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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.

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,

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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 1216 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" (Fig. 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%.

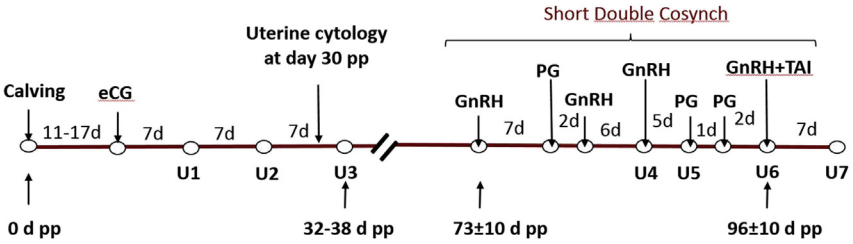

Fig. 1. Schematic diagram of eCG administration, uterine ultrasound assessments (U), and artificial insemination based on the "short double Cosynch" procedure. eCG, equine chorionic gonadotropin; GnRH, gonadotropin-releasing hormone; PG, prostaglandin; pp, post-partum; TAI, timed artificial insemination.