

Effects of equine chorionic gonadotropin on follicle development and pregnancy rates in suckled beef cows with or without calf removal¹

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ABSTRACT: Two experiments were conducted to evaluate the effects of eCG, temporary 72-h calf removal (CR), or both on dominant follicle (DF) diameter and pregnancy rates (PR) in suckled beef cows. For Exp. 1, we hypothesized that CR, eCG, or both at PGF_{2α} administration concomitant with synchronization of ovulation protocol would increase DF diameter and alter patterns of LH, estradiol (E), and progesterone (P4) secretion. Thirty-five multiparous, suckled crossbred beef cows were assigned randomly to a 2 × 2 factorial arrangement of 4 treatments: 1) cows received 100 µg GnRH and a controlled internal drug release (CIDR) insert containing 1.38 g of P4 (d -7) followed in 7 d by 25 mg PGF_{2α} and CIDR removal (d 0) followed in 72 h by GnRH and fixed-time AI (d 3; Control; *n* = 9); 2) similar to Control, but calves were removed from their dams for 72 h between d 0 and 3 (COCR; *n* = 9); 3) similar to Control, but cows received 400 IU eCG on d 0 (COeCG; *n* = 9); and 4) similar to COCR, but cows received 400 IU eCG on d 0 (eCGCR; *n* = 8). Blood sample collection and ovary scans were performed on d -14, -7, 0, 1, 2, 3, 4, and 10. Pregnancy rate, ovulation response by d 4, and peak concentrations of LH before 72 h after PGF_{2α} were greater

(*P* < 0.05) for cows exposed to CR (COCR and eCGCR) than for cows not exposed to CR (Control and COeCG). Follicle diameter on d 3 was greater (*P* = 0.02) for cows receiving eCG (COeCG and COeCG; 14.9 ± 0.5 mm) than for cows receiving no eCG (Control and COCR; 13.1 ± 0.5 mm). Concentrations of E were greater (*P* < 0.05) at 32 h for COCR (8.2 ± 1.0 pg/mL) and eCGCR (8.5 ± 0.9 pg/mL) than in Control (4.9 ± 1.2 pg/mL) and COeCG (4.6 ± 1.1 pg/mL) and at 44 h after PGF_{2α} for eCGCR (11.7 ± 1.6 pg/mL) compared with Control (6.9 ± 1.7 pg/mL), COCR (7.1 ± 1.5 pg/mL), and COeCG (7.5 ± 1.7 pg/mL). In Exp. 2, we determined whether administration of 200 IU eCG improved PR in suckled beef cows. The Control (*n* = 261) and COeCG (*n* = 252) treatments were similar to those previously described in Exp. 1; however, the interval from PGF_{2α} to fixed-time AI was 66 h and 200 IU of eCG were administered to the COeCG group. Pregnancy rates did not differ (*P* > 0.10) between COeCG (43%) and Control (50%). We conclude that eCG increased DF diameter and CR resulted in a greater percentage of cows experiencing LH peak before 72 h after PGF_{2α} and ovulation response; however, eCG failed to improve PR to timed AI.

Key words: artificial insemination, calf removal, equine chorionic gonadotropin, estrous synchronization

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INTRODUCTION

Equine chorionic gonadotropin is synthesized by endometrial cups, which originate from the trophoblastic epithelial cells during embryo development in horses (Allen and Moor, 1972). Synthesis of eCG begins approximately on d 40 and is maintained through d 120 of gestation. Equine chorionic gonadotropin has LH-like actions in the

equids and both LH and FSH-like actions in others species, including the bovine (Soumano and Price, 1997). The eCG glycoprotein is able to bind to FSH and LH receptors located within the granulosa and theca cells of the ovary (Murphy and Martinuk, 1991). For that reason, eCG has been used in combination with estrous synchronization and superovulation protocols in an attempt to improve follicle development and pregnancy rates (**PR**).

Treating cows with eCG in the absence of a dominant follicle (**DF**) promoted the growth of several follicles concomitantly and eventually resulted in multiple ovulations (Duffy et al., 2004). Greater DF growth rate was observed during the first 2 d after eCG treatment, resulting in an increased DF diameter at ovulation (Sá Filho et al., 2010). Cows in poor body condition and lacking normal estrous cycles (acyclic) seem to have an exaggerated response to an eCG treatment compared with cyclic cows in good body condition (Roche et al., 1992; Baruselli et al., 2004). This phenomenon may be a result of reduced concentrations of GnRH from the hypothalamus, reducing the release of LH from the pituitary, under those conditions (Yavas and Walton, 2000). Stimulation of follicle growth by eCG treatment increased the size of the DF at ovulation (Sá Filho et al., 2010) and increased concentrations of progesterone (**P4**) after fixed-time AI (Baruselli et al., 2004), thereby resulting in the subsequent formation of a larger corpus luteum (**CL**) postovulation. A larger CL synthesizes more P4 than a smaller CL (Vasconcelos et al., 1999, 2001); therefore, CL quality may be a viable reason for improved PR at fixed-time AI. In contrast, not all studies in which cows were treated with eCG have resulted in increased PR (Pinheiro et al., 2009); therefore, it is not known whether effects of eCG on follicle and CL development may improve pregnancy outcomes.

Calf removal (**CR**) also may stimulate GnRH release. It has been shown that suckling results in reduced LH secretion from the anterior pituitary gland (Radford et al., 1978; Dunlap et al., 1981). Suckling increased endogenous opioid concentrations in hypophyseal portal blood, inhibiting GnRH secretion and consequently pituitary FSH and LH release (Gordon et al., 1987). When used in combination with a fixed-time AI protocol, temporary CR has been reported to be an inexpensive and effective manipulation to improve response to GnRH treatment, increase DF diameter before fixed-time AI, and improve conception rates in suckled beef cows (Meneghetti et al., 2001; Baruselli et al., 2004; Duffy et al., 2004; Pinheiro et al., 2009; Sá Filho et al., 2009, 2010; Small et al., 2009).

In 2 experiments we hypothesized that eCG and CR would increase DF diameter, estradiol (**E**) concentrations,

and LH secretion, thereby increasing incidence of ovulation. The combination of both treatments (eCG plus CR) would further stimulate E and LH secretion, resulting in an additive effect for increased incidence of ovulation and improved synchrony of ovulation plus enhanced luteal volume and increased secretion of P4 by the subsequently formed CL, and that eCG treatment would improve PR in suckled beef cows.

MATERIALS AND METHODS

Experiment 1 was conducted at the North Florida Research and Education Center in Marianna, FL, in April 2009. Experiment 2 was conducted at 3 locations in Kansas, between April and June of 2009. Both experiments were performed in compliance with the University of Florida and Kansas State University Institutional Animal Care and Use Committee guidelines.

Animals and Treatments

In Exp. 1, 35 multiparous suckled crossbred beef cows with a mean BCS of 4.8 ± 0.7 (range 4 to 7) and 34 ± 7.3 d postpartum (range 19 to 40 d) were assigned randomly to a 2×2 factorial arrangement of 4 treatments: 1) cows received the 7-d Co-Synch + controlled internal drug release (**CIDR**) protocol (Larson et al., 2006), which included 100 μ g of GnRH intramuscularly (d -7; 2 mL of Cystorelin; Merial, Duluth, GA) and a CIDR (EAZI-Breed CIDR containing 1.38 g of P4; Pfizer Animal Health, New York, NY) insert followed in 7 d by 25 mg PG (d 0; 5 mL of Lutalyse; Pfizer Animal Health) and the CIDR was removed followed in 72 h (d 3) by fixed-time AI and a second 100 μ g injection of GnRH (**Control**; $n = 9$); 2) similar to Control, but calves were removed from their dams for 72 h between d 0 and 3 (**COCR**; $n = 9$); 3) similar to Control, but cows received 400 IU of eCG (Pregnenol; Bioniche Animal Health, Atlanta, GA) on d 0 (**COeCG**; $n = 9$); and 4) similar to COCR, but cows received 400 IU of eCG on d 0 (**eCGCR**; $n = 8$). During CR, calves were housed in an open-faced barn with ad libitum access to perennial peanut hay and water and were located no closer than 100 m from their dams (Fig. 1).

In Exp. 2, 513 suckled beef cows (purebred and crossbred Angus, Simmental, and Hereford), at 3 different locations (Location 1: 8 AI technicians, 33 AI sires, cow parity ranging from 1 to 9; Location 2: 4 AI technicians, 1 AI sire, cow parity ranging from 1 to 9; Location 3: 2 AI technicians, 6 AI sires, cow parity ranging from 1 to 12) were enrolled in a 7-d Co-Synch + CIDR protocol. Cows were assigned randomly to a nontreated Control ($n = 261$) or to receive eCG (COeCG; $n = 252$) at the time of PGF_{2 α} injection and CIDR removal (d 0); however,

the interval from $\text{PGF}_{2\alpha}$ to fixed-time AI was 66 h and 200 IU of eCG were administered to the COeCG group (Fig. 1). Body condition scores averaged 5.7 ± 0.71 (range 3 to 7) and cows were $71.9 \pm 16\text{d}$ postpartum (range 17 to 99) at the time of AI.

Ultrasound Scanning

Ovaries of all cows in Exp. 1 were examined on d -14, -7, 0, 1, 2, 3, 4, and 10 by transrectal ultrasonography (5.0-MHz linear array transducer, Aloka SSD-500; Aloka Co. Ltd, Wallingford, CT) and all structures were mapped to monitor changes in CL volume and follicular diameter. The vertical and horizontal diameters of the largest follicle on each ovary and all CL present were measured and recorded. Volume of CL tissue was calculated using the formula $V = 4/3\pi r^3$ in which $r = 1/2$ of the estimated diameter of the CL (average of vertical and horizontal diameter). If a CL had a fluid-filled cavity, the volume of the cavity was subtracted from the total volume of the CL. Pregnancy diagnosis in Exp. 1 was performed 33 d after fixed-time AI and in Exp. 2 was performed between 30 and 35 d and a second diagnosis between 60 and 65 d after fixed-time AI.

Blood Sample Collection

Blood samples were collected via coccygeal vessel puncture using 10 mL Vacutainer tubes with no additive, silicone-coated/ K_3EDTA interior (Becton & Dickinson Vacutainer Systems; Rutherford, NJ) on d -14, -7, every 4 h between d 0 and 3, every 2 h between d 3 and 4, and once on d 10 in Exp. 1. In Exp. 2, blood samples were collected on d -14, -7, 0, 3, and at both pregnancy diagnoses. Blood was refrigerated 24 to 30 h after collection and centrifuged for 20 min at $1,500 \times g$ at 4°C . After centrifugation a pipette was used to siphon plasma

into polypropylene vials (12 by 75 mm; Fisherbrand; Thermo Fisher Scientific Inc., Waltham, MA), which were stored at -20°C until analyses.

Analyses of Hormones

Concentrations of plasma P4 were analyzed by ELISA. The ELISA procedure was adopted from that previously described by Rasmussen et al. (1996). Quality controls were established using 100 μL plasma with a known P4 concentration of 2.5 ng/mL. Standards were determined with 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, and 20.0 ng/mL concentrations with a duplicate of each respective standard. Pooled samples revealed that the intra- and interassay CV were 5.4 and 12.7% for 14 plates, respectively.

Plasma concentrations of LH were analyzed by RIA, using methodology described previously by Perry and Perry (2008). Inter- and intra-assay CV were 5.3 and 8.0%.

A commercial RIA kit (Estradiol Double Antibody; Siemens Healthcare Diagnostics, Los Angeles, CA) previously validated for use in bovine (Siddiqui et al., 2009) was used to analyze plasma concentrations of estradiol. The intra- and interassay CV was 10 and 17%, respectively. Samples were analyzed in duplicate and reassayed when the CV between duplicates was >0.20 . Plasma from a cow exhibiting estrus and charcoal-stripped plasma from a male calf were used as positive and negative quality controls, respectively.

In Exp. 2, blood samples were assayed for P4 using a solid-phase, no-extraction RIA (Coat-a-Count Progesterone; Diagnostic Products Corporation, Los Angeles, CA; Stevenson, 2011). The intra- and interassay CV for 13 assays were 8.2 and 8.6%, respectively. Blood collected on d -14 and -7 was used to verify the functional presence of a CL (when concentrations of P4 exceeded 1 ng/mL) at the onset of treatments. If any 1 of the first 2 samples contained plasma P4 >1 ng/mL (typical of cows in the luteal phase of the estrous cycle), cows were assumed to be cycling before the onset of treatments (d -7). If concentrations in the first 2 samples were <1 ng/mL, cows were considered to be noncycling.

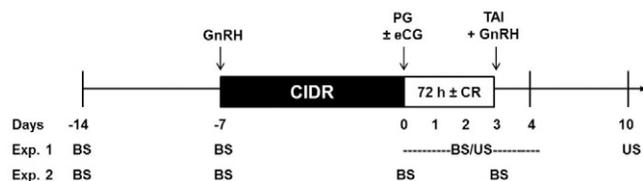


Figure 1. Experimental protocol: Cows received a 7-d CO-Synch + controlled internal device release (CIDR) protocol [100 μg of GnRH (d -7) and a CIDR insert followed in 7 d by 25 mg $\text{PGF}_{2\alpha}$ (PG; d 0) and CIDR removal followed in 72 h (d 3) by fixed-time AI (TAI) and an injection of GnRH]. In Exp. 1 cows were assigned to 1 of 4 treatments: 1) Control, 2) Control plus calf removal (CR) between d 0 and 3 (COCR), 3) Control plus an injection of 400 IU of eCG on d 0 (COeCG), or 4) Control plus an injection of 400 IU of eCG on d 0 and CR between d 0 and 3 (eCGCR). In Exp. 2 cows received the Control and COeCG treatments; however, AI was performed at 66 h after $\text{PGF}_{2\alpha}$ and 200 IU of eCG were administered on d 0. In Exp. 1 blood samples (BS) were collected on d -14, -7, every 4 h between d 0 and 3, every 2 h between d 3 and 4, and once on d 10. In Exp. 2, BS were collected on d -14, -7, 0, and 3. US = Ultrasound.

Statistical Analyses

Experiment 1 was designed as a 2×2 factorial arrangement with CR and eCG as main effects. Cow was considered to be the experimental unit. Continuous data (follicle diameter and concentration of P4, E, and LH) were analyzed by ANOVA for repeated measures using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC), in which day was the repeated factor and

cow was the subject. Categorical data (PR, proportion of ovulation, and proportion of cows with a detectable LH peak before 72 h after PGF_{2α}) were analyzed using the PROC GLIMMIX procedure of SAS, specified as binary distribution. The model included main effects for CR and eCG and their respective interactions. Follicle diameter and concentrations of hormones on d 0 were used as a covariate in their respective analyses of subsequent measurements in time. Within cow, the time of peak of LH was calculated to be the interval between basal concentrations of LH (the mean of 8 samples from 0 to 48 h after PGF_{2α}) and when the concentration of LH exceeded the basal concentrations by fivefold followed by a significant decrease in concentration before 72 h after PGF_{2α}.

Pearson correlations were calculated among variables using the CORR procedure of SAS to determine correlations between ovulation to first GnRH, ovulation before second GnRH, LH peak before second GnRH, and incidence of pregnancy.

Experiment 2 was designed as a completely randomized block design in which cow was the experimental unit. The LOGISTIC procedure of SAS was used to analyze categorical data (PR, pretreatment cycling status, rate of luteolysis, and rate of pregnancy loss). A backward stepwise regression model was used, and variables were sequentially removed from the model by the Wald statistic criterion when $P > 0.20$. The model for PR included treatment, technician, cycling status, location, parity, sire, and the respective interactions

as explanatory variables. The GLM procedure of SAS was used to analyze concentrations of P4 at the first and second pregnancy diagnosis. The model included treatment, cycling status, location, and parity as independent variables.

Pregnancy rate, incidence of ovulation, and proportion of cows having a detectable LH peak before 72 h after PGF_{2α} are reported as actual proportions. In contrast, all remaining values are reported as least squares mean \pm SE. Significance was established at $P < 0.05$ and a tendency was considered when $0.05 < P \leq 0.10$.

RESULTS

Experiment 1

There was no effect ($P = 0.62$) of treatments on ovulation rate between d 3 and 4, percentage of cows experiencing LH surge within 72 h after PGF_{2α} ($P = 0.57$), percentage of ovulation between d 3 and 10 ($P = 0.60$), and overall PR ($P = 0.25$; Table 1). The analysis of the main effects of CR and eCG indicated no difference ($P = 0.85$) on the percentage of cows expressing an LH surge before the second GnRH between cows receiving or not receiving the eCG treatment. In contrast, a greater ($P = 0.02$) percentage of cows exposed to CR had experienced an LH peak before the second GnRH than cows not exposed to CR (Table 1).

Table 1. Incidences of ovulation, percentage of cows experiencing LH peak, overall pregnancy rates (PR), and concentrations of progesterone (P4) in Exp. 1

Item	Treatment ¹				Main effects ²			
	Control	COCR	COeCG	eCGCR	No CR	CR	No eCG	eCG
	no./no. (%)				no./no. (%)			
Ovulation to first GnRH ³	4/9 (44)	3/9 (33)	3/9 (33)	2/8 (25)	7/18 (38)	5/17 (29)	7/18 (38)	5/17 (29)
Ovulation between d 3 and 4	1/9 (11)	6/9 (66)	3/9 (33)	5/8 (62)	5/18 (27) ^x	11/17 (64) ^y	8/18 (44)	8/17 (47)
LH peak < 72 h ⁴	1/9 (11)	5/9 (55)	2/9 (22)	4/8 (50)	3/18 (16) ^x	9/17 (52) ^y	6/18 (33)	6/17 (36)
Ovulation by d 10 ⁵	6/9 (67)	9/9 (100)	7/9 (78)	8/8 (100)	13/18 (72) ^x	17/17 (100) ^y	15/18 (83)	15/17 (88)
Overall PR ⁶	0/9 (0)	4/9 (44)	2/8 (25)	3/8 (37)	2/18 (12) ^x	7/17 (41) ^y	4/18 (22)	5/17 (29)
	ng/mL				ng/mL			
P4 on d 10 ⁷	1.4 \pm 0.4	2.7 \pm 0.4	2.7 \pm 0.4	2.8 \pm 0.4	2.1 \pm 0.3 ^w	2.8 \pm 0.3 ^z	2.1 \pm 0.3 ^w	2.8 \pm 0.3 ^z

^{x,y}Means within a row differ ($P < 0.05$).

^{w,z}Means within a row tend to differ ($0.05 < P < 0.10$).

¹Cows were assigned on d 0 to these treatments: Control = cows received 100 μ g GnRH and a controlled internal drug release (CIDR) insert containing 1.38 g of P4 (d -7) followed in 7 d by 25 mg PG and CIDR removal (d 0) followed in 72 h by GnRH and fixed-time AI (d 3); COCR = similar to control, but calves were removed from their dams for 72 h between d 0 and 3; COeCG = similar to control, but cows received 400 IU eCG on d 0; and eCGCR = similar to COCR, but cows received 400 IU eCG on d 0.

²Main effects of calf removal (CR; COCR and COeCG) or no CR (Control and COeCG treatments) and eCG (COeCG and eCGCR) or no eCG (Control and COCR treatments).

³Cows that experienced ovulation to the first GnRH injection assessed on d 0 by ultrasonography.

⁴Cows experiencing peak of LH within 72 h after prostaglandin injection.

⁵Percentage of cows that ovulated between d 3 and 10 assessed on d 10.

⁶PR = pregnancy rate to AI, determined on d 33 after administration of PGF_{2α}.

⁷Concentrations of P4 10 d after administration of PGF_{2α}.

Cows exposed to CR had greater ($P = 0.02$) proportion of ovulations between d 3 and 4 compared with cows not exposed to CR; however, cows treated with eCG had similar ($P = 0.82$) proportion of ovulations between d 3 and 4 compared with cows not treated with eCG (Table 1).

A greater ($P = 0.01$) percentage of cows exposed to CR had ovulated by d 10 compared with cows not exposed to CR. In contrast, cows receiving eCG or not receiving eCG had similar ($P = 0.60$) proportion of ovulation by d 10 (Table 1).

Cows that had a LH peak before the second GnRH had greater ($P < 0.0001$) PR than cows with a LH peak induced by GnRH. In addition, PR were greater ($P = 0.04$) for cows exposed to CR compared with cows not exposed to CR. Cows treated with eCG had similar ($P = 0.51$) PR than cows not treated with eCG (Table 1).

There was a negative correlation ($r = -0.42$, $P = 0.01$) between ovulation to the first GnRH and ovulation to the second GnRH. As expected, there was also a positive correlation ($r = 0.78$, $P < 0.001$) between LH peak before the second GnRH and ovulation rate between d 3 and 4 (12 of 16; 75% vs. 0 of 19; 0% for ovulation between d 3 and 4 for cows experiencing LH peak before or after second GnRH injection, respectively).

There was no effect ($P = 0.99$) of treatments on follicle diameter. In addition, follicle diameter on d 3 was not affected ($P = 0.26$) by the main effect of CR and was similar for cows exposed (14.2 ± 0.5 mm) or not exposed (13.5 ± 0.5 mm) to CR (Fig. 2). In contrast, follicle diameter on d 3 was greater ($P = 0.04$) for cows exposed to eCG (14.7 ± 0.6 mm) compared with cows

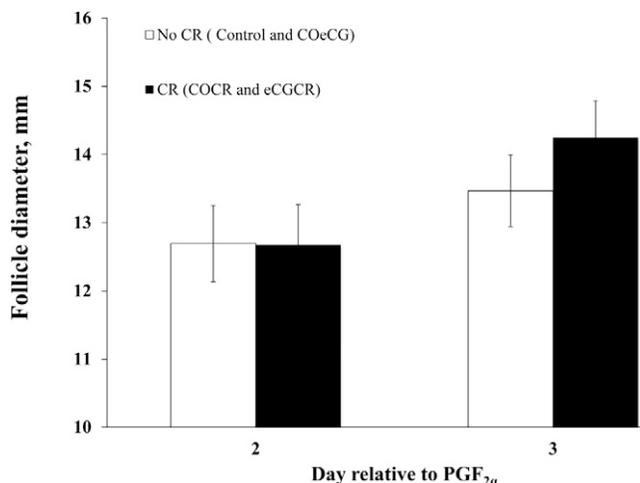


Figure 2. Experiment 1: Diameter of the largest follicle present on the ovary associated with cows receiving calf removal (CR) or no CR on d 0. Control = cows received 100 µg GnRH and a controlled internal drug release (CIDR) insert containing 1.38 g of P4 (d -7) followed in 7 d by 25 mg PGF_{2α} and CIDR removal (d 0) followed in 72 h by GnRH and fixed-time AI (d 3); COCR = similar to control, but calves were removed from their dams for 72 h between d 0 and 3; COeCG = similar to control, but cows received 400 IU eCG on d 0; and eCGCR = similar to COCR, but cows received 400 IU eCG on d 0.

not exposed to eCG (13.0 ± 0.5 mm; Fig. 3). Follicle growth rate on cows exposed to CR (0.77 ± 0.27 mm/d) were similar ($P = 0.14$) than cows not exposed to CR (0.23 ± 0.26 mm/d). In contrast, follicle growth rate was greater ($P = 0.02$) on cows exposed to eCG (0.93 ± 0.24 mm/d) compared with cows not exposed to eCG (0.07 ± 0.24 mm/d).

The releasing patterns and concentrations of hormones were affected by treatments. There was a significant interaction ($P = 0.04$) between CR and eCG on concentrations of E. The COCR (8.2 ± 1.0 pg/mL) and eCGCR (8.5 ± 0.9 pg/mL) treatments had greater ($P = 0.03$) concentrations of E at 32 h after PGF_{2α} than Control (4.9 ± 1.2 ng/mL) and COeCG (4.6 ± 1.1 ng/mL). In addition, eCGCR (11.7 ± 1.6 pg/mL) had greater ($P = 0.03$) concentrations of E at 44 h after PGF_{2α} compared with Control (6.9 ± 1.7 pg/mL), COCR (7.1 ± 1.5 pg/mL), and COeCG (7.5 ± 1.7 pg/mL) treatments (Fig. 4).

Concentrations of P4 on d 10 tended ($P = 0.08$) to be greater for cows exposed to CR compared with cows not exposed to CR and for cows exposed to eCG compared with cows not exposed to eCG (Table 1).

Experiment 2

Pregnancy rates at d 30 to 35 after fixed-time AI did not differ ($P > 0.10$) between Control and COeCG (Table 2). There was no effect ($P > 0.10$) of cycling status or the interaction treatment × cycling status on pregnancy rates (Table 2). Pregnancy rates at d 30 to 35

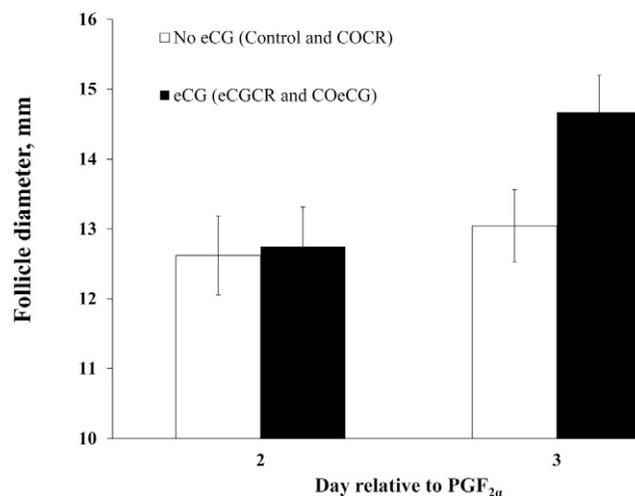


Figure 3. Experiment 1: Diameter of the largest follicle present on the ovary associated with cows receiving 400 IU of eCG or Control treatments on d 0. *Treatment with eCG differs from Control on d 3 ($P = 0.03$). Control = cows received 100 µg GnRH and a controlled internal drug release (CIDR) insert containing 1.38 g of P4 (d -7) followed in 7 d by 25 mg PGF_{2α} and CIDR removal (d 0) followed in 72 h by GnRH and fixed-time AI (d 3); COCR = similar to control, but calves were removed from their dams for 72 h between d 0 and 3; COeCG = similar to control, but cows received 400 IU eCG on d 0; and eCGCR = similar to COCR, but cows received 400 IU eCG on d 0.

after fixed-time AI differed ($P < 0.05$) by location and ranged from 34% (Location 2) to 59% (Location 3).

Concentrations of P4 were similar on d 30 to 35 of pregnancy and on d 60 to 65 after fixed-time AI for COeCG and Control groups, respectively (Table 2).

Pretreatment cycling status differed ($P < 0.01$) among locations (Location 1 = 76.5%, Location 2 = 54.3%, and Location 3 = 27.4% cycling; Table 3). For cows with increased (>1 ng/mL) P4 at CIDR insert removal, 97.4% experienced luteolysis by fixed-time AI, and 17.3% of cows had low (<1 ng/mL) P4 at CIDR removal and at fixed-time AI, and 1.2% with increasing P4 from insert removal to fixed-time AI.

Herd, cycling status, technician, and treatment influenced PR. Cycling cows were 1.5 times more likely [$P = 0.046$; 95% confidence interval (CI) = 1.01 to 2.27] to conceive compared with noncycling cows. Control cows were 1.5 times more ($P = 0.036$; CI = 1.03 to 2.13) likely to conceive than those treated with eCG. Cows at location 3 were 1.8 to 3.5 times more ($P = 0.004$; CI = 1.1 to 5.6) likely to conceive than cows at other locations. Pregnancy loss to d 67 did not differ between treatments (3.7 vs. 2.3% for eCG vs. Control, respectively).

DISCUSSION

Numerous reports have indicated that CR before fixed-time AI improved fertility in beef cows (Smith et

al., 1979; Kiser et al., 1980; Yelich et al., 1995; Geary et al., 2001b; Meneghetti et al., 2001; Pinheiro et al., 2009; Sá Filho et al., 2009). Endorphins are known to reduce GnRH neuronal activity thus decreasing the secretion of GnRH into the portal vessels that impact anterior pituitary function (Cox and Britt, 1982; Malven and Hudgens, 1987; Myers et al., 1989; Rund et al., 1989). Calf removal and the temporary cessation of the suckling stimulation reduce the release of endorphins into the hypothalamus, and consequently, GnRH pulses are increased (Edwards, 1985; Shively and Williams, 1989), resulting in increased LH pulse frequency. Increasing LH pulses stimulate DF growth, ovulation, and luteinization (Schallenberger et al., 1984; Walters et al., 1984), thereby increasing the opportunity for conception to occur.

Shively and Williams (1989) demonstrated that pulses of LH increase gradually from the time of CR to 48 h and remain elevated for as long as 144 h if CR is maintained. Calf removal before fixed-time AI in estrus- or ovulation-synchronization protocols improved conception in most (Smith et al., 1979; Kiser et al., 1980; Yelich et al., 1995; Geary et al., 2001b; Peres et al., 2009; Sá Filho et al., 2009) but not all reports (Geary et al., 2001a; Pinheiro et al., 2009). In Exp. 1, CR increased PR (41 vs. 12% for CR and no CR, respectively), perhaps because a greater percentage of cows exposed to CR had an LH surge before fixed-time AI (52 vs. 16%) and ovulation by 24 h after fixed-time

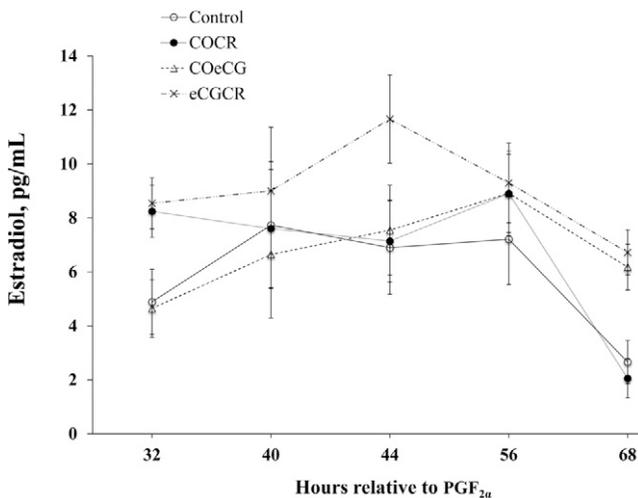


Figure 4. Experiment 1: Diameter of the largest follicle present on the ovary associated with Control, COCR, COeCG, and eCGCR treatments initiated at h 0. Least square means were adjusted based on h 28 used as covariate. *The COCR and eCGCR treatments differ from Control and COeCG ($P = 0.03$). **The eCGCR treatment differs from Control, COCR, and COeCG ($P = 0.03$). Control = cows received 100 μ g GnRH and a controlled internal drug release (CIDR) insert containing 1.38 g of P4 (d -7) followed in 7 d by 25 mg PGF_{2 α} and CIDR removal (d 0) followed in 72 h by GnRH and fixed-time AI (d 3); COCR = similar to control, but calves were removed from their dams for 72 h between d 0 and 3; COeCG = similar to control, but cows received 400 IU eCG on d 0; and eCGCR = similar to COCR, but cows received 400 IU eCG on d 0.

Table 2. Pregnancy rate at d 35 by location and cycling status and concentration of progesterone (P4) in pregnant cows at d 35 and d 67 in Exp. 2

Item	Treatment ¹	
	Control	COeCG
Location pregnancy rates ²	no./no. (%)	
Location 1	36/75 (48)	35/76 (46)
Location 2	33/96 (34)	28/88 (31)
Location 3	56/91 (65)	45/89 (50)
Total	130/262 (50)	108/253 (43)
Cycling status PR ³		
Anestrus	63/125 (50)	50/117 (43)
Cyclic	67/136 (49)	58/135 (43)
P4 ⁴	ng/mL (n)	
d 35	6.4 \pm 0.3 (126)	6.0 \pm 0.4 (100)
d 67	6.4 \pm 0.3 (123)	6.6 \pm 0.3 (97)

¹Cows were assigned to receive eCG (COeCR) or no eCG (Control) at the day of PGF_{2 α} . Control = cows received 100 μ g GnRH and a controlled internal drug release (CIDR) insert containing 1.38 g of P4 (d -7) followed in 7 d by 25 mg PG and CIDR removal (d 0) followed in 72 h by GnRH and fixed-time AI (d 3); COCR = similar to control, but calves were removed from their dams for 72 h between d 0 and 3; COeCG = similar to control, but cows received 400 IU eCG on d 0; and eCGCR = similar to COCR, but cows received 400 IU eCG on d 0.

²Pregnancy rate differs ($P < 0.05$) by location.

³PR = pregnancy rates.

⁴Concentrations of P4.

AI (64 vs. 27%). Every cow (12 of 35 cows) that had an LH surge by 72 h after PGF_{2α}, ovulated between 72 and 96 h after PGF_{2α}, and 62% of those cows conceived. It has been reported that fertilization was improved when the time of ovulation and AI were synchronized (Waberski et al., 1994; Roelofs et al., 2006). In addition, embryo quality was improved when AI was performed 24 to 12 h before ovulation in dairy cattle (Roelofs et al., 2006). Meneghetti et al. (2001) reported an increase in the diameter of the DF and incidence of ovulation when CR was performed before fixed-time AI. Our data indicate that follicle diameter on d 3 did not differ between cows either exposed or not exposed to CR. However, in cows where follicles had not ovulated by d 4, follicles of cows receiving CR had a greater diameter than those not exposed to CR (data not shown).

Equine chorionic gonadotropin, which binds to FSH and LH receptors of follicular cells to stimulate follicular growth (Soumano and Price, 1997), has been used as a complementary hormone to improve fertility in beef cows; however, reported results have been inconsistent. The inconsistent data on improvement of fertility by eCG application may be attributed to several factors, such as malnutrition, estrous cyclic status, and stage of the estrous cycle when the treatment was applied. Each of these conditions has been reported to result in different fertility outcomes. Cows that were nutritionally stressed or in low BCS had greater PR when treated with eCG, but eCG did not improve PR in cows with good BCS (Bó et al., 2003; Souza et al., 2009).

Previous reports indicated that follicle growth rate and PR was improved in cows receiving eCG and the effect was more pronounced in anestrus cows and cows in low BCS (Baruselli et al., 2004; Sá Filho et al., 2010; Sales et al., 2011). In addition, the treatment with eCG may stimulate more than 1 ovulation when cows are treated in the absence of a DF (Duffy et al., 2004). Moreover, the treatment with eCG increased concentrations of P4

after fixed-time AI, despite similar diameter of the pre-ovulatory follicle (Baruselli et al., 2004). In Exp. 1, eCG increased growth rate and the diameter of the DF but did not increase the proportion of cows that ovulated assessed on d 10 compared with cows that did not receive eCG. Concentrations of P4 7 d after fixed-time AI tended to be greater when eCG was administered, which concurs with results reported in dairy cattle (Souza et al., 2009). Our data concur with the report from Sales et al. (2011) that indicated that eCG increased follicle growth rate and diameter of the DF in *Bos indicus* beef cattle, but our data demonstrated no improvements in conception rates as were noted in *Bos indicus* cattle. In contrast, Pegorer et al. (2011) reported no improvements in follicle diameter, proportion of ovulation, or PR in *Bos indicus* beef heifers treated with eCG 2 d before fixed-time AI. However, Bó et al. (2003) reported that eCG administered concomitant with estradiol benzoate resulted in an increase in PR compared with cows only receiving eCG or estradiol benzoate and indicated that the increase in PR was more pronounced in anestrus cows. In Exp. 1, an additive effect of eCG and CR occurred for an increase in concentrations of E, but not in follicle diameter, percentage of cows with peak of LH before second GnRH, proportion of ovulation, or PR. In Exp. 2, the overall PR was not improved in suckled beef cows treated with 200 IU of eCG at the time of PG injection. Treatment with eCG in cows with BCS ≤ 4 seemed to have a positive impact on PR (35 vs. 47% for Control and eCG, respectively; data not shown) but an insufficient number of cows with low BCS precluded the possibility to detect a difference. Failure of eCG to improve PR could have occurred because an inadequate dose (200 IU) was applied in Exp. 2. Previous reports indicate that 300 (Pegorer et al., 2011; Sales et al., 2011) or 400 IU (Baruselli et al., 2004; Sá Filho et al., 2009, 2010; Small et al., 2009; Souza et al., 2009) of eCG were effective in enhancing PR in suckled beef cows.

Table 3. Ovarian function and pregnancy outcomes by location in Exp. 2

Location	n	Pattern of progesterone concentrations ¹						
		Pretreatment cycling status ²	Corpus luteum (CL) lysis ³	Premature CL lysis ⁴	Anestrus ⁵	Metestrus ⁶	Pregnancy rate per AI ⁷	Pregnancy loss ⁸
1	183	76.5 ^y	91.2	5.0	3.3 ^y	0.6	33.9 ^y	6.5 ^x
2	151	54.3 ^y	88.5	0.7	3.4 ^y	0.0	47.0 ^y	4.2 ^x
3	179	27.4 ^x	62.3 ^x	6.3 ^x	28.6 ^x	1.7	58.7 ^x	0.0 ^y

^{x,y}Means within columns with different superscript differ ($P \leq 0.05$).

¹Proportion of cows having high (≥ 1 ng/mL) or low (< 1 ng/mL) progesterone on d -14, -7, 0, or 3.

²Proportion of cows cycling before placement of controlled internal drug release insert on d -7.

³Proportion of cows having high-low concentrations of progesterone on d 0 and 3, respectively.

⁴Proportion of cows having low concentrations of progesterone on d 0 and 3.

⁵Proportion of cows having low progesterone on d -14, -7, 0, and 3.

⁶Proportion of cows having low-high concentrations of progesterone on d 0 and 3, respectively.

⁷Detected on d 35 posttimed AI.

⁸Between d 35 and 67 of pregnancy.

In addition, Bó et al. (2003) and Souza et al. (2009) reported that eCG improved PR in cows with poor BCS and short postpartum intervals; however, cows in Exp. 2 were in good BCS (mean = 5.7 ± 0.7) with relatively long postpartum intervals (mean = 71 ± 16 d) at fixed-time AI.

Surprisingly, in Exp. 1, we noted a negative correlation between ovulation to the first GnRH and ovulation to the second GnRH. Previous reports (Pursley et al., 1995; Thompson et al., 1999; Moreira et al., 2001; El-Zarkouny et al., 2004; Giordano et al., 2012) in beef and dairy cows have reported an increase in synchronization for cows that ovulate to an initial injection of GnRH. However, by design in this study, cows were early postpartum, anestrus cows. Therefore, response of cows in “deep” anestrus may differ from estrous cycling or mixed populations of estrous cycling and anestrus cows.

The variability in results among reports indicating various pregnancy outcomes after administration of eCG likely may arise from the use of different breeds and biotypes. It seems that eCG had greater effects in cows in anestrus, in poor BCS, with small follicles, or nursing their first calf. A majority of these reports were conducted using *Bos indicus* cattle (Baruselli et al., 2004; Sá Filho et al., 2009, 2010; Pegorer et al., 2011). *Bos indicus* cattle tend to have a greater incidence of anestrus and poorer BCS at initiation of the breeding season (Sá Filho et al., 2009) and generally have smaller follicles compared with *Bos taurus* cattle (Figueiredo et al., 1997; Sartori and Barros, 2011). Therefore, the impact of eCG may be greater in *Bos indicus* than in *Bos taurus* cattle. In contrast, eCG may have an impact in *Bos taurus* cattle exposed to suboptimal nutritional environments (Baruselli et al., 2004; Small et al., 2009; Souza et al., 2009).

Conclusion

Equine chorionic gonadotropin is a viable alternative to increase follicle growth rate and diameter before fixed-time AI. Calf removal was more effective at stimulating an LH surge and ovulation than eCG, and the combination of both treatments did not have a positive additive effect on fertility. The variability of pregnancy outcomes in response to eCG may be related to the dose and timing of eCG treatment, BCS, and days postpartum of the cows. More research is warranted to clarify the effect of eCG in low BCS cows.

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