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Equine chorionic gonadotropin increases fertility of grazing dairy cows that receive fixed-time artificial insemination in the early but not later postpartum period

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ABSTRACT

This study evaluated effects of equine chorionic gonadotropin (eCG) on fertility of 679 crossbred (*Bos taurus* x *Bos indicus*) lactating grazing cows synchronized for fixed-time AI (FTAI). At a random day of the estrous cycle cows received an intravaginal progesterone (P4) implant, 2 mg estradiol benzoate (EB) and 100 μg gonadorelin (D0-AM). On D7-AM, cows received 0.5 mg sodium cloprostenol and were randomly assigned into two treatments: eCG (n = 340; 400 IU eCG on D7), or Control (n = 339; no eCG). On D8-PM, P4 implants were removed and cows received 0.5 mg sodium cloprostenol and 1 mg EB. Insemination was performed on D10-AM. Pregnancy was diagnosed 30 and 60 d after AI. Treatment with eCG tended to increase pregnancy per AI (P/AI) compared to Control at 30 (37.8 vs. 30.2%; $P = 0.06$) and 60 (31.9 vs. 25.1%; $P = 0.08$) d. Pregnancy loss and twinning did not differ between groups. Treatment with eCG increased ($P < 0.05$) P/AI at 30 (39.0 vs. 25.2%) and 60 (32.8 vs. 21.3%) d for cows inseminated at ≤ 70 d in milk (DIM) but had no effect in cows receiving AI after 70 DIM. Thus, eCG on D7 of a FTAI protocol increased fertility of crossbred dairy cows inseminated in the early postpartum period.

1. Introduction

Reproductive efficiency is of economic importance for dairy herds worldwide because the lactation cycle is initiated and renewed by calving [1]. For most dairy herds it is crucial that cows become pregnant soon after the voluntary waiting period and this is particularly critical for herds with cattle that have lower persistency of milk production as lactation proceeds. Efficient use of artificial insemination (AI) can be an important tool to improve the genetics and future productivity of a herd as well as improve current reproduction and production of a dairy herd. However, the efficacy of an AI program depends on timely and consistent submission of cows for AI which can be limited by factors such as low efficiency of heat detection and anovular cows [2,3]. In order to minimize these problems, protocols have been developed that synchronize ovarian function, hormonal dynamics, and produce a synchronized ovulation allowing fixed-time AI (FTAI) [3]. Use of FTAI in dairy herds can lead to more pregnant cows at a shorter interval after the end of the voluntary waiting period due to a reduced interval from calving to first service, thereby reducing the calving-conception period [4–7].

Ovulation synchronization programs generally utilize combinations of GnRH and PGF2α [8] or a combination of progesterone (P4) and estradiol (E2) [9]. Nevertheless, the percentage of cows that become pregnant to a single FTAI (P/AI) has frequently been sub-optimal in lactating dairy cows [10–12]. Thus, research that increases fertility during FTAI protocols could provide tools for dairy producers to improve reproductive efficiency of their herds.

In beef cattle, especially in *Bos indicus*, equine chorionic gonadotropin (eCG), a glycoprotein secreted by the endometrial cups of pregnant mares [13], has been used in P4/E2-based FTAI programs [14,15]. Treatment with eCG at the time of P4 implant removal improved preovulatory follicle growth, increased the percentage of cows ovulating at the end of a FTAI protocol, and increased P/AI, especially in cows or heifers with low body condition score (BCS) or in non-cycling cows [16–19]. The benefits of eCG on reproductive function are attributed to the long half-life of eCG and the dual FSH- and LH-like activity of the molecule [13].

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In dairy cattle, few studies have evaluated the use of eCG during FTAI protocols and results have not shown the consistent advantages that have been reported for beef cattle. For example, Souza et al. [11] treated high producing dairy cows with eCG (400 IU) and reported an increase in corpus luteum (CL) volume and circulating P4 concentrations but no overall increase in P/AI due to eCG treatment. However, dairy cows with low BCS had an increase in P/AI in response to eCG in that study. Other studies reported no effect of eCG during a FTAI protocol on follicular and luteal dynamics or on P/AI in high producing Holstein cows [1,12]. Furthermore, there was no interaction between P/AI and other variables such as BCS and parity [1,12]. Nevertheless, two studies from New Zealand using grazing Friesian and Jersey-Friesian-cross dairy cattle reported positive effects of eCG on reproductive performance in anovular-anestrous cows that were synchronized for FTAI with P4/E2 [20] or GnRH-P4-based [21] programs. In one of these studies, the positive effect of eCG was only observed in cows that were early postpartum (<43 days in milk [DIM]) and not in cows that were later postpartum [21]. Thus, treatment with eCG during the final stages of FTAI protocols has been found to increase fertility in certain physiological situations, particularly in cows with lower BCS and earlier postpartum.

Crossbred cows (*Bos taurus* x *Bos indicus*) have been increasingly used for milk production in grazing systems in tropical countries to reduce the negative effects of heat stress and parasites on purebred *Bos taurus* dairy cattle. In this type of management system, the use of eCG during FTAI protocols may improve P/AI and thereby improve reproductive efficiency in these herds. Therefore, the objective of the present study was to evaluate the effect of eCG treatment on day 7 of the protocol on P/AI. Our hypothesis was that administration of 400 IU of eCG during the final stage of growth of the preovulatory follicle in a P4/E2-based FTAI protocol would increase P/AI of crossbred grazing dairy cows.

2. Material and methods

This experiment was conducted in two commercial dairy farms located in Pitangui, MG, Southeast Brazil between October 2013 and July 2014. The climate of Pitangui is considered tropical with an average temperature of 21.8 °C and annual rainfall of 1337 mm. The Animal Research Ethics Committee of College of Agriculture “Luiz de Queiroz” (ESALQ)/University of São Paulo approved all procedures involving cows in this study.

2.1. Cow management

A total of 679 crossbred Holstein x Gyr dairy cows was used, 492 multiparous and 187 primiparous, with an average milk production of 21.4 ± 7.6 kg/d, average DIM of 124.6 ± 97.0 (ranging from 38 to 498), lactation number of 2.46 ± 1.1 (ranging from 1 to 9), and number of AI of 2.1 ± 0.12 (ranging from 1 to 10). Cows had different percentages of crossbreeding ranging from 50% (1/2) Holstein to 96.7% (31/32) Holstein. Percentage of cows used in this experiment for each level of crossbreeding are: 1/2 = 9.0%; 3/4 = 22.0%; 5/8 = 2.6%; 7/8 = 33.0%; 15/16 = 26.8% and 31/32 = 6.6%. Both farms used a grazing system with cows having continuous access to pastures of Mombaça guinea grass (*Panicum maximum* Jacq. cv. ‘Mombaça’) with daily supplementation with a mixture of corn silage and a grain mix containing finely ground corn, soybean meal, citrus pulp, whole cottonseed, minerals, and vitamins. All cows had free access to water and were milked two times daily.

2.2. Experimental design

At a random day of the estrous cycle all cows received an intravaginal P4 implant (CIDR, Zoetis, São Paulo, Brazil), 2 mg estradiol benzoate (EB) i.m. (Gonadiol, MSD, São Paulo, Brazil), and 100 μg gonadorelin i.m. (Fertagyl, MSD, São Paulo, Brazil) in the morning (D0). Seven d later (D7) in the morning, all cows received 0.5 mg sodium cloprostenol i.m. (Sincrocio, OuroFino, Cravinhos, Brazil) and were randomly assigned into two groups: eCG (n = 340) that received 400 IU eCG i.m. (Novormon, MSD, São Paulo, Brazil); and Control (n = 339) that did not receive eCG. On the afternoon of D8, P4 implants were removed and all cows received 150 μg d-cloprostenol i.m. and 1 mg EB i.m. The FTAI was performed at D10 in the morning (Fig. 1) by one of nine technicians with frozen-thawed proven semen. Pregnancy diagnoses were performed at 30 and 60 d after AI by transrectal ultrasound examination (DP-2200 VET, Mindray, Shenzhen, China). Calving data were collected accessing a software for dairy farm management (Ideagri, Belo Horizonte, Brazil), including twinning. Cows that died or were sold after the 60 d diagnoses were not used in future analyses.

2.3. Statistical analysis

Categorical data were analyzed by logistic regression using the GLIMMIX procedure of SAS version 9.4 (SAS/STAT, SAS Institute Inc., Cary, USA) fitting a binary distribution. The models included the fixed effects of treatment, parity, sire, technician, number of AI (first AI vs. resynchronized AI), categorized milk yield within parity in the month of AI as above or below the mean value, categorized DIM as above or below 70 d, the interactions between treatment and parity, treatment and number of AI, and the random effect of cow. The Kenward-Roger method was used to calculate the denominator degrees of freedom to approximate the F tests in the mixed models. Model fitting was evaluated using the fit statistics.

Milk yield, DIM, number of AI, and number of lactation were analyzed using the GLIMMIX procedure of SAS with models fitting a Gaussian distribution. Data were tested for normality of residuals. The model included the fixed effects of treatment, parity, and interaction between treatment and parity, and the random effects of cows. The Kenward-Roger method was used to calculate the denominator degrees of freedom to approximate the F tests in the mixed models.

In all analyses, only variables with P < 0.20 were kept in the final model, unless the variable was essential. Differences were

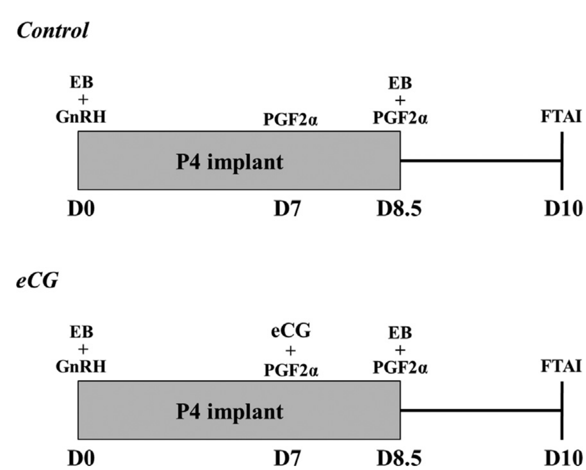

Fig. 1. Experimental design to evaluate the effect of 400 IU eCG on Day 7 of the protocol on pregnancy per AI of crossbred dairy cows submitted to FTAI. D0 = 2 mg estradiol benzoate (EB), 100 μg gonadotropin-releasing hormone (GnRH) and progesterone implant (P4); D7 = 0.5 mg prostaglandin F2α (PGF2α) and 400 IU equine chorionic gonadotropin (eCG); D8.5 = 1 mg EB and 0.5 mg PGF2α; D10 = fixed-time artificial insemination (FTAI).