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The effect of administering equine chorionic gonadotropin (eCG) and human chorionic gonadotropin (hCG) post artificial insemination on fertility of lactating dairy cows

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Abstract

The objective was to evaluate the effect of equine chorionic gonadotropin (eCG) and hCG post artificial insemination (AI) on fertility of lactating dairy cows. In Experiment 1, cows were either treated with eCG on Day 22 post AI (400 IU; n = 80) or left untreated (n = 84). On Day 29, pregnant cows were either treated with hCG (2500 IU; n = 32) or left untreated (n = 36). Pregnancy and progesterone were evaluated on Days 29 and 45. In Experiment 2, cows (n = 28) were either treated with eCG on Day 22 (n = 13) or left untreated (n = 15) and either treated with hCG on Day 29 (n = 14) or left untreated (n = 14). Blood sampling and ultrasonography were conducted between Days 22 and 45. In Experiment 3, cows were either treated with eCG on Day 22 post AI (n = 229) or left untreated (n = 241). Pregnancy was evaluated on Days 36 and 85. In Experiment 1, eCG on Day 22 increased ($P < 0.02$) the number of pregnant cows on Day 29 (50.0 vs. 33.3%) and on Day 45, the increase was higher ($P < 0.01$) in cows with timed AI (41.2 vs. 6.5%) than in cows AI at detected estrus (50.0 vs. 37.8%). Pregnancy losses were reduced by eCG and hCG, but increased in cows that did not receive eCG but were given hCG ($P < 0.01$). Treatment with hCG tended ($P < 0.06$) to increase progesterone in control cows, but not in cows treated with eCG. In Experiment 2, hCG increased ($P < 0.01$) the number of accessory CLs on Day 35 (28.5 vs. 0.0%) and tended ($P < 0.07$) to increase progesterone. In Experiment 3, eCG increased the number of pregnant cows ($P < 0.05$) on Days 36 and 85, but only in cows with low body condition (eCG = 45.6 and 43.5%; Control = 22.9 and 22.9%). In conclusion, eCG at 22 days post insemination increased fertility, primarily in cows with low body condition and reduced pregnancy losses when given 7 days before hCG; hCG induced accessory CLs and slightly increased progesterone, but hCG given in the absence of a prior eCG treatment reduced fertility.

Keywords: Reproduction; Gonadotropins; Fertility; Progesterone; Dairy

1. Introduction

Reproductive performance in lactating dairy cows is reduced by late embryonic and early fetal mortality, which average 13% [1] and 11% [2], respectively. Suboptimal concentrations of plasma progesterone in lactating dairy cows [3] is a major cause for impaired embryo development [4] and increased pregnancy

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losses [5]. In fact, cows with a number of corpora lutea exceeding the number of embryos (additional corpus luteum) were less likely to experience fetal loss [2]. Therefore, induction of accessory corpora lutea that increase progesterone concentrations in plasma [6] or administration of progesterone [7] reduced late embryonic and early fetal mortality.

Strategies, such as administration of human chorionic gonadotrophin (hCG) on Day 5 [8] and Day 7 [9] of the estrous cycle in dairy cows, have been used to induce accessory corpora lutea and increase plasma progesterone concentrations. Administration of hCG on Day 5 of the estrous cycle induced a high proportion of accessory corpora lutea (86.2%), elevated progesterone concentrations and specifically increased conception rate in cows with low body condition score [10]. In the late embryonic period, administration of a GnRH agonist (deslorelin) implant [11] at Day 27 after insemination in pregnant cows induced accessory CL only in half of the cows (53.7%), increased plasma progesterone concentrations, and reduced pregnancy losses between Days 45 and 90 of gestation, but only in cows with an accessory CL on Day 45. In non-lactating beef cows, hCG was able to induce replacement CL in the early fetal period after stimulation of follicular growth with FSH and monitoring the size of the follicles by ultrasonography [12].

Follicular growth occurs during early pregnancy, especially in the contralateral ovary to the pregnant horn [13–15]. Administration of low doses of equine chorionic gonadotrophin (eCG) can stimulate follicular growth in lactating dairy cows [16]. Therefore, administration of eCG followed by a treatment with hCG a few days later could be more effective in generating accessory corpora lutea and follicle luteinization that may enhance embryo and fetal survival. The hypothesis of this study was that treatment with eCG and hCG will increase progesterone concentrations and fertility in lactating dairy cows. The objective of Experiment 1 was to evaluate the effect of a treatment with eCG on Day 22 and hCG on Day 29 after AI on fertility. The objective of Experiment 2 was to evaluate eCG and hCG effects on ovarian structures and plasma progesterone concentration. The objective of Experiment 3 was to evaluate only the effect of an eCG treatment on Day 22 after AI on fertility.

2. Materials and methods

2.1. Experiment 1

The experiment was conducted in a dairy herd located in the North East of La Pampa province, Argentina, with 450 Holstein lactating cows during the winter of 2008. Cows were grazing a winter annual pasture (avena sativa) and supplemented with corn silage, alfalfa hay and a grain concentrate formulated to meet or exceed the requirements of lactating dairy cows [17]. Cows were milked twice a day and the rolling herd average was 7625 kg. Reproductive management included a voluntary waiting period of 50 days and a breeding program that combined AI at detected estrus (AIDE) and timed AI (TAI) utilizing the Select Synch and Cosynch protocols, respectively. Cows were treated with GnRH (10 μg im; buserelin, Receptal, Intervet, Argentina) and 7 days later were given cloprostenol (0.15 mg im; D-cloprostenol, Enzaprost, Biogenesis, Bago, Argentina; PGF) and AIDE (Select Synch Protocol). Cows that did not show estrus by 72 h after PGF were treated with GnRH and TAI (Cosynch Protocol). Detection of estrus was conducted by visual observation for 45 min during early morning, noon and evening, and overall estrus detection rate for the farm was 60%.

The day of AI was considered experimental Day 0. Cows that did not return to estrus by Day 22 (n = 164) were assigned randomly to receive either eCG (400 IU im; Novormon, Syntex, Argentina, n = 80) or remain untreated as controls (n = 84). On Day 29, pregnant cows (n = 68) were randomly assigned to receive either hCG (2500 IU im; Ovusyn, Syntex, Argentina; n = 32), or remain untreated as controls (n = 36). Intramuscular administrations of eCG and/or hCG were applied over the rump in all experiments. Pregnancy diagnosis was conducted by ultrasonography on Day 29 [18] and ultrasonography and transrectal palpation of the uterus [19] on Day 45 (Fig. 1). On Day 0, parity, days in milk, and type of AI were recorded. The distribution of cows by parity, days in milk, and type of AI for each treatment are shown (Table 1).

Blood samples were collected during the morning milking into vacuum tubes containing sodium heparin (Vacutainer, BD, Franklin Lakes, NJ, USA) by puncture of the median coccygeal vein or artery on Days 29 and 45 in a subset of cows (n = 60) that were pregnant on both days. Upon collection, samples were immediately placed on ice, and centrifuged (1100*g* for 20 min) within 12 h. Plasma was separated and stored at −20°C until assayed. Plasma progesterone concentrations were determined in duplicates by radioimmunoassay using the RIA kit (Diagnostic Products Corporation, Los Angeles, CA) with a sensitivity of 0.1 ng/mL and an intraassay coefficient of variation <9% for samples between 0.1 and 40 ng/mL.

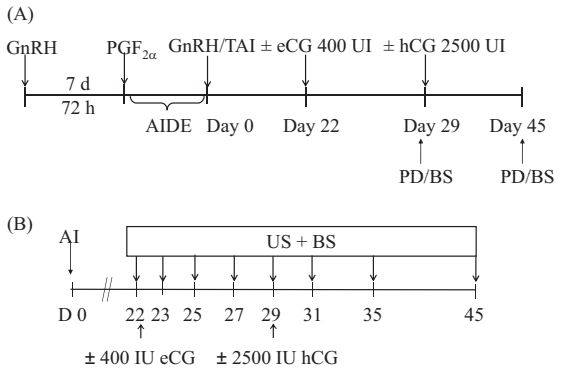

Fig. 1. (A) Design for Experiment 1. Number of cows at Day 22 were eCG = 80, Control = 84, and pregnant cows at Day 29 were eCG-Control = 20, eCG-hCG = 20, Control-hCG = 16, Control-Control = 12. (B) Design for Experiment 2. At Day 22 cows were assigned to either eCG = 13 or Control = 15; At Day 29 cows were assigned to eCG-Control = 7, eCG-hCG = 6, Control-hCG = 8, Control-Control = 7. AIDE= AI at detected estrus; TAI= timed artificial insemination; PD=pregnancy diagnosis, BS= blood sample, US= ultrasongraphy.

Response variables were number of pregnant cows on Days 29 and 45 and pregnancy losses between Days 29 and 45. Explanatory variables were treatment with eCG on Day 22 after AI, treatment with hCG on Day 29 of gestation, days in milk (quartiles: 1 = 44 - 91; 2 = 92 - 154; 3 = 155 - 288; 4 = 289 - 705), parity and type of AI (AIDE or TAI). The statistical model for number of pregnant cows on Days 29 included eCG on Day 22, type of AI, parity, days in milk and the interactions. Statistical model for number of pregnant cows on Day 45 and pregnancy losses between Days 29 and 45 included eCG on Day 22, hCG on Day 29, type of AI, parity, days in milk and the interactions. The effect of treatments on response variables were analyzed by logistic regression using backward elimination procedures [20] in Proc GENMOD of SAS system (SAS Institute, Inc., Cary, NC). Plasma progesterone concentration was analyzed by analysis of variance (repeated measures, Proc MIXED, SAS system, SAS Institute, Inc.) and the statistical model included Days, eCG on Day 22, hCG on Day 29, and the interactions. The cow was considered a random variable [21]. Variables with $P < 0.05$ were considered significant.

2.2. Experiment 2

This experiment was conducted in the same farm as Experiment 1 during May and June of 2009. Lactating dairy cows previously AIDE and not showing estrus by day 22 (n = 28) were assigned randomly to receive eCG (400 IU im; n = 13) or remain untreated (n = 15). On day 29, regardless of pregnancy status, cows were assigned to receive either hCG on day 29 (2500 IU im; n = 14) or remain untreated (n = 14, Fig. 1). Groups had the following number of cows: eCG - Control=7, eCG - hCG=6, Control-hCG = 8, Control-Control = 7. Parity, days in milk and body condition score were 3.3 ± 0.4, 282.7 ± 26.6 and 3.2 ± 0.1 and were not different among groups (P = 0.80, 0.57 and 0.98, respectively). Ultrasonography (Aloka 210, real-time, B-mode with 5.0 MHz linear transrectal transducer, Corometrics Medical Systems, Inc., North Wallingford, CT) was conducted on Days 22, 23, 25, 27, 29, 31, 35, and 45 to determine ovarian structures and number of pregnant cows. Blood samples were collected on Days 22, 23, 25, 27, 29, 31, 35, and 45 to determine plasma progesterone concentrations following the same procedures as Experiment 1.

Response variables were number of class 3 follicles (≥10 mm in diameter) on Day 29, size of the largest follicle (mm) between Days 22 and 29, number of accessory CL on Day 35, number of pregnant cows on Days 29 and 45, and plasma progesterone concentrations between Days 22 to 29, and 29 to 45. The effect of treatment on number of class 3 follicles and accessory CL was evaluated using the Poison distribution in Proc GENMOD of the SAS System (SAS Institute, Inc). Number of pregnant cows was not statistically analyzed, because of the small sample size. Size of the largest follicle and plasma progesterone concentration was analyzed by analysis of variance (repeated measures, Proc MIXED, SAS system, SAS Institute, Inc.). The statistical model for plasma progesterone concentration and size of the largest follicle between Days 22 to 29 included day, eCG treatment on Day 22, and the interaction of day*eCG. The statistical model for plasma progesterone concentrations between Days 29 to 45 included day, hor-

Table 1
Distribution of cows between treatment groups for Experiment 1.

	Treatment with eCG		P value
	Yes (n = 80) % (N)	No (n = 84) % (N)	
Parity			0.58
Primiparous	23.7 (19)	20.2 (17)	
Multiparous	76.3 (61)	79.8 (67)	
Days in milk			0.37
44–91	23.7 (19)	26.2 (22)	
92–154	26.3 (21)	23.8 (20)	
155–288	27.5 (22)	22.6 (19)	
289–705	22.5 (18)	27.4 (23)	
Type of AI			0.46
Timed AI	42.5 (34)	36.9 (31)	
AI at detected estrus	57.5 (46)	63.1 (53)	

mone treatment (i.e., eCG on Day 22 and hCG on Day 29) and the interaction of day × hormone treatment. Cow was considered as a random variable [20]. Variables with $P < 0.05$ were considered significant.

2.3. Experiment 3

The experiment was conducted during summer 2009 and winter 2010 in a dairy herd located in the North East of La Pampa province, Argentina, with 1700 Holstein lactating cows. During the winter, cows were grazing a winter annual pasture (avena sativa) and supplemented with corn silage, alfalfa hay and a grain concentrate formulated to meet or exceed the requirements of lactating dairy cows [17]. Cows were milked twice a day and the rolling herd average was 6500 kg. During the summer cows were grazing alfalfa and supplemented with corn silage and a concentrate formulated to meet or exceed the requirements of lactating dairy cows. Reproductive management included two breeding seasons (May to July and November to January), and the breeding program combined both, TAI protocols and AIDE after $PGF_{2\alpha}$ treatment. Cows inseminated during the last 4 wk of each breeding season (July and January) and that did not return to estrus by Day 22 ± 2 (n = 470) were assigned randomly to receive eCG (400 IU im; n = 229) on Day 22 post AI or remain non-treated (n = 241). Because breeding was seasonal, detection of estrus was conducted until the end of the breeding season, and estrus detection rate for the farm was 65%. Pregnancy diagnosis was conducted on Days 36 and 85 using transrectal palpation of the uterus [19]. The distributions of cows by time of the year, body condition score (Scale 1–5 [22]), parity, and days in milk are shown in Table 2.

Response variables were number of pregnant cows on Days 36 and 85, and explanatory variables were treatment (eCG or control), time of the year (summer or winter), body condition score (high = ≥2.75 or low = <2.75), parity (primiparous or multiparous) and days in milk (quartiles). The effects of explanatory variables and their interaction on pregnancy per AI were evaluated by logistic regression using the backward elimination procedures [20] in Proc GENMOD of the SAS system (SAS Institute, Inc).

3. Results

3.1. Experiment 1

Cows given eCG on Day 22 had a higher proportion ($P < 0.02$) of pregnant cows on Days 29 (50.0%; 40/80) compared to untreated control cows (33.3%; 28/84). Cows AIDE had a higher proportion ($P < 0.05$) of pregnant cows on Day 29 (48.5%; 48/99) than cows TAI (30.8%; 20/65). The interaction of eCG with type of insemination ($P < 0.01$) on the number of pregnant cows at Day 45 was attributed to a greater difference between eCG and control in number of pregnant cows to TAI (41.2%; 14/34 vs. 6.5%; 2/31) than cows AIDE (50.0%; 23/46 vs. 37.8%; 20/53). Treatment with eCG on Day 22 reduced ($P < 0.01$) pregnancy losses between Days 29 and 45 (7.5%; 3/40 vs. 21.4%; 6/28). An eCG*hCG interaction ($P < 0.01$) was indicative that treatment with hCG reduced pregnancy losses between Days 29 and 45 in cows treated with eCG on Day 22 (0.0%; 0/20 vs. 15.0%; 3/20) but increased pregnancy losses in cows not treated with eCG (33.3%; 4/12 vs. 12.5%; 2/16). Treatment with hCG on Day 29 tended ($P < 0.06$) to increase plasma progesterone concentrations (mean ± SEM) in control cows (10.51 ± 0.82 vs. 7.98 ± 0.74 ng/mL) but not in cows treated with eCG on Day 22 (9.60 ± 0.55 vs. 9.81 ± 0.64 ng/mL).

Table 2
Distribution of cows between treatment groups for Experiment 3.

	Treatment with eCG		P value
	Yes (n = 229) % (N)	No (n = 241) % (N)	
Time of the year			0.82
Summer	46.3 (106)	47.3 (114)	
Winter	43.7 (123)	42.7 (127)	
Body condition score			0.96
Low	20.1 (46)	19.9 (48)	
High	79.9 (183)	80.1 (193)	
Parity			0.73
Primiparous	38.9 (89)	37.3 (90)	
Multiparous	61.1 (140)	62.7 (151)	
Days in milk			0.13
66–121	26.2 (60)	24.1 (58)	
122–148	24.0 (55)	23.2 (56)	
149–169	27.9 (64)	24.5 (59)	
170–565	21.9 (50)	28.2 (68)	

3.2. Experiment 2

The number of pregnant cows on Day 29 was 69.2% (9/13) for cows treated with eCG on Day 22 and 80.0% (12/15) for untreated control cows. The number of pregnant cows at Day 29 was 71.4% (5/7) for eCG–Control, 66.7% (4/6) for eCG-hCG, 87.5% (7/8) for Control-hCG and 71.4% (5/7) for Control-Control. The number of pregnant cows at Day 45 was 57.1% (4/7) for eCG-Control, 50.0% (3/6) for eCG-hCG, 50.0% (4/8) for Control-hCG and 57.1% (4/7) for Control-Control. Treatment with eCG did not increase the num-

ber of Class 3 follicles (eCG = 1.6 ± 0.27; Control = 2.1 ± 0.25; $P < 0.17$) on Day 29. In addition, there was no effect ($P = 0.29$) of treatment with eCG on Day 22 on the size of the largest follicle (eCG = 14.7 ± 0.6 mm; Control = 15.6 ± 0.6 mm). Treatment with hCG increased ($P < 0.01$) the number of accessory CLs by Day 35 (28.5%; 4/14 vs. 0.0%; 0/14). Treatment with eCG on Day 22 did not affect plasma progesterone concentration from Days 22 to 29 ($P < 0.29$, Fig. 2a), and treatment with hCG on Day 29 tended ($P < 0.07$) to increase plasma progesterone concentration between Days 31 to 35 (Fig. 2b). There was no eCG or eCG*hCG time trends detected. Cows that did not receive eCG and were treated with hCG seem to have higher pregnancy losses, but sample size was not appropriate to evaluate this type of response (37.5%; 3/8 vs. 16.7%; 1/6).

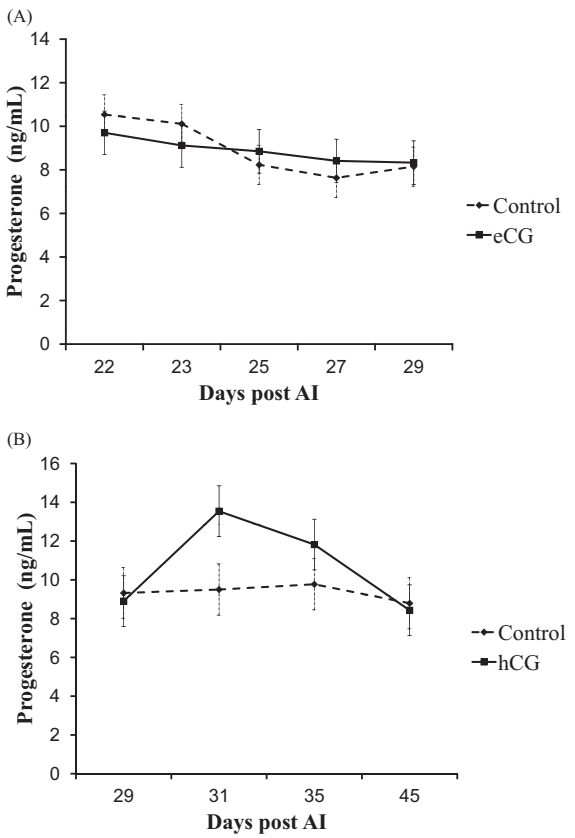

Fig. 2. (A) Plasma progesterone concentration (mean ± SEM) between Days 22 to 29 in cows treated with eCG on Day 22 (n = 13) and Controls (n = 15) in Experiment 2 ($P < 0.29$). (B) Plasma progesterone concentration (mean ± SEM) between Days 29 to 45 in cows treated with hCG (n = 14) on Day 29 and Controls (n = 14) in Experiment 2 ($P < 0.07$).

3.3. Experiment 3

The number of pregnant cows on Days 36 and 85 was not different between treatments [eCG = 36.2% (83/229) and 32.3% (74/229); Control = 30.7% (74/241) and 29.0% (70/241)]. However, there was a significant interaction ($P < 0.05$) between treatment and body condition score. Treatment with eCG increased the number of pregnant cows on Days 36 and 85 in cows with low body condition score [eCG = 45.6%; 21/46 and 43.5% (20/46); Control = 22.9% (11/48) and 22.9% (11/48)], but not in cows with high body condition score [eCG = 33.9% (62/183) and 29.7% (54/183); Control = 32.6% (63/193) and 30.6% (59/193)]. Treatment did not affect pregnancy losses [eCG = 10.8% (9/83); Control = 5.4% (4/74)] for cows in either low or high body condition score.

4. Discussion

The hypothesis of the study was that treatments with eCG and hCG given at specific stages after AI in lactating dairy cows will increase accessory CL formation and elevate plasma progesterone concentrations, which would increase the number of pregnant cows and reduce pregnancy losses. Treatment with eCG on Day 22 after AI increased the number of pregnant cows, mainly in cows timed inseminated (Experiment 1) and cows with low body condition score (Experiment 3). Treatment with hCG on Day 29 of gestation reduced pregnancy losses between Days 29 and 45 in cows treated with eCG on Day 22 after AI, but increased pregnancy losses in cows not treated with eCG on Day 22 (Experiment 1). Treatment with eCG on Day 22 did not affect the size of the largest follicle or the number of class 3 follicles on Day 29. However, treatment with hCG on Day 29 increased the number of cows with accessory CLs on Day 35 (Experiment 2). There was a tendency for eCG to increase plasma progesterone concentrations on Day 29 (Experiment 1). However, when a more intensive sampling was conducted, plasma progesterone concentration was not affected (Experiment 2).

Administration of eCG in lactating dairy cows at the time of luteolysis in a TAI protocol tended to increase both CL volume and plasma progesterone and increased pregnancy rate in cows induced to ovulate with GnRH and cows with low body condition score [23]. In the present study, eCG was administered on Day 22 after AI to stimulate follicular growth and hCG was then administered 7 days later (Day 29) to induce ovulation of those follicles in order to form accessory corpora lutea. The fact that cows treated with eCG and

hCG had reduced pregnancy loss was expected since cows with an additional CL should have higher progesterone and increased embryo survival. The risk of pregnancy losses is highly reduced in cows with more than one CL [2,24]. However, the efficacy of hCG administered on Day 29 post insemination to induce accessory CLs was low (28.5%) in both cows treated with eCG on Day 22 or controls. Approximately 90% of beef cows formed an accessory CL after administration of 2.5 mg of FSH for 3 days followed by 2500 IU of hCG on the third day, and 50% of beef cows formed accessory CL after treatment with 1000 IU of hCG in cows with a follicle of at least 10 mm detected by ultrasonography [12]. In Experiment 1, treatment with eCG increased the number of pregnant cows regardless of the treatment with hCG 7 days later. However, this finding should be interpreted with caution, since pregnancy was not determined on Day 22 and, despite the random assignment of cows, a greater distribution of pregnant cows at Day 22 in the eCG group due to chance cannot be excluded. This was the reason for the design of Experiment 3. In Bos indicus suckled beef cows, administration of eCG at the end of a progesterone treatment increased progesterone during the following diestrus phase [25]. The treatment of eCG on Day 22 after AI coincides with maximal follicular growth after estrus in lactating dairy cows [26,27], which could induce differentiation and/or luteinization of follicles and stimulation of the CL. However, in this study, eCG failed to stimulate concentrations of progesterone in plasma during the period prior to injection of hCG or to accentuate progesterone concentrations after injection of hCG. Since eCG binds to LH receptors in the gonadal tissue of the cow [28], perhaps the dose or the timing of the eCG injection was not optimal to increase concentrations of progesterone in plasma.

The interaction between treatment with eCG and type of AI in Experiment 1 indicated that the beneficial effect of eCG on conception rate was greater in cows TAI. In this experiment, estrus was synchronized using GnRH and $PGF_{2\alpha}$ 7 days later. Cows detected in estrus were AI, and cows not showing estrus received another dose of GnRH and were TAI 72 h after $PGF_{2\alpha}$ injection. Perhaps TAI cows were more likely to be in anestrus, ovulated smaller follicles, formed a less functional CL, and therefore may have benefitted from the effect of eCG. Similarly, administration of hCG on Day 5 post AI increased conception rate especially in cows with low body condition score [10]. This was in agreement with our findings of Experiment 3, in which eCG increased pregnancy rates, but only in cows with low body condition score.

Since the administration of a GnRH analogue in the late embryonic period (Day 27 of gestation) reduced pregnancy losses between Days 45 and 90 in cows that formed accessory corpora lutea but only 55% of the cows responded to this treatment [11], it was decided to use the hCG on Day 29 to increase the occurrence of accessory corpora lutea formation. The interaction detected between eCG on Day 22 after AI and hCG on Day 29 of gestation on pregnancy losses between Days 29 and 45 was not expected. We expected that hCG will induce ovulation or luteinization of follicles that were stimulated by eCG 7 days earlier, and therefore reduce pregnancy losses. In fact, in Experiment 1, hCG reduced pregnancy losses in cows that were previously treated with eCG, but increased pregnancy losses in cows that were not treated with eCG on Day 22. In Experiment 2, a greater percentage of cows lost pregnancy after treatment with hCG on Day 29 without prior treatment with eCG, but sample size was insufficient to evaluate this response. A potential interaction of exogenous eCG and/or hCG during the process of implantation in the cow should not be overlooked. The LH/hCG receptor is expressed in the human endometrium, and hCG was able to stimulate secretion of leukemia inhibitory factor (LIF) and inhibit production of interleukin 6 (IL-6) indicative of a local immune modulation of human endometrial epithelial cells [29].

In conclusion, treatment with eCG during late embryonic period increased the percentage of pregnant cows, especially in those with low body condition score. Treatment with hCG on Day 29 of gestation reduced pregnancy losses only in cows previously treated with eCG, but increased losses in control cows. Since plasma progesterone was not significantly increased in this experiment, the mechanism by which eCG and hCG may be stimulating embryo survival in lactating dairy cows is not clear. Future studies should investigate the underlying mechanisms by which eCG may stimulate fertility of lactating dairy cows.

Acknowledgments

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