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Conception rate in Holstein cows treated with GnRH or hCG on the fifth day post artificial insemination during summer

[*Taxa de concepção de vacas Holandesas tratadas com GnRH ou hCG no quinto dia após a inseminação artificial no verão*]

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ABSTRACT

Lactating Holstein cows (n=158), at 213±112 days in milking and averaging 26±9kg of milk per day, were randomly assigned to one of three treatment groups: control (CG, n=52, saline), GnRH (GG, n=55, 100μg gonadorelin), and hCG (HG, n=51, 2500IU) given five days after artificial insemination (AI). Rectal temperature was taken at the moment of AI and blood samples were collected five, seven, and 12 days after AI. Pregnancy was determined between 42 and 49 days after AI. Concentration of progesterone (P4) in serum (ng/ml, mean±SE) for CG, GG, and HG were, respectively, 2.7±0.4, 2.5±0.4, and 3.2±0.5 on day 5; 4.8±0.4, 4.2±0.4, and 5.7±0.5 on day 7; and 5.2±0.4, 6.9±0.4, and 8.5±0.5 on day 12 after AI. P4 concentration had proportional increase in serum between days 5 and 7 after AI (CG: 178%, GG: 168%, and HG: 178%), suggesting that the treatments did not induce a luteotropic effect on the existing corpus luteum (CL). Concentrations of P4 increased between days 7 and 12 in cows treated with GnRH and hCG (GG: 164%, and HG: 149%, P<0.01); but not in control cows (GC: 18%, P=0.31), suggesting that a new CL was formed. Treatments with GnRH or hCG increased conception rates in cows with rectal temperature below 39.7°C (CG: 10.1%, n=26, GG: 36.8%, n=27; and HG: 32.8%, n=21), but not in cows with rectal temperature above 39.7°C (CG: 15.2%, n=26; GG: 17.8%, n=28; and HG: 24.4%, n=30). These data suggest that high body temperature masked the positive impact of treatment with GnRH or hCG on day 5 after AI on conception.

Keywords: dairy cow, conception, progesterone, hCG, GnRH

RESUMO

Vacas da raça Holandesas em lactação (n=158) aos 213±112 dias de lactação e produção de 26±9kg leite/dia, foram aleatoriamente distribuídas em três grupos: controle (GC, n=52, salina); GnRH (GG, n=55, 100mcg de gonadorelina); e hCG (GH, n=51, 2500UI de hCG) aplicado no dia 5 após a inseminação artificial (IA). A temperatura retal foi verificada no momento da IA, e as amostras de sangue coletadas nos dias 5, 7 e 12 após a IA. A concepção foi determinada entre os dias 42 e 49 após IA. As concentrações séricas de progesterona (P4 - ng/ml, média±EPM) para GC, GG, e GH foram, respectivamente: no dia 5: 2,7±0,4, 2,5±0,4 e 3,2±0,4; no dia 7: 4,8±0,4, 4,2±0,4 e 5,7±0,5; e no dia 12 após a IA: 5,2±0,4, 6,9±0,4 e 8,5±0,5. O aumento proporcional na concentração sérica de P4 entre os dias 5 e 7 após IA (GC: 178%, GG: 168%, e GH: 178%) sugere que os tratamentos não induziram efeito luteotrópico no corpo lúteo (CL) existente. O aumento na P4 sérica entre os dias 7 e 12 nos animais tratados com GnRH ou hCG (GG: 164% e GH: 149%, P<0,01) em relação aos animais controle (GC: 18%, P=0,31), sugere a indução de novo CL. Os tratamentos com GnRH ou hCG aumentaram as taxas de concepção nas vacas com temperatura retal abaixo de 39,7°C (GC: 10,1%, n=26; GG: 36,8%, n=27 e

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GH: 32,8%, n=21), mas não em vacas com temperatura retal acima de 39,7°C (15,2% n=26; 17,8%, n=28 e 24,4%, n=30). Os resultados sugerem que a alta temperatura corporal pode mascarar os efeitos positivos do tratamento com GnRH ou hCG no dia 5 após a IA, na concepção.

Palavras-chave: vaca leiteira, concepção, progesterona, hCG, GnRH

INTRODUCTION

Dairy cows with high milk production have low conception rates, which causes major economic losses (Nebel and McGilliard, 1993), and have lower progesterone (P4) serum concentrations, probably due to higher progesterone clearance (Vasconcelos et al., 1999). Low P4 concentration in blood would partially explain the low fertility in high producing dairy cows, as P4 concentrations after artificial insemination (AI) are positively associated with embryo maturity and functionality (Garret et al., 1988), which is critical for inhibiting luteolysis and maintaining pregnancy (Mann et al., 1995; Meyer et al., 1995). Therefore, it is possible to conclude that part of the embryonic losses in dairy cattle may be due to inadequate maternal luteal function (Lamming et al., 1989; Mann et al., 2001). Conception can also be significantly compromised by high ambient temperature influencing body temperature, which is typical of thermal stress (Ealy et al., 1993). Lactating cows are more sensitive to high temperatures due to the heat generated by increased metabolism associated with high feed intake and milk production (Fuquay, 1981); thereby, suggesting a negative interaction between milk production and heat stress on reproductive performance of dairy cows (Al-Katanani et al., 1999).

The use of GnRH (Lewis et al., 1990; Schmitt et al., 1996a) or hCG (Mancio et al., 1999; Santos et al., 2001) after AI increases P4 secretion due to the luteotropic effect (Kerbler et al., 1997; Santos et al., 2001) and/or induction of an accessory corpus luteum (aCL) (Schmitt et al., 1996a; Fonseca et al., 2001; Santos et al., 2001); thus, increasing progesterone concentration and, potentially, also conception.

The objective of the present study was to evaluate the effects of GnRH or hCG given five days after AI on serum P4 concentrations at days five, seven, and 12 after AI, and on conception rate in lactating Holstein cows during the summer.

MATERIAL AND METHODS

The experiment was carried out in the state of São Paulo, Brazil, from of January to April.

Lactating Holstein cows (n=158) at 213±112 days in milk (DIM) and averaging 26±9kg of milk per day were used. They were fed a total mixed ration of corn silage and grains that was balanced according to nutritional requirements based on milk production. Cows were housed by lactation number, days postpartum, and milk production, and were milked three times daily.

Cows were inseminated 12h after observed in spontaneous estrus with semen from eight sires by two AI techniques. Rectal temperature (RT) was taken at the moment of AI and ambient temperature and humidity were daily measured throughout the study. Milk production was weekly evaluated for individual cows and the utilized value was the mean of the production in the weeks before and immediately after the treatments.

Treatments were administered in the morning of day 5 after AI, which was also day 5 of the estrous cycle (day 0 = estrus). Cows were randomly assigned to one of the three treatment groups that received: IM injection of saline (control group – CG, n=52); IM injection of GnRH (GG group, n=55, 100µg gonadorelin[1]); and IM injection of hCG (HG group, n=51, 2500IU hCG[2]). Twenty-two animals from each treatment were randomly selected to have blood sampled in the morning of days 5, 7, and 12 after AI by puncture of the median coccygeal vein or artery with evacuated tubes with no additives. The blood samples were refrigerated at 4°C for 24h and then centrifuged (1200 x g for 15 minutes) for separation of serum. Serum was frozen and stored at -20°C until radioimmunoassay for P4 determination (Knickerbocker et al., 1986). Three assays were

[1]GnRH, Cystorelin®, Merial - São Paulo, Brazil.
[2]hCG, Vetecor®, Serono Veterinária - São Paulo, Brazil.

performed and intra-assay coefficients of variation were 7.6, 8.2, and 5.3, with inter-assay coefficient of variation of 7.9 and assay sensitivity of 0.3ng/ml.

Pregnancy was diagnosed by palpation per rectum of the uterine contents between days 42 and 49 after AI.

Concentrations of progesterone in serum were analyzed using the Mixed procedure of SAS (User's…, 2003), according to the repeated measurements within the same experimental unit. The model included the effects of treatment, day of blood collection, RT, milk production, parity (primiparous and multiparous), and interactions related to each pair of factors. Rectal temperature and milk production were categorized as above or below the mean values of 39.7°C and 26.0kg/day, respectively.

Conception rate was also analyzed by the Mixed procedure of SAS (User's…, 2003), and the model included the effects of treatment, RT, milk production, lactation number, and the respective interactions. Sire and inseminator were initially included in the model, but because no significance caused by then was observed, they were removed.

RESULTS

The mean ambient temperature throughout the study was 26.2±4.4°C, with a maximum of 39.8°C and minimum of 20.0°C. Mean relative humidity was 84%, and varied between 56 and 92%.

No difference in P4 serum concentration (ng/ml) on day 5 before treatment was observed among the groups; however, on day 7, there was a difference between the GG and HG animals ($P<0.01$, Table 1). On day 12, P4 serum concentration in HG group was significantly higher than in GG animals, and both were significantly higher than controls ($P<0.01$, Table 1). P4 serum concentration increased between days 5 and 7 in all three groups ($P<0.01$). There was no increase in the concentration of P4 in the CG animals between days 7 and 12; whereas, there was significantly higher ($P<0.01$) concentration of P4 on day 12 relative to day 7 in animals of GG and GH groups (Table 1).

Table 1. Concentration of progesterone in serum (ng/ml) on days 5, 7, and 12 after artificial insemination (AI) in cows submitted to the three treatments on day 5 after AI (mean±SEM)

Day/Group	Control (n=22)	GnRH (n=22)	hCG (n=22)
Day 5	2.7±0.43aB	2.5±0.44aC	3.2±0.45aC
Day 7	4.8±0.43abA	4.2±0.44bB	5.7±0.45aB
Day 12	5.2±0.43cA	6.9±0.44bA	8.5±0.45aA

Values in the rows with different lower case letters are statistically different ($P<0.01$).
Values in the columns with different upper case letters are statistically different ($P<0.01$).

Concentrations of P4 in serum between days 5 and 7, and days 7 and 12 were calculated considering hormone concentration as 100% on days 5 and 7, respectively as shown in Table 1. Increases in P4 from day 5 to 7 were proportional for CG (178%), GG (168%), and HG cows (178%, Fig. 1). On day 12, serum P4 concentrations were significantly higher ($P<0.01$) than day 7 for GG (164%) and HG cows (149%), with no significant increase in CG cows (18%, $P=0.31$) (Fig. 1). Rectal temperature, milk production, and lactation number had no effect on concentrations of P4 in serum on days 5, 7, and 12 after AI.

No interaction ($P<0.10$) effect of treatment x RT at AI was observed on conception rate. Treated cows (GG and HG groups) had higher conception rate than CG cows when RT was <39.7°C at AI, but when RT was ≥39.7°C at AI, treatment did not influence conception (Table 2). Milk yield and lactation number did not influence conception rates.

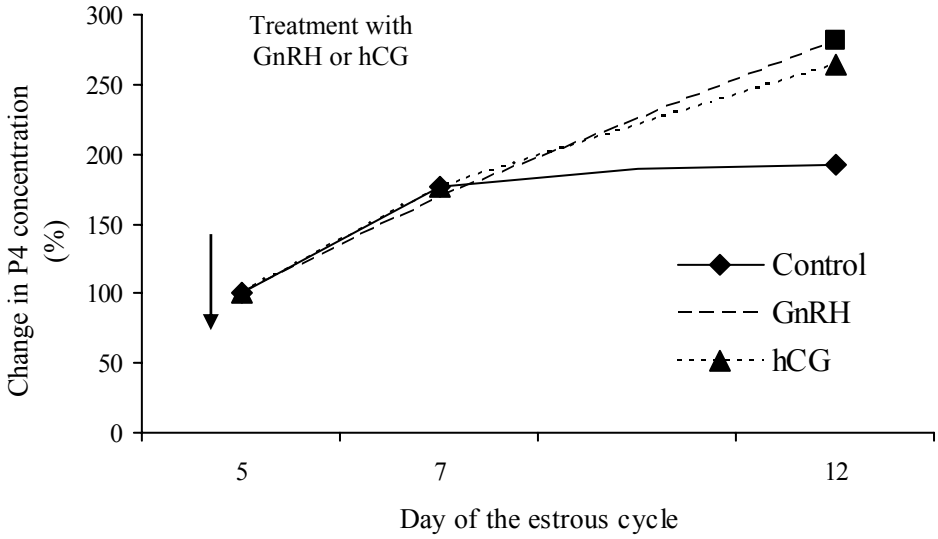

Figure 1. Percentage increase in serum concentrations of progesterone (P4) between days 5 to 7 and 7 to 12 after AI in control, GnRH, and hCG groups, considering concentrations of P4 on day 5 as 100%.

Table 2. Conception rate (adjusted means) according to treatment on day 5 after artificial insemination (AI) and rectal temperature at AI

Treatment	Rectal temperature	
	<39.7°C	>39.7 °C
Control (n=52)	10.1 (3/26)aB	15.2 (4/26)aA
GnRH (n=55)	36.8 (9/27)aA	17.8 (6/28)bA
hCG (n=51)	32.8 (7/21)aA	24.4 (7/30)aA

Values in the rows with different lower case letters are statistically different (P<0.10)
Values in the column with different upper case letters are statistically differents (P<0.10).

DISCUSSION

Changes in progesterone concentrations from day 5 to 7 after AI were proportional in all three groups, which suggest that a possible luteotropic effect of hCG and GnRH was not detected in treated animals. This finding is in agreement with Schmitt et al. (1996a), who evaluated P4 concentration and the original CL morphology and functionality on day 17 in cows treated with GnRH or hCG on day 5 after AI, and did not find increase in P4 concentration or cellular alterations that justified the luteotropic effect. However, Kerbler et al. (1997) observed luteotropic effects as determined by increases in P4 concentrations when cows were treated with 1500IU of hCG on day 5 after AI. In the current study, administration of 100μg gonadorelin or 2500IU hCG on day 5 after AI, significantly increased progesterone concentrations between days 7 and 12 relative to control cows. This response may be the results of induced ovulation of the first-wave dominant follicle and formation of an accessory CL, as cows on day 5 of the estrous cycle should have a dominant follicle capable of ovulation in response to either LH (Xu et al., 1995; Sartori et al., 2001) or GnRH/hCG administration (Schmitt et al., 1996a; Diaz et al., 1998).

The highest increase in serum concentrations of P4 at day 12 in HG cows than GG cows can be attributed to the higher steroidogenicity of CL induced by hCG than the induced by GnRH (Chenault et al., 1990; Schmitt et al., 1996a; Schmitt et al., 1996b; Fonseca et al., 2001). The longer half-life in blood (30h) of hCG (Chenault et al., 1990) and the slower turnover of LH receptors activated by hCG on the surface of granulosa cells are probably responsible for a greater gonadotropic stimulation of the ovulatory follicle upon treatment on day 5. On the other

hand, treatment with GnRH increases plasma LH concentrations for only 5h, considerably shorter that a spontaneous ovulatory surge of LH, which may explain the difference in steroidogenesis of the induced CL when cows were treated with hCG compared with GnRH (Schmitt et al., 1996a). The effects of hCG on the follicular cells and subsequent differentiation into luteal cells with higher steroidogenic probably explain the highest concentrations of P4 when ovulation was induced by hCG as compared with GnRH.

Rectal temperature at AI did not influence concentrations of progesterone in serum at days 5, 7, and 12 of the estrous cycle. Studies in the literature have suggested that high ambient temperatures, characteristic of thermal stress, can cause decreased steroidogenesis in follicular cells (Badinga et al., 1993), and decreased systemic concentrations of P4 (Wolfenson et al., 1993). However, findings of this study observed that treatments were effective in increasing progesterone concentrations between days 7 and 12 after AI independently upon the rectal temperature of the cow, which suggests that changes in body temperature were unlikely to affect response to treatment and influence serum concentrations of progesterone in dairy cows.

In this study, increased conception was observed after treatment with hCG or GnRH in cows with rectal temperatures <39.7°C at AI, but not in those with rectal temperature ≥39.7°C at AI ($P<0.10$). This higher conception rate can be attributed to an increased concentration of P4 in serum during mid diestrus as result of the induced CL. Increases in endogenous progesterone concentrations are associated with increased embryo development and pregnancy maintenance, especially during the early stages of diestrus (Mann et al., 2001). The accessory CL formed after induction of ovulation of the first-wave dominant follicle on day 5 or 7 of the estrous cycle (Schmitt et al., 1996b; Diaz et al., 1998; Santos et al., 2001) and consequent increase in P4 concentration is expected to minimize the luteolytic cascade by endometrial cells during the period of maternal recognition of pregnancy, which favors pregnancy maintenance (Meyer et al., 1995; Kerbler et al., 1997; Mann et al., 2001). Pregnancy rates were improved in dairy cows treated with hCG on day 5 after AI, and pregnancy rates increased with increased concentrations of progesterone or with more CL on day 14 after AI (Santos et al., 2001). These data agree with those of Schmitt et al. (1996a) and Diaz et al. (1998), who found increased concentration of P4 in plasma between days 6 and 13 of the estrous cycle due to the presence of an induced CL on day 5.

The failure of GnRH and hCG treatments to improve conception in animals with RT ≥39.7°C at AI is probably due to reduced fertilization or compromised early embryo development caused by high body temperature (Hansen and Arechiga, 1999). Fertilization and embryo development are most susceptible to high body temperature from estrus to the first 24h after AI (Ealy et al., 1993). The effects of heat stress on fertilization and early embryo development are likely to negate the possible benefits of increasing progesterone concentrations by inducing an accessory CL. Similarly, Santos et al. (2001) observed a benefit to hCG treatment on conception rates in cows inseminated during the cool season, but not during the heat stress season. If thermal stress compromised embryo development, the resulting underdeveloped embryos are less capable of blocking luteolysis (Garret et al., 1988). These results support the hypothesis that losses due to elevated temperatures are due to effects on either fertilization or early embryonic development and increased progesterone during mid diestrus is not able to overcome this detrimental effect.

Milk production and lactation number did not influence concentrations of P4 in serum concentration and conception rates, although reports in the literature suggest, in some cases, a negative association between milk production and progesterone concentrations or conception in dairy cows (Nebel and McGilliard, 1993; Vasconcelos et al., 1999).

Results of this study indicated that treatment with hCG or GnRH to induce ovulation of a first wave dominant follicle on day 5 of the estrous cycle successfully increased concentrations of progesterone during mid luteal phase in lactating dairy cows. The positive effects of treatments on progesterone concentration in serum of lactating dairy cows were observed in spite of exposure to thermal stress. However, treatment with GnRH or hCG to induce an accessory CL and increase concentration of progesterone in serum were only effective at improving pregnancy when cows had low rectal temperature (<39.7°C),

showing that the harmful effects of high RT at AI prevailed over the benefits of the progesterone increase. Strategies to decrease embryonic loss during early pregnancy, other than enhancing progesterone concentrations during diestrus are warranted when cows experience high body temperatures.

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