

The ovulatory response to human chorionic gonadotropin administration on day 4 post timed artificial insemination improved fertility in repeat breeder cows

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Abstract

This study focused on determining the success of inducing endogenous progesterone source (by formation of accessory corpus luteum) by human chorionic gonadotropin (hCG) administration on day 4 post timed artificial insemination (TAI) and on evaluating the effect of the day of hCG administration (day 4 or 5 post TAI) on pregnancy rates in repeat breeder cows according to their responses to hCG administrations. Non-pregnant cyclic lactating Holstein dairy cows with more than three services despite no clinical reproductive abnormalities were enrolled in the study. All cows (n = 192) received the Ovsynch protocol (GnRH-7d-PGF_{2α}-56h-GnRH-16/18h-TAI) and hCG administration (1500 IU, i.m.) was performed in group hCG4 (n = 64) on day 4 and in group hCG5 (n = 68) on day 5 post TAI. The control group (CON, n = 60) did not receive any hCG treatment. The responses (ovulation rate) to hCG (~64%) were similar on days 4 and 5 post TAI. Pregnancy per AI (P/AI) at 31 days was similar among the groups (48.3% in CON, 48.4% in hCG4, and 42.6% in hCG5). Regardless of the day of the treatment (hCG4 + hCG5), the cows had an ovulatory response to hCG (54.1%), and a greater P/AI (P = 0.007) at 31 days than those without a response (29.8%). The administration of hCG on either day 4 or 5 post TAI did not increase overall pregnancy rates, however, the ovulatory response to the post TAI hCG administration on day 4 improved fertility in repeat breeder cows. Further studies are needed with a larger number of animals to reveal this potentially beneficial effect.

Cattle, repeat breeding, hCG, post TAI

Repeat breeder (RB) cows are generally defined as cows that have no pregnancy after more than three services despite normal cyclicity and healthy genital tracts (Zemjanis 1980). Repeat breeding causes important financial loss on dairy farms because of increased calving intervals, culling rates, and insemination costs. Since there are multiple factors that correlate with repeat breeding in cows, the specific reasons remain speculative (Gustafsson and Emanuelson 2002). Most prominent aetiological factors include oestrus detection errors, infections, and hormonal disturbances leading to embryonic mortalities (Gustafsson and Emanuelson 2002; Opsomer 2014). Hormonal disturbances such as delayed progesterone increases (Shelton et al. 1990; Bage et al. 2002; Moss et al. 2002; Kendall et al. 2009) and decreased total concentrations of progesterone after ovulation (Shelton et al. 1990; Bage et al. 2002) may induce embryonic mortalities which occur mostly 6 to 8 days after timed artificial insemination (TAI) in RB cows (Shelton et al. 1990). Since progesterone plays a critical role in embryonic development and continuation of pregnancy, several studies have focused on either exogenous or endogenous progesterone supplementation during the post-TAI period to increase the pregnancy rate (Tefera et al. 2001; Mann et al. 2006; Khoramian et al. 2011; Wiltbank et al. 2011). Endogenous progesterone can be stimulated by induction of ovulation of the dominant follicle generated

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from the first follicular wave after TAI using either gonadotropin releasing hormone (GnRH) or human chorionic gonadotropin (hCG) administration to produce an accessory corpus luteum (CL) (Helmer and Britt 1986; Santos et al. 2001; Stevenson et al. 2007; Beltran and Vasconcelos 2008). It is reported that administration of hCG on day 5 post TAI increases progesterone levels (Thatcher et al. 2001; Beltran and Vasconcelos 2008), embryo development (Nascimento et al. 2013) and pregnancy rates (Thatcher et al. 2001; Beltran and Vasconcelos 2008; Shabankareh et al. 2010; Khoramian et al. 2011; Nascimento et al. 2013). In similar studies focused on RB cows, a beneficial effect of hCG administration on day 5 post TAI on pregnancy rates was reported (Kendall et al. 2009; Khoramian et al. 2011; Pandey et al. 2016). Thus, supporting pregnancy by inducing endogenous progesterone source as early as possible may result in improved fertility. Likewise, studies evaluating hCG administration on day 4 post TAI are limited in both lactating dairy cows (Tefera et al. 2001; Fischer-Tenhagen et al. 2010) and RB (Alnimer and Shamoun 2015) cows. Thus, more knowledge is needed about the effect of earlier administration of hCG on day 4 post TAI on dairy cows' fertility. With this aim, this study focused to determine the success of inducing endogenous progesterone source by hCG administration one day 4 post TAI and also to evaluate the effect of the day of hCG administration (day 4 or 5 post TAI) on pregnancy rates in RB cows according to their responses to hCG administrations.

Table 1. Feed ingredients for the total mixed ration.

Ingredient	Amount % of DM
Corn silage (32% DM)	31.18
Alfaalfa hay (16% protein)	11.61
Wheat straw	1.85
Triticale silage (34% DM)	5.12
Wet orange pulp	3.93
Wet corn gluten feed (42% DM)	9.35
Sodium bicarbonate	0.64
Toxin binder	0.04
Yeast (<i>Saccharomyces cerevisiae</i>)	0.02
Magnesium oxide	0.20
Dry corn gluten feed	0.54
Corn gluten (65% protein)	0.79
Hydrogenised rumen bypass fat	1.57
Dairy Min/Vit complex	0.04
Cotton seed meal (38% protein)	8.32
Crushed corn grain	2.29
Distillers' dried grains	5.45
Soy bean meal (48% protein)	1.90
Barley	1.16
Corn	0.63
Sunflower seed meal (38% protein)	1.00
Molasses (sugar beat)	0.84
Calcium carbonate	0.48
Salt	0.32
Bakery byproducts	4.43
Wheat middlings	6.31

DM - dry matter

Materials and Methods

Animals

Cyclic lactating Holstein dairy cows that had been subjected to more than three services with no clinical reproductive abnormalities (RB cows, n = 214) from a commercial dairy farm were enrolled in the present study for over a year's period. The cows were kept in free-stall dairy barns and fed a total mixed ration (Table 1, NRC 2001) with access to water *ad libitum*. Data regarding the milk yield and reproductive health of each cow were obtained from the management program of the dairy farm (Alpro 2000, DeLaval, Tumba, Sweden). Cows with a history of metritis were not included in the study. The average milk yield of each cow was noted from 7 days before to after AI. All cows were scored at the beginning of the study using the 5-point body condition scoring (BCS) system (Ferguson et al. 1994). The treatment groups were formed equally according to BCS, milk production, days in milk (DIM), service and lactation numbers (Table 2). This research was approved by the Lalahan Livestock Central Research Institute Animal Care Committee (No: 2009 / 27).

Treatment groups

All cows (n = 214) received the Ovsynch protocol, which includes GnRH administration (Buserelin acetate, i.m., 10 µg, Oviren[®], Topkim, İstanbul, Turkey) and prostaglandin F_{2α} (PGF_{2α}) administration (Dinoprost, 25 mg, i.m., Enzaprost[®], Ceva, İstanbul, Turkey) 7 days later. The second GnRH administration was performed 56 h after PGF_{2α} administration, and all cows were inseminated at a fixed time (TAI) 16 h to 18 h after the second GnRH injection. Twenty-two out of 214 cows that had early ovulation (lack of a follicle at TAI) or no ovulation (no CL at 4 d after TAI) were defined as non-synchronized cows and were excluded from the study. After TAI, all synchronized cows

Table 2. General results, ovarian responses to the treatments and pregnancy rates of repeat breeder cows.

Item	CON	hCG4	hCG5	<i>P</i> value
BCS	2.87 ± 0.05	2.87 ± 0.05	2.81 ± 0.04	0.58
Milk production (kg/day)	31.6 ± 0.79	30.2 ± 0.76	31.4 ± 0.74	0.38
DIM	228.8 ± 6.7	237.6 ± 6.6	232.0 ± 6.3	0.64
Service number	3.36 ± 0.09	3.48 ± 0.08	3.42 ± 0.08	0.61
Lactation number	2.20 ± 0.16	2.36 ± 0.15	2.32 ± 0.15	0.76
Follicle size at TAI, mm	15.49 ± 0.32	15.26 ± 0.31	15.94 ± 0.30	0.28
Follicle size at 4 d post TAI, mm	12.28 ± 0.54	11.63 ± 0.40	11.52 ± 0.40	0.51
Ovulation to first GnRH, % (n/total)	70.0% (42/60)	68.8% (44/64)	77.9% (53/68)	0.44
Ovulation to hCG, % (n/total)	-	64.1% (41/64)	64.7% (44/68)	0.94
Pregnancy/AI, % (n/total)				
At 31 d	48.3% (29/60)	48.4% (31/64)	42.6% (29/68)	0.74
At 62 d	45.0% (27/60)	42.2% (27/64)	39.7% (27/68)	0.64
Pregnancy loss	6.9% (2/29)	12.9% (4/31)	6.9% (2/29)	0.44

hCG4 and hCG5 included repeat breeder cows treated with hCG administration on day 4 or 5 post TAI, respectively. CON included untreated repeat breeder cows.

DIM - days in milk; BCS - body condition score; TAI - timed artificial insemination; AI - artificial insemination

(n = 192) were randomly assigned to three groups. The cows in the hCG4 group (n = 64) received hCG (1500 IU, i.m., Chorulon®, MSD, İstanbul, Turkey) on day 4 post TAI, the cows in the hCG5 group (n = 68) received 1500 IU hCG i.m. on day 5 post TAI, and the cows in the control (CON) group (n = 60) did not receive any treatment post TAI.

Ultrasonographic examinations

Evaluations of ovaries were performed via transrectal ultrasonography with a 7.5 MHz transducer (Honda HS 2000, Honda®, Tokyo, Japan). Cows that had at least one CL on the ovaries were included in the study, and the size of the largest follicle in the ovaries was also measured. Follicular diameter was determined by using the mean diameters in two directions at the right angle of the largest frozen image of the follicle. In addition, ultrasonographic examinations were performed on the day of PGF_{2α} administration (to determine ovulation after the first GnRH injection), on the day of TAI (to determine the size of the ovulatory follicle), on the day of hCG administration (4 d after TAI, to determine ovulation after the second GnRH injection and to measure the follicle size) and 7 days after hCG administration (to determine ovulation after hCG administration). Ovulations in response to the GnRH and hCG treatments were verified by the disappearance of the dominant follicle and the appearance of a new CL. Pregnancy checks were performed 31 and 62 days after TAI by ultrasonography.

Statistical analysis

Statistical analyses were performed using SAS (version 9.4, SAS Inst. Inc., Cary, NC). The data were evaluated with PROC LOGISTIC, PROC GLM and PROC FREQ analyses in the SAS program. The statistical model included the effects of treatments, parity, DIM, BCS, service number, average milk production, responses to the first and second GnRH and hCG administrations, and follicle sizes at the time of TAI and at 4 days post TAI. To analyse the differences between groups in milk production, DIM, BCS, and follicle size at the time of TAI and 4 days post TAI, the PROC GLM program was used. Chi-square results were obtained using the PROC FREQ procedure for independent tests to analyse the differences in the GnRH administration response and pregnancy/artificial insemination (P/AI, 31 and 62 days) between the groups. The effects of covariant factors on P/AI (31 and 62 days) were determined with the LOGISTIC procedure. For all statistical analyses, *P* values < 0.05 were accepted as significant while those < 0.10 were considered to indicate a tendency towards significance.

Results

General results

The response to first GnRH administration in Ovsynch (72.4%) and the maximum follicle size at the time of TAI (~ 15.5 mm) were found to be similar (*P* > 0.05) among the groups (Table 2).

The follicle diameter at day 4 post TAI was not different ($P = 0.51$) among the groups (12.28 ± 0.54 mm in CON, 11.63 ± 0.40 mm in hCG4, and 11.52 ± 0.40 mm in hCG5). The ovulatory responses to hCG administration were similar ($P = 0.94$) between the cows that received hCG on day 4 (64.1%) vs. on day 5 (64.7%) post TAI (Table 2). Pregnancy per AI (P/AI) at 31 days was similar ($P = 0.74$) among the groups (48.3% in CON, 48.4% in hCG4, and 42.6% in hCG5; Table 2). Pregnancy/AI at 62 days was also similar ($P = 0.64$) among the groups (45.0% in CON, 42.2% in hCG4, and 39.7% in hCG5). Pregnancy loss was similar ($P = 0.44$) among the groups (6.9% in CON, 12.9% in hCG4, 6.9% in hCG5). According to the logistic regression, BCS, DIM, milk production, service number, and parity did not have effects on P/AI ($P > 0.05$).

Effect of response to hCG administration on P/AI

When hCG-treated cows were evaluated, regardless of the day of the treatment, the cows that were responsive to hCG (54.1%, 46/85) had a greater ($P = 0.007$) P/AI at 31 days than the cows that were not responsive (29.8%, 14/47). Additionally, P/AI at 62 days was higher ($P = 0.008$) in the cows that were responsive to hCG (49.4%, 42/85) than in the cows that were not responsive (25.5%, 12/47). However, pregnancy loss was similar ($P > 0.05$) between the cows that were responsive to hCG (8.7%, 4/46) and the cows that were not responsive (14.3%, 2/14).

The pregnancy/AI values at 31 and 62 days were not different ($P = 0.43$, $P = 0.92$) either in responsive cows between the hCG4 and hCG5 groups (58.5% vs. 50.0%) or in nonresponsive cows between the hCG4 and hCG5 groups (30.4% vs. 29.2%; Table 3).

Pregnancy per AI at 31 days was higher ($P = 0.03$) in the cows that were responsive (58.5%) than in those that were not responsive to hCG administration (30.4%) in the hCG4 group, while P/AI tended to be higher ($P = 0.09$) in responsive cows (50.0%) than in nonresponsive cows (29.2%) in the hCG5 group (Table 3). Pregnancy per AI at 62 days was higher ($P = 0.01$) in the responsive cows (53.7%) than in the nonresponsive cows (21.7%) in the hCG4 group, but there was no difference between these cows in the hCG5 group (Table 3). Embryonic loss was not different between the groups (Table 3).

Table 3. Results of pregnancy/AI at 31 and 62 d pregnancy in cows according to their response to hCG administration between the hCG groups.

	31-d pregnancy			62-d pregnancy			Pregnancy loss		
	hCG(+)	hCG(-)	<i>P</i> value	hCG(+)	hCG(-)	<i>P</i> value	hCG(+)	hCG(-)	<i>P</i> value
hCG4	58.5	30.4	0.03	53.7	21.7	0.01	8.3	28.6	0.16
% (n/total)	(24/41)	(7/23)	(22/41)	(5/23)	(2/24)	(2/7)			
hCG5	50.0	29.2	0.09	45.5	29.2	0.19	9.0	0	0.41
% (n/total)	(22/44)	(7/24)		(20/44)	(7/24)		(2/22)	(0/7)	
<i>P</i> value	0.43	0.92		0.45	0.56		0.93	0.13	

hCG4 and hCG5 included repeat breeder cows treated with hCG administration on day 4 or 5 post TAI, respectively.

AI - artificial insemination; TAI - timed artificial insemination

Discussion

The success of inducing endogenous progesterone source which is determined by ovulatory response to post-TAI administration of hCG on days 5–7 is accepted as adequate, with response rates of 60 to 100% in lactating dairy cows (Santos et al. 2001; Nascimento et al. 2013; Torres et al. 2013), but the response to hCG on day 4 post TAI has not yet been documented. In the present study, the response to hCG administration on

day 4 (64.1%) was similar to that on day 5 (64.7%) and was within the ranges observed in other studies (Santos et al. 2001; Nascimento et al. 2013; Torres et al. 2013). Likewise, the follicle sizes (11.56 mm on average) measured on day 4 in all cows seemed appropriate for induction of ovulation by hCG (Sartori et al. 2001). Thus, administration of hCG on day 4 post TAI is similarly effective in inducing ovulation and generating an accessory CL on day 5 which is commonly used.

Administration of hCG either 4 or 5 days after TAI to RB cows did not increase pregnancy rates or decrease embryonic loss compared to the control regimen in the present study. Previous studies including small numbers of RB cows (Khoramian et al. 2011; Pandey et al. 2016) have reported a beneficial effect of hCG treatment on day 5 post TAI. However, Kendall et al. (2009) reported no improvement in fertility after hCG treatment in RB cows, similar to our findings. While no effect of hCG on overall PR was found in this study, it has been reported that hCG treated cows with progesterone concentrations between 2 to 4 ng/ml at 5 days post TAI have higher pregnancy rates than control cows (Kendall et al. 2009). Similarly, in the present study, higher pregnancy rates at both 31 and 62 days post TAI were observed in cows responsive to hCG administration than in nonresponsive cows, regardless of the day of hCG administration. Since these responsive cows with accessory CL could be assumed advantageous in terms of progesterone levels compared to nonresponsive cows, post TAI hCG treatment either on day 4 or 5 may be accepted as beneficial in responsive RB cows in this study.

Embryonic loss was not different among the groups, similar to the results of Tefera et al. (2001) who reported that promoting luteal function post AI had no effect on embryonic mortality in lactating dairy cows. However, our results conflict with the other studies focused on hCG administration on day 4/5 post AI that reported beneficial effects in both RB cows (Alnimer and Shamoun 2015) and lactating dairy cows (for only summer months, Fischer-Tenhagen et al. 2010).

The pregnancy rates on days 31 and 62 were higher in cows that had an ovulatory response to hCG administration on day 4 compared to cows not responsive to the treatment. Such a difference was not observed between responsive and nonresponsive cows in group hCG5. The increased pregnancy rates in responsive cows in group hCG4 may have been due to one day earlier administration of hCG which is consistent with the previous studies that reported that advancing hCG administration from day 5 to day 4 post AI may improve embryo development and hence fertility due to earlier progesterone increase (Mann et al. 2006; Nascimento et al. 2013). The earlier administration of hCG may have also had the effect of improving the size and luteal activity of the primary CL (Rizos et al. 2012), besides its primary effect of inducing accessory CL, since ovulatory responses to hCG were similar between the groups in the present study.

In conclusion, although administration of hCG either on day 4 or 5 post TAI did not increase overall pregnancy rates compared to untreated RB cows that served as control, the ovulatory response to the post TAI hCG administrations improved fertility regardless of the day of hCG administration. Furthermore, hCG administration on day 4 post TAI appeared to be more beneficial than that on day 5, not only exhibiting similar efficiency in inducing the formation of an accessory CL as the endogenous progesterone source, but also producing better pregnancy rates in the cows that had an ovulatory response. Further studies are needed with a larger number of RB cows to explore this potential beneficial effect.

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