Effects of postbreeding gonadotropin treatments on conception rates of lactating dairy cows subjected to timed artificial insemination or embryo transfer in a tropical environment

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ABSTRACT

The objective of experiment 1 was to evaluate the effects of treatments with human chorionic gonadotropin (hCG) or GnRH 7 d after induced ovulation on reproductive performance of lactating dairy cows submitted to timed artificial insemination (TAI) or timed embryo transfer (TET). A total of 834 potential breedings were used from 661 lactating Holstein cows (37.3 ± 0.3 kg of milk/d). Cows had ovulation synchronized and were assigned randomly to receive TAI on d 0 or TET on d 7. Within each group, cows were assigned randomly to receive on d 7 no additional treatment (control; $n_{TAI} = 156$; $n_{TET} = 126$), a 100 μ g i.m. injection of GnRH ($n_{TAI} = 155$; $n_{TET} = 124$), or a 2,500 IU i.m. injection of hCG ($n_{TAI} = 151$; $n_{TET} = 122$). Postbreeding treatment affected the percentages of pregnant cows at TET on d 28 (control: 38.1%; GnRH: 52.4%; hCG: 45.1%) and on d 60 (control: 32.5%; GnRH: 41.1%; hCG: 38.5%), but postbreeding treatment did not affect percentages of pregnant cows at TAI on d 28 (control: 30.1%; GnRH: 32.2%; hCG: 32.4%) or on d 60 (control: 25.6%; GnRH: 27.1%; hCG: 29.8%). The objective of experiment 2 was to evaluate the effect of a treatment with GnRH 7 d after TET on reproductive performance of lactating dairy cows that received a previous GnRH treatment at TET. A total of 285 potential breedings were used from 257 lactating Holstein cows (35.1 ± 0.8 kg of milk/d). Cows had ovulation synchronized and were assigned for TET on d 7. Immediately after TET, all cows were treated with a 100 μ g i.m. injection of GnRH. On d 14, cows were assigned randomly to receive (G7–14; $n = 147$) or not (G7; $n = 138$) an additional injection of GnRH. Pregnancy diagnosis were performed on d 28 and 60. The additional treatment with GnRH on d 14 did not affect the percentages of pregnant cows

on d 28 (G7: 48.5%; G7–14: 42.9%) or on d 60 (G7: 39.8%; G7–14: 37.4%). In conclusion, treatment with GnRH or hCG 7 d after induced ovulation increased conception rates in lactating dairy cows submitted to TET, but not in cows submitted to TAI. Moreover, treatment with GnRH 7 d after TET did not enhance reproductive performance of lactating dairy cows that received a previous GnRH treatment at TET.

Key words: artificial insemination, embryo transfer, gonadotropin-releasing hormone, human chorionic gonadotropin

INTRODUCTION

Several studies have indicated a reduction in reproductive efficiency associated with an increase in genetic merit for milk production in dairy cows (Butler, 1998; Lucy, 2001; Washburn et al., 2002). Some potential reasons for the decline in reproductive performance of the modern dairy cow may be reduced circulating concentrations of ovarian steroids (Vasconcelos et al., 1999), poor oocyte quality and early embryonic development (Sartori et al., 2002a), and increased average body temperature (Berman et al., 1985; Kadzere et al., 2002). Although Sartori et al. (2006) did not find differences on conception rates between dairy cows receiving AI or embryo transfer (ET), others have suggested that, compared with AI, pregnancy rates are enhanced with ET (Putney et al., 1989; Drost et al., 1999; Demetrio et al., 2007) because transfers are made only with embryos considered competent to be transferred and that had by-passed the most critical thermosensitive periods of the oocyte or early embryo (1 to 3 d after fertilization; Ealy et al., 1993). Thus, use of ET may minimize the challenges of poor oocyte quality, unsuccessful early embryonic development, and low tolerance to heat of the modern dairy cow.

Circulating progesterone (P4) controls LH pulsatility (Bergfeld et al., 1996), follicular dynamics (Stock and Fortune, 1993), oviductal and uterine environments

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(Thatcher et al., 2001; Green et al., 2005), and embryonic development (Mann and Lamming, 2001), whereas reduced circulating concentrations of P4 after fertilization are associated with increased early conception failure (Mann et al., 1999), poor embryo development (Walton et al., 1990), and reduced subsequent pregnancy rates (Stronge et al., 2005; Mann et al., 2006; Demetrio et al., 2007). Interestingly, in lactating dairy cows submitted to AI, conception rate was positively affected by the size of ovulatory follicle (Sartori et al., 2006) and serum concentrations of P4 7 d after ovulation (Demetrio et al., 2007), but these effects were not significant in cows submitted to ET. Treatment with gonadotropins such as human chorionic gonadotropin (**hCG**) and GnRH, 5 to 7 d after ovulation has the potential to stimulate luteal function (Hoyer and Niswender, 1985), induce ovulation of the dominant follicle and formation of accessory corpus luteum (**CL**; Santos et al., 2001), and increase the occurrence of cows experiencing 3-wave follicular cycles (Diaz et al., 1998), which was associated with enhanced conception rates (Ahmad et al., 1997; Santos et al., 2001). Several studies have evaluated strategic treatments with gonadotropins to improve pregnancy rates in dairy cows after AI (Ambrose et al., 1999; Peters et al., 2000; Santos et al., 2001; Willard et al., 2003; Bartolome et al., 2005). In contrast, limited information exists to evaluate those treatments in dairy cows submitted to ET. Thus, this manuscript describes 2 experiments, in which the objective of experiment 1 was to evaluate the effects of treatments with hCG or GnRH 7 d after induced ovulation on conception rates of lactating dairy cows submitted to timed AI (**TAI**) or ET (**TET**), and the objective of experiment 2 was to evaluate the effect of treatment with GnRH 7 d after TET on reproductive performance of lactating dairy cows that received a previous GnRH treatment at TET.

MATERIALS AND METHODS

Experiments were conducted at a commercial dairy farm located in Araras, Brazil (22°21' S; 47°23' W; altitude = 614 m). All animal procedures followed the recommendations of the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 1999). Cows were housed according to parity in 12 freestall barns with access to an adjoining sod-based paddock. Cows were cooled by intermittent sprinkling and forced ventilation to minimize the effects of heat stress. Cows were fed *ad libitum* a TMR based on corn silage, Bermuda grass (*Cynodon dactylon* 'Coast-Cross'), ground corn, cottonseed, soybean meal, and a mineral and vitamin mix, which was balanced to meet or exceed the nutritional requirements of lactating

dairy cows (NRC, 2001). Cows were milked 3 times daily in a parallel parlor milking system. Daily milk yield for each cow was recorded automatically.

Experiment 1

Animals and Treatments. The hypothesis of this experiment was that treatment with hCG or GnRH given 7 d after induced ovulation would improve conception rates of lactating dairy cows subjected previously to TAI or TET. A protocol for the synchronization of ovulation was performed 993 times in 684 lactating Holstein cows from May 2007 to May 2008. Once a month, cows that were ≥ 60 DIM, or cows diagnosed as non-pregnant after breeding received an intravaginal insert containing 1.9 g of progesterone (**CIDR**, Pfizer Animal Health, São Paulo, Brazil) and 100 μg (i.m.) of gonadorelin (GnRH; 1 mL of Fertagyl, Intervet/Schering-Plough Animal Health, São Paulo, Brazil) on d -10, 25 mg (i.m.) of dinoprost as tromethamine salt ($\text{PGF}_{2\alpha}$; 5 mL of Lutalyse, Pfizer Animal Health) concurrent with CIDR withdrawal on d -3, and 1 mg (i.m.) of estradiol cypionate (0.5 mL, Pfizer Animal Health) on d -2. At the time of assignment, cows averaged ($\pm\text{SEM}$) 169.8 ± 4.4 (range from 60 to 471) DIM, yielding 37.3 ± 0.3 (range from 8.0 to 65.5) kg of milk/d, with BCS of 3.0 ± 0.4 [range from 2.0 to 4.5, on a 1 (emaciated) to 5 (obese) scale; Wildman et al., 1982], and lactation number of 2.1 ± 0.1 (range from 1 to 8), and had been bred 1.9 ± 0.1 (range from 0 to 8) times previously. On d -2, concurrent with the treatment with estradiol cypionate, cows were assigned randomly to receive either TAI or TET. The TAI cows were inseminated artificially on d 0 by a single experienced technician using commercial frozen-thawed semen from 1 of 18 sires, and an embryo was placed into the uterine horn of TET cows ipsilateral to the CL by an experienced veterinarian. The embryos used in this study (77.2% fresh and 22.8% frozen) were collected from superovulated Holstein heifers and nonlactating cows, and according to the International Embryo Transfer Society guidelines for grading embryos (Wright, 1998). Embryos consisted of morula (stage 4; 62.3%), early blastocyst (stage 5; 22.6%), blastocyst (stage 6; 12.0%), or expanded blastocyst (stage 7; 3.1%) of excellent (grade 1; 69.4%) or good (grade 2; 30.6%) quality. Ovaries of cows in both groups were evaluated by transrectal ultrasonography (Aloka SSD-500 with a 7.5-MHz linear-array transducer, Aloka, Tokyo, Japan) on d 7, and only cows that had a CL were analyzed further. Moreover, cows with reproductive abnormalities such as endometritis, pyometra, and uterine-ovarian adhesions were excluded. Thus, the final database contained 834 protocols in which ovulation was synchronized successfully [syn-

chronization rate = 84.0% (834/993)] performed in 661 cows. Within treatment, cows were assigned randomly to receive (1) no additional treatment (control; $n_{TAI} = 156$; $n_{TET} = 126$), (2) 100 μg (i.m.) of GnRH on d 7 (GnRH; $n_{TAI} = 155$; $n_{TET} = 124$), or (3) 2,500 IU (i.m.) of hCG (2.5 mL of Chorulon, Intervet/Schering-Plough Animal Health) on d 7 (hCG; $n_{TAI} = 151$; $n_{TET} = 122$). Cows were considered to be pregnant when an embryo was detected by transrectal ultrasonography on d 28 and a fetus on d 60. Percentages of pregnant cows on d 28 and 60 were calculated by dividing the number of pregnant cows on those days by the number of cows having a CL on d 7.

Sample Collection. Rectal temperature was measured in all cows using a digital thermometer (G-Tech, São Paulo, Brazil) after milking on d 7 (0600 to 1000 h). In all cows, blood samples were collected on d 7, via coccygeal vein or artery, into commercial blood collection tubes (Vacutainer, 10 mL, Becton Dickinson, Franklin Lakes, NJ), placed on ice immediately, maintained at 4°C for 12 h, and centrifuged at $1,500 \times g$ for 15 min at room temperature for serum collection. Serum was stored at -20°C until P4 analysis. On d 14, blood samples were collected from a subgroup of 183 cows. Serum concentrations of P4 on d 7 and d 14 were analyzed using the Coat-A-Count solid-phase ^{125}I RIA kit (Diagnostic Products Corp., Los Angeles, CA). The intraassay CV was 6.1% and the assay sensitivity was 0.01 ng/mL.

Experiment 2

Animals and Treatments. The hypothesis of this experiment was that treatment with GnRH given 7 d after TET (14 d after induced ovulation) would improve reproductive performance of lactating dairy cows that had received a previous GnRH treatment at TET. The rationale was that removing a dominant follicle near the time of maternal recognition of pregnancy would delay luteolysis and increase embryo survival. A protocol for the synchronization of ovulation was performed 350 times in 274 lactating Holstein cows from July to November 2008. Once a month, cows that were ≥ 60 DIM or cows detected nonpregnant after breeding were assigned to receive an ovulation synchronization protocol similar to that described in experiment 1. At the time of assignment, cows averaged ($\pm$ SEM) 192.9 ± 7.6 (range from 60 to 479) DIM, yielding 35.1 ± 0.8 (range from 66.8 to 10.2) kg of milk/d, with BCS of 3.1 ± 0.1 (range from 2.5 to 4.5), and lactation number of 2.1 ± 0.1 (range from 1 to 6), and had been bred 2.1 ± 0.1 (range from 0 to 8) times previously. Ovaries of all cows were evaluated by transrectal ultrasonography on d 7, and cows having no CL or any reproductive abnor-

malities were excluded from further analysis. Thus, the final database contained 285 successful protocols for synchronization of ovulation [synchronization rate = 81.4% (285/350)] performed in 227 cows. Immediately after ovarian examination, cows that had a CL received one fresh embryo into the uterine horn ipsilateral to the CL, by an experienced veterinarian, and 100 μg (i.m.) of GnRH. The embryos used in this study were collected from superovulated Holstein heifers and non-lactating cows, and comprised morula (29.1%), early blastocyst (26.3%), blastocyst (35.2%), or expanded blastocyst (9.4%) of excellent (78.6%) or good (21.4%) quality. On d 14, cows were assigned randomly to receive an additional administration of GnRH (G7-14; $n = 147$) or not (G7; $n = 138$). Cows were considered pregnant when an embryo was detected by transrectal ultrasonography on d 28 and a fetus on d 60. Percentages of pregnant cows on d 28 and 60 were calculated by dividing the number of pregnant cows on those days by the number of cows that received an embryo.

Sample Collection. Rectal temperatures were measured in all cows using a digital thermometer (G-Tech) after milking on d 7 and d 14 (0600 to 1000 h).

Statistical Analyses

Experiment 1. This study was a completely randomized design. Initial analyses were performed, using ANOVA (SAS Institute Inc., Cary, NC), to determine that the distribution of cows by parity (primiparous and multiparous), BCS, DIM, number of previous breedings, milk yield, season (spring, summer, fall, and winter), rectal temperature on d 7, and serum concentration of P4 on d 7 were similar between breeding techniques (TAI and TET) and among postbreeding treatments (control, GnRH, and hCG). Additional analyses were performed to determine that (1) the distribution of cows by sire was similar among treatments within cows submitted to TAI, and (2) the distribution of recipient cows by embryo donor, embryonic type (fresh and frozen), embryonic stage (morula, early blastocyst, blastocyst, and expanded blastocyst), and embryonic quality (excellent and good) was similar among postbreeding treatments within cows submitted to TET. A large correlation was detected between DIM and the number of previous breedings, and effects of season on rectal temperature on d 7, and of postbreeding treatment on serum concentration of P4 on d 14 were observed. To avoid multicollinearity, only postbreeding treatment and DIM remained in the models. Furthermore, season and rectal temperature on d 7 were not used concurrently in a same model.

Pregnancy data were analyzed using the GLIMMIX procedure (SAS Institute Inc.) with a binomial distribu-

tion and logit link function for statistical calculations, and Gaussian distribution with identity link function to determine least squares means. Explanatory variables such as breeding technique, postbreeding treatment, and parity were used in the models as class variables. Explanatory variables considered covariates, such as BCS, DIM, and milk yield, were previously submitted to univariate analysis, and were included in the multivariate models when found to be significant. Effects of rectal temperature on d 7 on dependent variables were analyzed either as continuous or categorized (≤ 37.9 , 38.0–38.4, 38.5–38.9, 39.0–39.4, and $\geq 39.5^\circ\text{C}$) variables. Because results of analyses were similar between either methods of inclusion, the categorized values were used to generate frequency tables. All data were analyzed using barn as a random variable. For comparison of the percentage of pregnant cows on d 28 and 60, the final models contained the effects of breeding technique, postbreeding treatment, parity, rectal temperature on d 7, breeding technique $\times$ postbreeding treatment, breeding technique $\times$ parity, and breeding technique $\times$ rectal temperature on d 7. A secondary analysis was performed to evaluate possible interactions between breeding technique, rectal temperature on d 7, and milk yield. For this analysis, the UNIVARIATE procedure (SAS Institute Inc.) was previously used to determine the median for milk yield. Cows were ranked and assigned to groups according to median milk production (≤ 37.7 vs. > 37.7 kg) and rectal temperature on d 7 (≤ 39.0 vs. $> 39.0^\circ\text{C}$) and the final model contained the effects of breeding technique, milk yield, rectal temperature on d 7, and all interactions.

Continuous dependent variables were analyzed by PROC MIXED using barn as a random variable and the Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. The model statement used for air temperatures (mean, maximum, and minimum) and relative humidity contained the effects of season. Two model statements were used for milk yield: the first model contained the effects of season, breeding technique, parity, and breeding technique $\times$ parity, and DIM was included as covariate; the second model contained the effects of rectal temperature on d 7, breeding technique, parity, and rectal temperature on d 7 $\times$ parity, and DIM was included as covariate. The model statement used for rectal temperature on d 7 contained the effects of season, breeding technique, parity, and breeding technique $\times$ parity, and milk yield was included as a covariate. The model statement used for serum concentration of P4 on d 7 contained the effects of breeding technique, postbreeding treatment, parity, breeding technique $\times$ postbreeding treatment, and breeding technique $\times$

parity, and milk yield was included as a covariate. The model statement used for serum concentration of P4 on d 14 contained the effect of postbreeding treatment, and serum concentration of P4 on d 7 was included as a covariate. Continuous data were expressed as least squares means $\pm$ SEM. For all analyses, when an effect of postbreeding treatment was found, means were compared by 2 orthogonal contrasts (contrast 1: control vs. GnRH + hCG; contrast 2: GnRH vs. hCG).

Experiment 2. This study was a completely randomized design. Initial analyses were performed, using ANOVA (SAS Institute Inc., Cary, NC), to determine that the distribution of cows by parity (primiparous and multiparous), BCS, DIM, number of previous breedings, milk yield, embryo donor, embryonic stage, embryonic quality, rectal temperature on d 7, and rectal temperature on d 14 were similar between treatments. A large correlation was detected between DIM and the number of previous breedings, and between rectal temperature on d 7 and rectal temperature on d 14. Thus, only DIM and rectal temperature on d 7 remained in the models.

Pregnancy data were analyzed using the GLIMMIX procedure (SAS Institute Inc.) with a binomial distribution and logit link function for statistical calculations, and Gaussian distribution with identity link function to determine least squares means. All data were analyzed using barn as a random variable. Procedure UNIVARIATE (SAS Institute Inc.) was previously used to determine the median for milk yield. Cows were ranked and assigned to groups according to median milk production (≤ 36.3 vs. > 36.3 kg) and rectal temperature on d 7 (≤ 39.0 vs. $> 39.0^\circ\text{C}$), and the final model contained the effects of treatment, milk yield, rectal temperature on d 7, parity, and milk yield $\times$ rectal temperature on d 7. In both experiments, effects were considered significant when $P < 0.05$, whereas tendencies were considered when $0.10 > P \geq 0.05$.

RESULTS

Experiment 1

Effects of season on climate conditions, milk yield, and rectal temperatures are described in Table 1. Effects of season were detected ($P < 0.0001$) for mean, minimum, and maximum air temperatures, as well as for relative humidity. Maximum air temperatures were greater than 25°C during every month of the study. Regarding climate conditions, mean rectal temperature on d 7 was greater ($P < 0.01$) during summer than during fall and winter, but similar to that in spring, whereas rectal temperature on d 7 during spring did not differ ($P > 0.10$) from that in other seasons. Milk yield did

Table 1. Meteorological data,¹ milk yield, and rectal temperature in lactating Holstein cows by season at Araras, SP, Brazil (latitude = 22°21' S; longitude = 47°23' W; altitude = 614 m)

Season (year)	Air temperature, °C			Relative humidity, %	Milk yield, kg	Rectal temperature on d 7, °C
	Mean	Maximum	Minimum			
Winter (2007)	18.8 ± 0.2 ^a	26.2 ± 0.3 ^a	11.7 ± 0.3 ^a	65.1 ± 1.0 ^a	40.5 ± 0.8 ^a	38.6 ± 0.1 ^a
Spring (2007)	23.4 ± 0.2 ^b	29.1 ± 0.3 ^b	18.0 ± 0.3 ^b	69.8 ± 1.0 ^b	39.7 ± 0.9 ^a	38.8 ± 0.1 ^{ab}
Summer (2007 and 2008)	24.2 ± 0.2 ^c	30.3 ± 0.3 ^c	19.0 ± 0.3 ^c	83.2 ± 1.0 ^c	35.5 ± 0.9 ^b	39.1 ± 0.1 ^b
Fall (2008)	19.4 ± 0.2 ^a	27.8 ± 0.3 ^d	12.8 ± 0.3 ^d	78.7 ± 0.9 ^d	36.7 ± 0.4 ^c	38.6 ± 0.1 ^a
<i>P</i> -value	<0.001	<0.001	<0.001	<0.001	<0.001	0.008

^{a-d}Means with different superscripts differ ($P < 0.05$).

¹Obtained weekly from a local meteorological station (Centro Meteorológico Militar de Pirassununga-DTCEAYS-Comando da Aeronáutica, Pirassununga-SP, Brazil).

not differ ($P > 0.10$) between winter and spring, but decreased ($P < 0.001$) from spring to summer, and then increased ($P < 0.001$) from summer to fall.

Serum concentrations of P4 on d 7 were similar among postbreeding treatments ($P > 0.10$). In contrast, postbreeding treatment affected serum concentrations of P4 on d 14, which were greater ($P < 0.001$) in gonadotropin-treated cows than in control cows but did not differ between GnRH and hCG treatments (Table 2). Percentages of pregnant cows on d 28 and 60 were greater in TET than in TAI cows (d 28: $P < 0.01$; d 60: $P < 0.05$; Table 3). Postbreeding treatments affected the percentage of cows pregnant from ET (d 28 and d 60: $P < 0.05$) but not from AI (d 28 and d 60: $P > 0.10$; breeding technique $\times$ postbreeding treatment, $P < 0.05$; Table 3). Rectal temperature on d 7 affected the percentages of pregnant cows at TAI (d 28: $P < 0.05$; d 60: $P < 0.10$) but not at TET (d 28 and d 60: $P > 0.10$; breeding technique $\times$ rectal temperature, $P < 0.1$; Table 4). Percentages of pregnant cows at TAI were greater in cows with rectal temperature $\leq 39.0^\circ\text{C}$ on d 7 than in those with $> 39.0^\circ\text{C}$ (d 28 and d 60: $P < 0.05$), but no differences between categorized rectal

temperatures on d 7 occurred in cows submitted to TET (d 28 and d 60: $P > 0.10$; breeding technique $\times$ categorized rectal temperature, $P < 0.1$; Table 5). Percentages of pregnant cows were greater in primiparous than in multiparous submitted to TAI (d 28: $P < 0.05$; d 60: $P < 0.01$), but similar between primiparous and multiparous submitted to TET (d 28 and d 60: $P > 0.10$; breeding technique $\times$ parity, $P < 0.05$; Table 6).

Experiment 2

No effects of treatment were detected on the percentages of pregnant cows on d 28 [G7: 48.5% (67/138); G7-14: 42.9% (63/147); $P = 0.322$] and 60 [G7: 39.8% (55/138); G7-14: 37.4% (55/147); $P = 0.349$]. A tendency for greater percentage of pregnant cows on d 28 was observed in cows producing > 36.3 kg of milk ($P < 0.10$), but no effects of milk yield were found on the percentage of pregnant cows on d 60 ($P > 0.10$; Table 7). The percentages of pregnant cows on d 28 and d 60 were greater in cows with rectal temperature $\leq 39.0^\circ\text{C}$ on d 7 than in those with $> 39.0^\circ\text{C}$ ($P < 0.05$; Table 7).

Table 2. Serum concentrations of progesterone in lactating Holstein cows receiving no treatment (control), 100 μg of GnRH, or 2,500 IU of human chorionic gonadotropin (hCG) on d 7 (d 0 = induced ovulation; experiment 1)

Treatment	Progesterone, ¹ ng/mL	
	on d 7 (n = 834)	on d 14 (n = 183)
Control	2.91 ± 0.07	4.85 ± 0.36
GnRH	2.94 ± 0.07	6.24 ± 0.35
hCG	2.88 ± 0.07	6.76 ± 0.36
<i>P</i> -value		
Treatment	0.901	0.001
Contrast 1 (control vs. GnRH + hCG)	NS	<0.001
Contrast 2 (GnRH vs. hCG)	NS	0.329

¹Serum concentrations of progesterone on d 7 were evaluated on samples collected from 834 cows; serum concentrations of progesterone on d 14 were evaluated in a subgroup of 183 cows.

Table 3. Percentage of pregnancy in lactating Holstein cows submitted to timed AI (TAI) on d 0 or embryo transfer (TET) on d 7 and receiving no treatments (control), 100 µg of GnRH, or 2,500 IU of human chorionic gonadotropin (hCG) on d 7 (experiment 1)

Breeding technique	Postbreeding treatment	Pregnant cows, ¹ % (n/n)	
		on d 28	on d 60
TAI	Control	30.1 (47/156)	25.6 (40/156)
	GnRH	32.2 (50/155)	27.1 (42/155)
	hCG	32.4 (49/151)	29.8 (45/151)
TET	Control	38.1 (48/126)	32.5 (41/126)
	GnRH	52.4 (65/124)	41.1 (51/124)
	hCG	45.1 (55/122)	38.5 (47/122)
<i>P</i> -value			
Breeding technique		0.003	0.015
Postbreeding treatment		0.129	0.238
Breeding technique × postbreeding treatment		0.045	0.048
TAI: Contrast 1 (control vs. GnRH + hCG)		0.941	0.204
TAI: Contrast 2 (GnRH vs. hCG)		0.899	0.961
TET: Contrast 1 (control vs. GnRH + hCG)		0.033	0.011
TET: Contrast 2 (GnRH vs. hCG)		0.108	0.586

¹Pregnancy was evaluated by ultrasonography on d 28 and 60. Percentage of pregnant cows was calculated by dividing the number of pregnant cows by the number of cows having a corpus luteum on d 7.

DISCUSSION

Many experiments have assessed the effects of treatments with gonadotropins after AI on fertility in dairy cattle, with contradictory results (Peters et al., 2000; Santos et al., 2001; Willard et al., 2003; Bartolome et al., 2005; Stevenson et al., 2007). In contrast, few studies have evaluated these hormonal treatments on fertility of lactating dairy cows after ET. The rationale of treatments with gonadotropins during the early luteal phase is to induce ovulation of the first postovulation

dominant follicle, which will increase circulating concentrations of progesterone, due to the formation of an accessory CL (Santos et al., 2001), and may change the pattern of follicular development, increasing the occurrence of 3-wave follicular cycles (Diaz et al., 1998). These effects of gonadotropin treatment will potentially enhance conception rates (Ahmad et al., 1997; Santos et al., 2001). In the current studies, administering 100 µg of GnRH or 2,500 IU of hCG 7 d after the induced ovulation in lactating dairy cows increased serum concentrations of P4 on d 14. In contrast, treatment

Table 4. Milk yield, days postpartum, and percentage of pregnancy according to the categorized rectal temperature on d 7 in lactating Holstein cows submitted to timed AI (TAI) on d 0 or embryo transfer (TET) on d 7 after induced ovulation (experiment 1)

Dependent variable	Categorized rectal temperature on d 7, °C					<i>P</i> -value (temperature)
	≤37.9	38.0–38.4	38.5–38.9	39.0–39.4	≥39.5	
Milk yield, kg						
Cows submitted to TAI	36.7 ± 2.4	38.4 ± 0.9	37.8 ± 0.7	37.1 ± 1.0	36.8 ± 2.1	0.544
Cows submitted to TET	37.5 ± 3.2	37.5 ± 1.2	37.3 ± 0.7	37.8 ± 0.9	37.9 ± 2.0	0.819
Pregnant cows, ¹ % (n/n)						
at TAI on d 28	52.4	37.4	29.3	26.7	21.0	0.047
	(11/21)	(46/123)	(58/198)	(27/101)	(4/19)	
at TET on d 28	46.1	48.0	44.4	44.0	40.0	0.959
	(6/13)	(47/98)	(72/162)	(37/84)	(6/15)	
<i>P</i> -value (breeding technique)	0.705	0.101	0.002	0.009	0.161	
Pregnant cows, ¹ % (n/n)						
at TAI on d 60	47.6	32.5	25.2	23.8	15.8	0.052
	(10/21)	(40/123)	(50/198)	(24/101)	(3/19)	
at TET on d 60	38.5	39.8	37.0	35.7	33.3	0.904
	(5/13)	(39/98)	(60/162)	(30/84)	(5/15)	
<i>P</i> -value (breeding technique)	0.594	0.245	0.008	0.053	0.137	

¹Pregnancy was evaluated by ultrasonography on d 28 and 60. Percentage of pregnant cows was calculated by dividing the number of pregnant cows by the number of cows having a corpus luteum on d 7.

Table 5. Percentages of pregnancy by breeding technique, categorized milk yield, and categorized rectal temperature on d 7 in lactating Holstein cows (experiment 1)

Breeding technique ¹	Categorized milk yield, kg	Categorized rectal temperature, °C	Pregnant cows, ² % (n/n)	
			on d 28	on d 60
TAI	≤37.7	≤39.0	34.1 (56/164)	28.6 (47/164)
		>39.0	21.3 (10/47)	19.1 (9/47)
	>37.7	≤39.0	34.8 (73/210)	30.9 (65/210)
		>39.0	17.1 (7/41)	14.6 (6/41)
TET	≤37.7	≤39.0	43.3 (71/164)	37.2 (61/164)
		>39.0	42.5 (20/47)	38.3 (18/47)
	>37.7	≤39.0	48.8 (59/121)	37.2 (45/121)
		>39.0	45.0 (18/40)	37.5 (15/40)
<i>P</i> -value				
Breeding technique			0.003	0.015
Milk yield			0.582	0.394
Rectal temperature			0.047	0.031
Breeding technique × milk yield			0.820	0.942
Breeding technique × rectal temperature			0.079	0.060
TAI (≤39.0 vs. >39.0°C)			0.021	0.013
TET (≤39.0 vs. >39.0°C)			0.481	0.390
≤39.0°C (TAI vs. TET)			<0.001	0.002
>39.0°C (TAI vs. TET)			<0.001	0.005
Milk yield × rectal temperature			0.651	0.797
Breeding technique × milk yield × rectal temperature			0.857	0.558

¹Timed AI (TAI) were performed on d 0, whereas timed embryo transfers (TET) were on d 7.

²Pregnancy was evaluated by ultrasonography on d 28 and 60. Percentage of pregnant cows was calculated by dividing the number of pregnant cows by the number of cows having a corpus luteum on d 7.

with gonadotropins on d 7 increased the percentage of pregnant cows on d 28 and 60 only when cows were submitted to TET; no effects of treatments on fertility were detected when cows were submitted to TAI. Furthermore, additional treatment with 100 µg of GnRH 7 d after TET in lactating dairy cows that had received a previous GnRH treatment concurrently with TET did not enhance their reproductive performance.

Ovulation rates were very high (96%) when GnRH was administered between d 5 and 9 of the estrous

cycle (Vasconcelos et al., 1999). In a previous study with Holstein heifers and cows receiving gonadotropins 5 d after estrus, 91% of the cows treated with 3,000 IU of hCG and 93% of cows treated with 8 µg of buserelin formed accessory CL (Schmitt et al., 1996a), whereas others reported that treatments with 3,300 IU of hCG (Santos et al., 2001; Stevenson et al., 2007) or 100 µg of GnRH (Willard et al., 2003; Stevenson et al., 2007) administered 5 or 7 d after induced ovulation increased the percentage of lactating dairy cows having multiple

Table 6. Milk yield, rectal temperature on d 7, serum concentrations of progesterone on d 7, and percentages of pregnancy by parity and breeding technique in lactating Holstein cows (experiment 1)

Parity	Breeding technique ¹	Milk yield, kg	Rectal temperature on d 7, °C	Progesterone on d 7, ng/mL	Pregnant cows, ² % (n/n)	
					on d 28	on d 60
Primiparous	TAI	37.3 ± 0.7	38.7 ± 0.1	2.95 ± 0.09	38.7 (70/181)	34.8 (63/181)
	TET	37.0 ± 0.7	38.8 ± 0.1	2.87 ± 0.09	46.2 (73/158)	40.5 (64/158)
Multiparous	TAI	38.0 ± 0.5	38.6 ± 0.1	2.85 ± 0.07	27.0 (76/281)	22.8 (64/281)
	TET	37.8 ± 0.6	38.7 ± 0.1	2.81 ± 0.08	44.4 (95/214)	35.0 (75/214)
<i>P</i> -value						
Parity		0.273	0.181	0.743	0.019	0.008
Breeding technique		0.322	0.168	0.761	0.003	0.015
Parity × breeding technique		0.503	0.848	0.868	0.044	0.035
Primiparous (TAI vs. TET)		NS	NS	NS	0.075	0.102
Multiparous (TAI vs. TET)		NS	NS	NS	<0.001	0.001
TAI (primiparous vs. multiparous)		NS	NS	NS	0.013	0.006
TET (primiparous vs. multiparous)		NS	NS	NS	0.792	0.529

¹Timed AI (TAI) were performed on d 0, whereas timed embryo transfers (TET) were on d 7.

²Pregnancy was evaluated by ultrasonography on d 28 and 60. Percentage of pregnant cows was calculated by dividing the number of pregnant cows by the number of cows having a corpus luteum on d 7.

Table 7. Percentage of pregnancy by categorized milk yield and rectal temperature on d 7 (d 0 = induced ovulation) in lactating Holstein cows submitted to timed embryo transfer on d 7 (experiment 2)

Categorized milk yield, kg	Categorized rectal temperature, °C	Pregnant cows, ¹ % (n/n)	
		on d 28	on d 60
≤36.3	≤39.0	42.6 (43/101)	34.6 (35/101)
	>39.0	31.1 (14/45)	26.7 (12/45)
>36.3	≤39.0	56.9 (58/102)	50.0 (51/102)
	>39.0	40.5 (15/37)	32.4 (12/37)
<i>P</i> -value			
Milk yield		0.079	0.1524
Rectal temperature		0.021	0.037
Milk yield × rectal temperature		0.801	0.502

¹Pregnancy was evaluated by ultrasonography on d 28 and 60. Percentage of pregnant cows was calculated by dividing the number of pregnant cows by the number of cows that received an embryo.

CL. In agreement with many previous reports (Schmitt et al., 1996a; Diaz et al., 1998; Santos et al., 2001; Willard et al., 2003; Stevenson et al., 2007), administration of gonadotropins during early diestrus in experiment 1 increased circulating concentrations of P4 after treatments. The increase of circulating concentrations of P4 after treatments with gonadotropins during early and mid estrus has been mostly associated with the formation of an accessory CL (Schmitt et al., 1996b; Diaz et al., 1998). In contrast with some studies in dairy cows in which hCG caused a greater luteotropic effect than GnRH (Schmitt et al., 1996a,b; Stevenson et al., 2007; Beltran and Vasconcelos, 2008), serum concentration of P4 on d 14 was similar between cows treated with hCG or GnRH in the current study.

The role of postovulatory circulating concentrations of P4 on fertility of ruminants has been extensively investigated. Several studies indicated a positive interrelationship between circulating P4 after AI and pregnancy rates in dairy (Santos et al., 2001; Vasconcelos et al., 2001) and beef (Peres et al., 2009) cattle. Interestingly, in a study comparing AI and ET in lactating Holstein cows in Brazil (Demetrio et al., 2007), circulating concentrations of P4 on d 7 of the estrous cycle were positively related to conception rate at AI but not at ET. Reproductive efficiency of dairy cows has decreased as genetic merit for milk production has increased (Butler, 1998; Lucy, 2001; Washburn et al., 2002), and this negative correlation has been attributed to the fact that high-producing dairy cows have decreased circulating concentrations of P4 (Vasconcelos et al., 1999; Sartori et al., 2002b), perhaps because a greater DM intake increases liver blood flow and metabolic clearance rate of steroids (Sangsrivong et al., 2002; Vasconcelos et al., 2003). During early pregnancy, P4 influences the endometrial secretion of nutrients, growth factors, immunosuppressive agents, enzymes, ions, and steroids that are critical for embryo development (Graham and Clarke, 1997) and production of

adequate quantities of interferon- τ (Mann et al., 1999). Because administration of gonadotropins between d 5 and 7 of the estrous cycle increases circulating concentrations of P4 and increases the percentage of cows experiencing 3-wave follicular cycles (Schmitt et al., 1996a), the potential exists for a positive effect of these treatments on pregnancy rates. Because high-producing dairy cows have reduced circulating concentration of P4, they are more likely to be responsive to treatments that increase luteal function, such as administration of gonadotropins during the early diestrus (Thatcher et al., 2006). Moreover, the alteration in the number of follicular waves during the estrous cycle has been found to benefit the likelihood of conception (Ahmad et al., 1997), perhaps because cows with 3-wave cycles have a delay in development of potential estrogenic follicles and a longer luteal lifespan than those with 2-wave cycles (Diaz et al., 1998; Araújo et al., 2009), which is favorable to early embryo survival.

In experiment 1, the percentages of pregnant cows on d 28 and 60 were increased by treatments with gonadotropins in cows submitted to TET, but not in those submitted to TAI. In a study performed in Florida during November to April (Bartolome et al., 2005), treatments with GnRH on d 5 or 15, or both, after TAI did not improve pregnancy rates, whereas Santos et al. (2001), in California, found a positive effect of treatment with 3,300 IU of hCG 5 d after estrus on conception during October to March, but treatment had no effect during the warmer months of May to September. During the summer in Brazil, Beltran and Vasconcelos (2008) observed that treatments with hCG or GnRH 5 d after AI improved conception rates only in cows having rectal temperature <39.7°C at the time of treatments, and a study in Florida during summer heat stress failed to show improvement in conception rate following treatment 3,000 IU of hCG on d 5 after AI (Schmitt et al., 1996a). Thus, although studies have reported conflicting results on conception rate of lactating dairy cows

submitted to AI, it seems that heat load is an important factor affecting success of postbreeding treatments with gonadotropins. In contrast, even in cows submitted to ET, in which conception is less affected by heat load, results have been contradictory. In agreement with the results of experiment 1, Nishigai et al. (2002) verified a positive effect of treatment with hCG 6 d after estrus on conception rate at ET in beef cows. In contrast, in a study with lactating cows in California from August to April, treatment with hCG on d 5 of the estrous cycle did not improve pregnancy establishment after frozen-thawed ET (Galvão et al., 2006).

The approach of ET permits all recipients to have a viable embryo in the reproductive tract at d 7, and therefore, recipients may be more likely to respond to the postbreeding treatments. In a previous study, circulating concentrations of P4 did not affect conception at ET (Demetrio et al., 2007); therefore, it is likely that the beneficial effect of treatment with gonadotropins on d 7 was due to changes on follicular wave patterns (increased percentage of cows experiencing 3 follicular wave cycles and delayed luteolysis). On the other hand, in cows submitted to AI, heat stress may have affected conception rates by decreasing oocyte competence (Al-Katanani et al., 2002), fertilization rate (Sartori et al., 2002a), and early embryo development and survival (Ealy et al., 1993; Ryan et al., 1993; Ju et al., 1999; Sartori et al., 2002a). In previous studies, conception rates of cows decreased sharply when maximum daily temperature exceeded 30°C (Badinga et al., 1985), and an increase of 0.5°C above basal uterine temperature at the time of insemination and 14.5 ± 4.6 h after insemination resulted in decreases of conception rates of 12.8 and 6.9%, respectively (Gwazdauskas et al., 1973). Because cows exposed to heat stress before ovulation or during the first 2 to 3 d after fertilization experience low fertilization rates or high percentages of early embryonic death (Hansen and Aréchiga, 1999), it is unlikely that increasing circulating concentrations of P4 or changing the follicular wave pattern by treatment with gonadotropins after AI would improve conception rate of heat-stressed dairy cows, which agrees with previous research (Schmitt et al., 1996a; Santos et al., 2001).

In experiment 2, an additional treatment with GnRH 7 d after TET did not increase the percentage of pregnant cows on d 28 and 60, which agrees with Bartolome et al. (2005) who found no effects of treatment with GnRH on either d 5 or d 15 after TAI on pregnancy rates in lactating dairy cows. This lack of response may be due to a low ovulation rate to the treatment with GnRH on d 14, because cows were likely to have high circulating concentrations of P4 on d 14 and a negative relationship between circulating concentrations of P4 and GnRH-induced LH surge was described in

beef cows (Stevenson et al., 2000), or due to an inefficiency of the second treatment with GnRH to delay luteolysis, even if it caused ovulation. We were unable to determine why pregnancy rates were affected by rectal temperature in experiment 2 but not in TET cows of experiment 1, but a possible reason is the fact that experiment 1 was conducted over a full year and experiment 2 was conducted only during winter and spring.

The greater pregnancy rates at AI in primiparous than in multiparous cows observed in experiment 1 are in agreement with previous reports (Chebel et al., 2004; Santos et al., 2009). It is important to note that this result was not due to milk yield, rectal temperature, or circulating concentrations of P4, because these variables did not differ between primiparous and multiparous cows. Chebel et al. (2004) suggested that the greater conception rate in primiparous cows might be partially explained by a greater incidence of postparturient diseases in multiparous compared with primiparous cows, such as retained placenta and milk fever, because cows experiencing those diseases were less likely to conceive than those not experiencing them. In contrast, when cows were submitted to ET, no effects of parity were observed on the percentages of pregnant cows, indicating that multiparous cows may experience a decrease in oocytes or early embryo competence. In cows exposed to heat stress, embryos were found to grow less (Biggers et al., 1987), likely reducing their capacity to produce IFN- τ . Considering that cows were exposed to the effects of heat load throughout the experimental period, we speculate that in a multiparous cow, that has a larger uterus, the production of IFN- τ by the heat-stressed embryo is not sufficient to prevent luteolysis. On the other hand, in a primiparous cow, lesser concentrations of IFN- τ may be sufficient to decrease PGF_{2 α} release during the critical period, because of the smaller uterus. The similarity between primiparous and multiparous cows submitted to TET may result from the fact that both received an embryo that was developed into a non-heat-stressed female during the first 6 d after fertilization, whereas in cows submitted to TAI, embryos were exposed to heat stress during a period of greater thermosensitivity. The reason why primiparous cows had greater pregnancy rates than multiparous cows cannot be explained by the current experimental design. Further studies evaluating the interrelationships between uterine and conceptus sizes on conception are required.

Decreased reproductive performance of dairy cows has been attributed, at least partially, to the high metabolic demands associated with high milk yield (Lucy, 2001). In contrast, research studies reported both negative (Vasconcelos et al., 2006) and positive effects (Nebel and McGilliard, 1993) of milk production on reproduc-

tive variables measured on dairy herds. Although Lucy (2001) suggested that an additive effect between body temperature and milk production might exist in regards to the decreased reproductive performance of dairy cattle, reduced pregnancy rates due to increased rectal temperatures in the present study were independent of milk production. Moreover, during winter and spring, when milk yield increased compared with summer and fall, pregnancy rates did not decrease. Thus, it is possible that the relationship observed by Lucy (2001) is a function of the higher incidence of postparturient problems and greater susceptibility to heat stress, among other factors, in high-producing dairy cows, and not necessarily the greater milk production per se affecting fertility. In fact, Santos et al. (2004) reported in their review that milk production alone does not affect late embryonic and fetal losses in dairy cows and concluded that there is little or no indication that milk production is a risk factor for reduced pregnancy establishment and maintenance in dairy cattle. These conclusions are consistent with those from the current study, in which rectal temperature was a factor of greater effect on conception rates than milk yield, indicating that high-producing dairy cows may not experience reduced fertility if management conditions and body temperatures are adequate, as others also reported (Al-Katanani et al., 1999; Lopez-Gatius, 2003).

CONCLUSIONS

In conclusion, in lactating Holstein cows under tropical climate, treatment with either 100 µg of GnRH or 2,500 IU of hCG on d 7 after induced ovulation improved pregnancy rates at TET, but not at AI (experiment 1). Because severe embryonic damage occurs very early after AI when body temperature is high, postbreeding treatments with gonadotropins are likely to improve conception rate at TAI if a high percentage of cows have a good quality embryo in their reproductive tract. Moreover, treatment with GnRH 7 d after TET did not enhance reproductive performance of cows that received a previous GnRH treatment concurrent with TET (experiment 2). The results of the current study also indicate that in a tropical climate use of in vivo-produced fresh embryos is an efficient strategy to improve reproductive performance of high-producing dairy cows because, their results are less affected by some variables, such as rectal temperature, parity, and circulating P4 compared with that achieved by AI.

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