Synchronization of ovulation using GnRH or hCG with the CO-Synch protocol in suckled beef cows[1]

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ABSTRACT: The objectives of this study were to evaluate replacing GnRH with hCG and the effects of 48-h calf removal (CR) on pregnancy rates of cows synchronized with the CO-Synch protocol. Suckled beef cows (n = 467) at two locations were assigned to treatment by breed, age, and calving date. Treatment included either GnRH with (n = 121) or without CR (n = 117) or hCG with (n = 115) or without CR (n = 114) using the CO-Synch protocol. On d 0 and 9, cows received either hCG (2,500 IU, i.m.) or GnRH (100 μg, i.m.), and on d 7 all cows received $PGF_{2\alpha}$ (25 mg). At one location, blood samples were collected from all cows (n = 203) on d −14, −7, 0, 7, 9, and 16. Calves were removed on d 7 and returned on d 9 (48 h) from approximately half of the cows that received GnRH or hCG. Cows that were detected in estrus between d 6 and 9 were bred approximately 12 h later and received no further injections. Cows not observed in estrus by d 9 received a second injection of either GnRH or hCG and were timed-inseminated. The AI pregnancy rates for GnRH-treated cows with or without CR and hCG-treated cows with or without CR were 46, 49, 35, and 34%, respectively (P = 0.44). Pregnancy rates of cows differed by treatment × age interaction (P = 0.07), hormone (P = 0.09), and hormone × age (P = 0.01) but not by CR (P = 0.66) or CR × age (P = 0.33). Among 2-yr-olds, pregnancy rates were higher for cows treated with hCG without CR than for cows that received GnRH with calf removal, whereas cows treated with hCG with CR and GnRH without CR were intermediate. In addition hCG-treated 2-yr-olds had higher pregnancy rates than GnRH-treated 2-yr-olds regardless of calf presence, but the reverse was true for older cows. Overall, GnRH-treated cows (48%) had a higher (P = 0.09) pregnancy rate than hCG-treated cows (34%). Among anestrous cows, GnRH and hCG were similar (P = 0.40) in their ability to induce ovulation and corpus luteum formation after the first and second injections of GnRH (31 and 76%, respectively) or hCG (39 and 61%, respectively). More (P = 0.001) hCG-treated cows exhibited short estrous cycles following timed AI. We conclude that hCG is not a suitable replacement for GnRH to synchronize ovulation with the CO-Synch protocol in multiparous cows, although further evaluation among primiparous cows is warranted using hCG with the CO-Synch protocol.

Key Words: Artificial Insemination, Beef Cattle, GnRH, hCG, Ovulation, Synchronization

 J. Anim. Sci. 2001. 79:2536–2541

Introduction

Gonadotropin-releasing hormone (GnRH), together with prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**) has been used successfully to synchronize estrus and ovulation among dairy and beef cows. Administration of GnRH or a GnRH agonist caused ovulation with formation of a functional corpus luteum by d 7 and emergence of a synchronized ovarian follicular wave within 2 to 4 d (Britt et al., 1974; Zaied et al., 1980; Thatcher et al., 1989). Administration of $PGF_{2\alpha}$ 6 to 7 d after an injection of GnRH or its agonist was used to synchronize estrus (Thatcher et al., 1989; Twagiramungu et al., 1992; Wolfenson et al., 1994). Pursley et al. (1995) and Silcox et al. (1995) synchronized ovulation within an 8-h window among cows and heifers with an additional injection of GnRH 48 h after the $PGF_{2\alpha}$. The GnRH/$PGF_{2\alpha}$/GnRH protocol with timed arti-

[1]The authors wish to acknowledge financial support of this research from the National Association of Animal Breeders and the donations of Cystorelin from Merial, hCG from Steris Laboratories, and Lutalyse from Pharmacia & Upjohn. We also wish to thank CSU beef herd employees Dave Schutz, Dave Schafer, and Eric Downing for their assistance with data collection.

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Received November 21, 2000.

Accepted May 25, 2001.

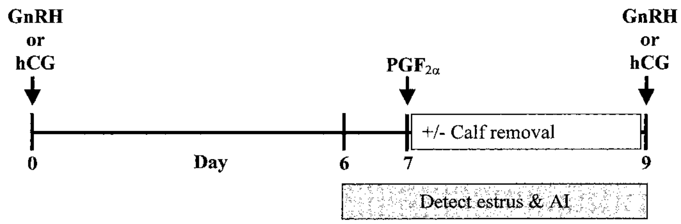

Figure 1. Illustration of the CO-Synch protocol that cows received with GnRH (100 μg; i.m.) or hCG (2,500 IU; i.m.) on d 0 and 9, $PGF_{2\alpha}$ (25 mg; i.m.) on d 7, and with or without 48-h calf removal from d 7 to 9.

ficial insemination (AI) 20 to 24 h later was termed the Ovsynch protocol (Pursley et al., 1994). Timed AI at the same time as the second GnRH injection (CO-Synch) required one less handling of cows but yielded lower (49%) pregnancy rates than AI 24 h later (Ovsynch; 57%) in beef cows (Geary and Whittier, 1998). Temporary calf removal from the $PGF_{2\alpha}$ injection to the second GnRH injection increased pregnancy rates of beef cows receiving the CO-Synch or Ovsynch protocols (Geary et al., 2001). Subsequent data from our laboratory have revealed no difference in pregnancy rates between Ovsynch- and CO-Synch-treated beef cows.

Human chorionic gonadotropin (hCG) and GnRH have similar effects on the ovary (Rajamahendran and Sianangama, 1992; Fricke et al., 1993), but hCG acts independently of the pituitary gland. We hypothesized that hCG could serve as a suitable replacement for GnRH to decrease the cost, improve the ovulatory response, and replace any beneficial effects of 48-h calf removal in beef cows receiving the CO-Synch protocol. Thus, our objective was to evaluate the effect of hCG vs GnRH with or without 48-h calf removal on conception rates in beef cows using the CO-Synch protocol.

Materials and Methods

Primiparous and multiparous lactating beef cows (n = 467) from two locations (eastern Colorado and southwestern Colorado) received the CO-Synch protocol with GnRH or hCG (Figure 1). Cows at the eastern Colorado location were primarily Hereford × Red Angus crossbred cows, and cows at the southwestern Colorado location were Angus, Hereford, or System 1 composites (7/16 Hereford, 1/8 Angus, 1/8 Red Angus, 1/16 each of Charolais, Red Poll, Pinzgauer, Red Holstein, and Brown Swiss). Cows were assigned to treatment using a randomized complete block design with a 2 × 2 (hormone; GnRH, or hCG × calf removal; ± 48-h calf removal) factorial. Blocking factors within location were breed, age, postpartum interval (**PPI**), and body condition score (**BCS**). Table 1 contains a description of BCS and PPI for cows within treatment and location. On d 0, cows received either an i.m. injection of hCG (2,500 IU, Chorionic Gonadotropin; Steris Laboratory, Phoenix, AZ) or GnRH (100 μg, Cystorelin; Merial, Iselin, NJ). Visual observation (3 times daily, approximately 0600, 1200, and 1800) was used to identify estrual cows beginning on d 6. On d 7, all cows received an i.m. injection of $PGF_{2\alpha}$ (25 mg, Lutalyse; Pharmacia & Upjohn, Kalamazoo, MI). Calves were removed (48 h) from d 7 to 9 from approximately half of the GnRH- and hCG-treated cows. Thus, the four treatments were GnRH with calf removal, GnRH without calf removal, hCG with calf removal, and hCG without calf removal using the CO-Synch protocol. Cows not observed in estrus by d 9 received a second injection of GnRH or hCG and were timed-inseminated on d 9. Cows that were observed in estrus received no further injections and were bred approximately 12 h later. Transrectal ultrasonography was used to determine pregnancy at both locations approximately 40 d following the timed insemination.

At the eastern Colorado location, three blood samples were collected from all cows (n = 203) on d −14, −7, and 0 for analysis of progesterone to identify estrual status (anestrous cows and approximate stage of the estrous cycle among cyclic cows) at initiation of treatment. The percentages of cycling cows within treatments at the eastern Colorado location are presented in Table 1. Three additional blood samples were collected on d 7, 9, and 16 and were assayed for progesterone to evaluate corpus luteum presence, regression, and ovulatory response, respectively. All blood samples were collected via jugular venipuncture and allowed to clot for 24 h at 4°C. The blood was centrifuged at 2,500 × g for 20 min and serum was stored at −20°C until it was assayed for progesterone by radioimmunoassay (Niswender, 1973). Blood samples with progesterone concentrations greater than 1 ng/mL were considered indicative of corpus luteum presence and cyclicity. At the eastern Colorado location, additional visual observation (three times daily) was used to identify cows in estrus from d 0 to 34. Cows exhibiting estrus between d 14 and 26 (5 to 17 d after induced ovulation and AI) were considered to have short interestrous intervals (short estrous cycles).

At the southwestern Colorado location (n = 264 cows), blood samples were not collected and subsequent estrous data beyond d 9 were not recorded. Pregnancy diagnosis with ultrasound 42 d after AI was used to differentiate between cows that had conceived to AI or natural service following a short estrous cycle. Cows identified as having a short estrous cycle were pregnant less than 35 d as determined by ultrasound and confirmed to be at least 100 d pregnant 110 d after AI by rectal palpation. This method of identifying short estrous cycles would underestimate the actual number of cows that exhibited short estrous cycles because only those cows that conceived to the repeat breeding would be included. However, treatment should not have affected conception to the repeat breeding, so data should not be biased.

Effects of treatments on pregnancy rates, short estrous cycles, estrous response, and serum progesterone were analyzed with CATMOD and GLM procedures. Interaction effects of treatment × age and location on pregnancy rates and treatment × age at the eastern Colorado location on ovulation rates were also analyzed with CATMOD

Table 1. Average (mean ± SE) body condition score (BCS) and postpartum interval (PPI) for cows within each treatment and location and percentage cycling at the eastern Colorado location for each treatment

	Eastern Colorado Location				Southwestern Colorado Location		
Treatment[a]	n	BCS	PPI, d	Cycling, %	n	BCS	PPI, d
GnRH	51	5.2 ± 0.1	77 ± 2.2	25[b]	66	4.7 ± 0.1	76 ± 1.8
GnRH + CR	53	5.3 ± 0.1	76 ± 2.5	40[c]	68	4.5 ± 0.1	75 ± 1.8
hCG	49	5.3 ± 0.1	77 ± 2.3	29[bc]	65	4.7 ± 0.1	76 ± 1.8
hCG + CR	50	5.2 ± 0.1	79 ± 2.4	33[bc]	65	4.6 ± 0.1	76 ± 1.7

[a]GnRH = gonadotropin-releasing hormone, hCG = human chorionic gonadotropin, and CR = calf removal between d 7 and 9.
[b,c]Different superscripts within a column indicate significant differences ($P < 0.10$).

and GLM procedures in SAS (SAS Inst. Inc., Cary, NC). Differences in serum progesterone concentrations were analyzed using GLM and LSM procedures in SAS.

Results and Discussion

Location had no effect ($P = 0.77$) on pregnancy rate, so data were pooled for further analyses. The pregnancy rate for cows synchronized using GnRH with or without calf removal and hCG with or without calf removal was 46, 49, 35, and 34%, respectively ($P = 0.44$; Figure 2). Pregnancy rates of cows differed or tended to differ by treatment × age interaction ($P = 0.07$; Table 2), hormone ($P = 0.09$), and hormone × age ($P = 0.01$; Table 3) but not by calf removal ($P = 0.66$; Table 3), age ($P = 0.81$; Table 2), or calf removal × age ($P = 0.33$; Table 3). Among primiparous cows, pregnancy rates were higher for cows treated with hCG without calf removal than for cows that received GnRH with calf removal, whereas those treated with hCG with calf removal and GnRH without calf removal were intermediate (Table 2). In addition, hCG-treated 2-yr-olds tended ($P = 0.18$) to have higher

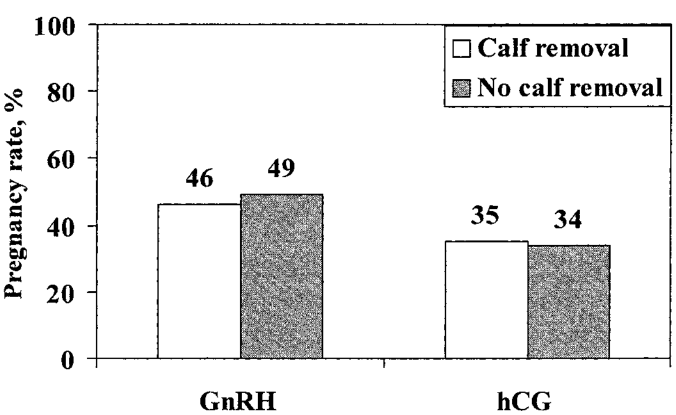

Figure 2. Pregnancy rate of cows (pooled across two locations) that were synchronized with the CO-Synch protocol using GnRH or hCG with or without 48-h calf removal. See Figure 1 for description of treatments and times. There were no differences ($P = 0.44$) in pregnancy rate between treatments.

pregnancy rates than GnRH-treated 2-yr-olds regardless of calf presence. Multiparous cows had higher pregnancy rates to the GnRH treatments than to hCG treatments independent of calf removal (Table 3). The percentages of 2-, 3-, and 4+-yr-old cows at eastern Colorado that were cycling at the onset of treatment were 25, 3, and 41%, respectively. More 2-yr-old cows (97%) ovulated following treatment than 3- (64%) or 4+- (68%) yr-old cows ($P = 0.04$). Virgin heifers at both locations are typically bred about 1 mo before the older cows, and thus the primiparous cows had longer postpartum intervals than the other age groups. Because hCG acts directly at the ovary, perhaps this longer postpartum interval was an important factor in the ovarian response to hCG. Alternatively, follicular growth may have occurred more rapidly in these younger cows such that their ovaries had larger follicles that were capable of ovulating and forming a healthy corpus luteum in response to the second hCG injection. Differences ($P = 0.04$) in ovulation rates existed among cows by treatment and age, but not other variables or interactions. Fewer ($P = 0.04$) cows receiving hCG with calf removal ovulated (54%) than cows receiving other treatments (86, 78, and 88% for hCG, GnRH, and GnRH with calf removal, respectively). Further studies are warranted with 2-yr-olds to confirm whether the CO-Synch protocol using hCG without calf removal is a superior protocol for synchronizing ovulation, as indicated by these observations.

The fact that temporary calf removal (48 h) had no effect ($P = 0.66$) on synchronized pregnancy rate of GnRH- or hCG-treated cows was surprising and differed from earlier results (Geary et al., 2001). Estrual status before synchronization was only determined in cows from the eastern Colorado location. Although cows from both locations were blocked by PPI and BCS before treatment, there tended ($P = 0.09$) to be more cows treated with GnRH with calf removal than cows treated with GnRH without calf removal cycling at the onset of treatment (Table 1). It is unlikely that estrual status was involved in the lack of response to calf removal because pregnancy rates did not differ among treatments (37, 38, 47, and 45% for hCG, hCG + calf removal, GnRH, and GnRH + calf removal, respectively) at the eastern Colorado location. In addition, we have reported previously that 48-h

Table 2. Pregnancy rate (least squares means) of cows pooled across two locations by age that received the CO-Synch protocol using gonadotropin-releasing hormone (GnRH) or human chorionic gonadotropin (hCG) with or without 48-h calf removal (CR) from the time of $PGF_{2\alpha}$ to GnRH or hCG

		Treatment[a]				
Age, yr	Age[b]	hCG	hCG + CR	GnRH	GnRH + CR	SE
2	44.6	59.4[c]	43.3[cd]	43.7[cd]	31.9[d]	10.7
3	39.7	29.9[cd]	31.0[cd]	47.1[cd]	50.9[ce]	13.2
4	40.0	27.2[d]	31.8[d]	51.0[ce]	49.8[ce]	5.9
Treatment[b]		38.8	35.4	47.3	44.2	5.6

[a]See Figure 1 for treatment times and amounts.
[b]Differences between treatment (P = 0.44) and age (P = 0.81) were not significant.
[c,d,e]Different superscripts indicate age × treatment interactions (P = 0.07).

calf removal with the CO-Synch protocol increased pregnancy rates in both cyclic and anestrous cows (Geary et al., 2001). Similar to earlier findings (Geary et al., 2001) is our observation that calf removal seems to be detrimental to pregnancy rates of primiparous cows (Table 3). Although pregnancy rates were numerically lower among primiparous cows that received calf removal, the percentage of these cows ovulating was not different (P = 0.37). From these data, we hypothesize that calf removal suppresses pregnancy rates of primiparous cows by altering the timing of ovulation rather than by preventing ovulation altogether. Pregnancy rates of multiparous cows receiving calf removal in the present study, unlike earlier results, were not different (P > 0.10).

The pregnancy rate of GnRH-treated cows (48%) tended to be higher (P = 0.09) than the pregnancy rate of hCG-treated cows (34%). Hormone treatment (GnRH or hCG) had no effect (P = 0.70) on pregnancy rates of cyclic (53 and 40%, respectively) or anestrous (43 and 36%, respectively) cows. The percentage of anestrous cows was similar (P = 0.27) between treatments at the beginning of treatment at the eastern Colorado location. Based on serum progesterone concentrations at the time of $PGF_{2\alpha}$ injection among cows from the eastern Colorado location, the ability of the first injection of GnRH or hCG to induce ovulation among anestrous cows was also similar (P = 0.40; 31 and 39%, respectively). Based on serum progesterone concentrations on d 7 following timed AI among cows from the eastern Colorado location, ovulation to the second GnRH or hCG injection was induced in a high percentage of anestrous cows (76 and 61%, respectively; P = 0.40) and did not vary (P = 0.35) by calf removal. Among both anestrous and cyclic cows, ovulation to the second GnRH or hCG injection did not differ (P = 0.28) between GnRH-treated (82%) and hCG-treated (63%) cows. In comparison to GnRH, initiation of a new wave of follicular growth might occur later following an hCG injection because of the lack of FSH stimulation. Thus, ovulation may have occurred less frequently among hCG-treated cows due to the presence of a smaller dominant follicle 9 d after the initial hCG injection. The tendency (P = 0.28) for decreased ovulation rate among hCG-treated cows was likely a primary reason pregnancy rates were lower. The time interval from hormone injection to ovulation was not determined but may also have accounted for some of the difference in pregnancy rate to the timed AI.

The lower pregnancy rate of hCG-treated cows may have been partially due to an increased incidence of short estrous cycles after breeding and elevated progesterone

Table 3. Effects of hormone and 48-h calf removal on pregnancy rate (least squares means) of cows pooled across two locations that received the CO-Synch protocol using gonadotropin-releasing hormone (GnRH) or human chorionic gonadotropin (hCG) on d 0 and 9 with or without 48-h calf removal from the time of $PGF_{2\alpha}$ (d 7) to the second GnRH or hCG injection[a]

	Hormone			Calf removal		
Age, yr	hCG	GnRH	SE	No	Yes	SE
2	51.5[c]	38.9[cd]	6.6	52.6	38.5	6.8
3	28.0[d]	47.8[c]	8.6	36.8	38.9	8.5
4	29.7[d]	50.3[c]	4.1	39.2	40.8	4.1
Overall[b]				42.9	39.4	3.9

[a]Hormone × calf removal interaction was not detected (P > 0.10); therefore, main effect values are presented.
[b]Hormone × age interaction was detected (P = 0.01), thus no overall value is shown. Calf removal × age interaction was not detected (P = 0.33), allowing effects of calf removal to be examined across ages (P = 0.66).
[c,d]Values with different superscripts within rows or columns differ (P < 0.10).

levels at the time of breeding. Short estrous cycles were observed more frequently ($P = 0.001$) after AI among hCG-treated cows (23%) than among GnRH-treated cows (11%). This is similar to the percentage of short estrous cycles observed in heifers (16%; Schmitt et al., 1996b) but lower than the percentage of short estrous cycles among early anestrous cows (29%; Thompson et al., 1999) that received the GnRH/$PGF_{2\alpha}$/GnRH injections. Among the cows that were anestrous prior to treatment, 21% of the hCG-treated and 12% of the GnRH-treated cows experienced short estrous cycles following AI. Pratt et al. (1982) reported a tendency for more anestrous cows that received GnRH than hCG to return to estrus within 8 to 12 d. Diaz et al. (1998) further reported formation of a smaller dominant follicle after hCG administration. The short lifespan of the corpora lutea induced by the second hCG injection in the present study may have been due to some inadequacy in the follicle that was induced to ovulate or luteinize. Sheffel et al. (1982) reported that follicles less than 10 mm in diameter formed corpora lutea in response to hCG. It is possible that our hCG protocol resulted in ovulation of a smaller follicle after the second injection of hCG and formation of a less viable corpus luteum.

Cows that experienced short estrous cycles following their second hCG injection had higher ($P < 0.05$) mean serum progesterone concentrations on d 7 ($PGF_{2\alpha}$ injection; 4.1 ng/mL) and d 9 (timed insemination; 1.0 ng/mL) than hCG-treated cows without short estrous cycles (1.6 and 0.2 ng/mL, respectively) or GnRH-treated cows (1.3 and 0.3 ng/mL, respectively). Human chorionic gonadotropin was used in this study because of its LH agonistic properties and its ability to act directly at the ovary (Seguin et al., 1976). Others have reported elevated progesterone production among cyclic heifers receiving hCG during metestrus (Shipley et al., 1988; Diaz et al., 1998). Thus, follicular growth may also have been retarded from d 7 to 9 among hCG-treated cows due to elevated serum progesterone (Hansel and Convey, 1983). Elevated progesterone levels at the time of insemination (and induced ovulation) among hCG-treated cows with short estrous cycles observed in the present study may have further suppressed basal and pulsatile LH secretion, which resulted in formation of a corpus luteum with decreased steroid production and lifespan. Schmitt et al. (1996a) reported only 6% short estrous cycles in heifers that received GnRH for the first injection and hCG for the last injection of the Ovsynch protocol.

Serum progesterone was higher ($P = 0.02$) on d 7 in hCG-treated cows (2.2 ng/mL) than in GnRH-treated cows (1.3 ng/mL), indicating that hCG may have caused hypertrophy of any preexisting corpus luteum (Veenhuizin et al., 1972; Fricke et al., 1993) or formation of a larger accessory corpus luteum (Schmitt et al., 1996a). Progesterone concentrations were similar ($P = 0.30$) on d 9 for the hCG-treated (0.41 ng/mL) and GnRH-treated (0.27 ng/mL) cows. These results are similar to those of other investigators (Wiltbank et al., 1961; Donaldson and Hansel, 1965; Veenhuizin et al., 1972) who reported increased concentration of progesterone after an injection of hCG compared with GnRH. In the present study, hCG-treated cows that conceived to the timed insemination had lower ($P < 0.01$) progesterone concentrations on d 7 and 9 (1.4 and 0.2 ng/mL, respectively) than cows that did not conceive (2.7 and 0.6 ng/mL, respectively). Among GnRH-treated cows, progesterone concentrations on d 7 (1.6 vs 1.0 ng/mL) and d 9 (0.2 vs 0.4 ng/mL) did not differ ($P = 0.20$) between cows that did or did not conceive, respectively, to the timed AI. Our timing of insemination may have been too late for optimal pregnancy rates among GnRH-treated cows that did not conceive because they were likely in estrus early after $PGFF_{2\alpha}$. However, our timing of insemination may have been too early for the hCG-treated cows that did not conceive because these cows may not have completed luteolysis and follicular maturation.

Nine percent of the hCG-treated cows had serum progesterone concentrations greater than 1 ng/mL at the time of mass mating, compared with 4% of GnRH-treated cows ($P = 0.20$). Elevated progesterone at this time suggests they were inseminated before complete luteolysis. Whether this incomplete luteolysis was due to hCG-induced corpora lutea being less responsive to $PGF_{2\alpha}$ or requiring more time for luteolysis is not known. Elevated progesterone at the time of breeding decreased spermatozoa transport to the ovum in ewes (Quinlivan and Robinson, 1969; Hawk and Conley, 1972; Hawk and Echternkamp, 1972) and may have contributed to the lower pregnancy rate among hCG-treated cows in the present study.

Estrous response from d 6 to 9 was higher ($P < 0.004$) among GnRH-treated cows (18%) than among hCG-treated cows (10%). Conception rates of cows detected in estrus from d 6 to 9 were similar ($P = 0.51$) between the two hormone treatments (GnRH, 66%; hCG, 53%). The percentage of cows detected in estrus within 24 h before $PGF_{2\alpha}$ for GnRH- and hCG-treated cows was 7 and 2%, respectively. Eighteen cows (9%) at the eastern Colorado location were detected in estrus before the $PGF_{2\alpha}$ injection. Five of these cows had received hCG and 13 of these cows had received GnRH for their first injection. All five of the hCG-treated cows were previously anestrous, whereas only seven of the GnRH-treated cows were previously anestrous. These data are supported by Pratt et al. (1982), who reported elevated progesterone in 15% of anestrous cows 4 d, but not 7 d, after hCG administration. Among the six cyclic cows that were detected in estrus prior to $PGF_{2\alpha}$, 83% were between d 14 and 17 of their estrous cycles when the first injection of GnRH was administered (d 0). The present study had a limited number of cyclic cows; however, these results are supported by those of Geary et al. (2000), who observed the majority of cows detected in estrus prior to $PGF_{2\alpha}$ with the GnRH/$PGF_{2\alpha}$ protocol to be late in their luteal phase at the time of GnRH. Therefore, hCG reduced the occurrence of an early estrus among cows, which is desirable for a timed insemination protocol.

The percentage of cows detected in estrus between d 6 and 9 was higher ($P < 0.001$) at the eastern Colorado location (GnRH = 25% and hCG = 16%) than at the southwestern Colorado location (GnRH = 7% and hCG = 2%). Body condition scores at the time of breeding were lower ($P < 0.05$) at the southwestern Colorado location (4.6 ± 0.8) than at the eastern Colorado location (5.3 ± 0.6) and may have contributed to the lower estrous response. Based on BCS (Table 1), we would have expected a lower percentage of the southwestern Colorado cows to be cycling at the onset of treatment. No difference ($P = 0.77$) in pregnancy rate was observed between locations.

Implications

Although hCG may be less expensive than GnRH and is often used as a replacement for GnRH to treat cystic ovaries, it is not a suitable replacement for GnRH to synchronize ovulation with the current CO-Synch protocol in multiparous cows. Further studies with hCG in primiparous cows are needed to confirm the present findings that it is better than GnRH among cows of this age group. Among all cows, replacing GnRH with hCG resulted in numerically lower estrous responses and ovulation rates, numerically higher progesterone levels at the time of insemination, and a higher frequency of short estrous cycles that resulted in lower pregnancy rates. In addition, 48-h calf removal did not improve the pregnancy rates of cows receiving either GnRH or hCG using the CO-Synch protocol in this study.

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