of milk production level [65].

In conclusion, our results do not support the idea that early post-partum administration of eCG to high-yielding dairy cows that calved under heat stress can enhance the conception rate after the first timed AI. Other strategies may prove more effective for mitigating the reduction in post-partum fertility caused by heat stress.

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Conflicts of interest

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Figure caption

Figure 1. Schematic diagram of eCG administration, uterine ultrasound assessments (U), and artificial insemination based on the "short double Cosynch" procedure. eCG, equine chorionic gonadotropin; GnRH, gonadotropin-releasing hormone; PG, prostaglandin; pp, post-partum; TAI, timed artificial insemination.