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PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Influence of hCG Administration after AI on Conception Rates and Serum Progesterone Concentration in Cattle

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Abstract: The objectives of this study were to determine the effect of administration of exogenous hCG 5 days after Artificial Insemination (AI) on serum progesterone concentration and conception rate in lactating dairy cows. In this experiment 5 days after AI the cows were alternately assigned randomly to treatment group (n = 70) received an injection of 3000 IU hCG (Chorulon, each vial contained 1500 IU, Intervet, Holland) and control group (n = 88), received no treatment. On day 5 and 12 blood samples from 20 of cows (10 in each group) were obtained to measure serum progesterone concentrations. The study population was stratified by parity, milk yield production, number of AI and Days in milk. Cows that were detected in estrous after days 18-24 were reinseminated and recorded as no pregnant (open) to the prior AI. The uteri of cows not observed in estrous were palpated per rectum 45-50 days after insemination to determine pregnancy status. Pregnancy proportion in treatment and control groups were 27.5 and 35.2%, respectively. The results demonstrated that there was no significant differences between two groups ($p < 0.05$). Mean serum progesterone concentrations did not differ between treatment and control groups on day 5 (2.94 ± 0.65 versus 2.98 ± 0.36 ng mL⁻¹). On day 12 serum progesterone concentrations were greater ($p < 0.05$) in the treatment group compared with control group (16.07 ± 3.64 versus 8.71 ± 0.86 ng mL⁻¹). Regardless of treatment, parity, milk yield production, number of AI and days in milk had no effect on the pregnancy rate. In conclusion, 3000 IU of hCG 5 days after insemination did not improve pregnancy rate in dairy cows, despite the fact that serum progesterone concentration were higher in treatment group.

Key words: hCG, dairy cows, pregnancy rate

INTRODUCTION

Reproductive efficiency in dairy cows has declined over last several years and is considerably lower than desired (Lucy, 2001; Washburn *et al.*, 2002; de Vries and Risco, 2005). In cycling cows, several studies have shown that cows which have lower progesterone concentrations during subsequent diestrous have a lower conception rate (Lamming *et al.*, 1989; Mann and Lamming, 1999). Maintenance of pregnancy is dependent on secretion of progesterone during early pregnancy. Luteal deficiency during the first 3 weeks of pregnancy has been hypothesized as a cause of pregnancy failure (Bulter *et al.*, 1996; Mann and Lamming, 2001; Santose *et al.*, 2004). Progesterone supplementation during the luteal phase of the estrous cycle could increase pregnancy rate in cattle (Robinson *et al.*, 1989; Macmillan *et al.*, 1991; Sianangama and Rajamahendran,

1992). Inducing formation of an additional CL during luteal phase of the estrous cycle with a gonadotropin challenge could be a strategy to increase concentrations of progesterone in plasma during the luteal phase (Schmitt *et al.*, 1996).

Various hormonal treatments have been used in attempts to improve pregnancy rate in cattle. Human chorionic gonadotropin has been given in single or multiple injections, in doses ranging from 200 to 10000 IU and at times ranging from 0 to 35 days after AI or mating (Helmer and Britt, 1986; McDermott *et al.*, 1986; Young and Swanson, 1988). Differences in pregnancy rate between hCG-treated and control cattle ranged from -18 to +13% points.

The mechanism of action of hCG is via the induction of an accessory CL by ovulating the dominant follicle of the first follicular wave, as well as having a stimulatory effect on the production of progesterone by the primary

CL (Schmitt *et al.*, 1996). On day 5 of the estrous cycle, granulose cells of the dominant follicle contain LH receptors such that hCG will induce ovulation and formation of an accessory CL. Therefore, administration of hCG 5 days after AI has the potential to increase progesterone secretion during early pregnancy and to alter ovarian follicular dynamics so that cows have three follicular waves within the period approaching the time of CL maintenance (Schmitt *et al.*, 1996; Diaz *et al.*, 1998).

The objective of present study was to investigate the effect of treating lactating Holstein dairy cows with 3000 IU hCG i.m., 5 days after artificial insemination, on their conception rate and serum progesterone concentration.

MATERIALS AND METHODS

This study was carried out in a large commercial dairy herd, 600 Holstein cows in milk located in suburbs of Esfahan province during a period from February to May 2006. Cows were milked three times a day at 0600, 1400 and 2100 h. Milk yield per cow was recorded monthly. The rolling, 305 days, herd average of the farm was 9000 kg of milk. The parity of cows in this herd ranged between one and seven. The herd was under veterinary health care throughout the year. All cows at 25-30 days after calving were checked for uterus recovery and ovaries at regular reproductive visit every two weeks. The animals were kept in a free stall barn with the concrete floor. The cows on the basis of their milk yield production and body condition scoring were fed a total mixed ration. Two experienced persons observed animals 24 h per day, for estrus detection, with an emphasis on two times of each day, at evening (just before dusk) and at morning (just after sunrise). Cows were inseminated at estrous by an

farm technician. In this experiment 5 days after AI the cows were alternately assigned randomly to treatment group (n = 70) received an i.m. injection of 3000 IU, hCG (Chorulon, each vial contained 1500 IU, Intervet, Holland) and control group (n = 88), received no treatment. On day 5 and 12 blood samples was collected from the coccygeal vessels of 20 cows (10 from each group) for progesterone analysis. The study population was stratified by parity, milk yield production, number of AI and days in milk. Cows that were detected in estrous after 18 day were reinseminated and recorded as no pregnant (open) to the prior AI. The uteri of cows not observed in estrous were palpated per rectum 45-50 days after insemination to determine pregnancy status. The differences in pregnancy proportion between treated and control cows were analyzed by using Chi-Square and Fisher Exact Test. Mean serum concentrations in the control and treatment group were compared at days 5 and 12 by using the Student's-test. Mean serum concentrations are expressed as Mean±SE. The analysis of data was performed using the program Sigma Stat version 2.03.

RESULTS

In total 158 cows in study. Of 70 cows in treatment group, 19 cows and of 88 cows in control group 31 cows were pregnant at rectal examination. There was no significant difference in the conception rate between treatment and control groups (p<0.05). The effect of this treatment on pregnant outcome was statistically indifferent among parity, milk yield production, days in milk and number of AI (p<0.05; Table 1).

Mean serum concentrations did not differ between treatment and control groups on days 5 (2.94±0.65 versus

Table 1: The effect of an injection of 3000 IU hCG on day 5 following AI on conception rate in lactating Holstein dairy cows, matched by parity, milk yield production, days in milk and number of AI.

		Cows (n = 158)			
		Treatment group (n = 70)		Control group (n = 88)	
Variables	Level	No. of pregnant (%)	No. of open (%)	No. of pregnant (%)	No. of open (%)
Parity	1	2 (20)	8 (80)	5(22.77)	17(77.28)
	2	8 (33.33)	16 (66.67)	5 (21.73)	189(78.27)
	≥3	9 (25)	27 (75)	17 (39.53)	26 (60.47)
Milk yield (kg)	<30	6 (35.29)	12 (64.71)	6 (31.57)	13 (68.43)
	30-35	2 (15.38)	11(84.62)	3 (23.07)	10 (76.92)
	>35	11 (28.2)	28 (71.8)	22 (39.28)	34 (60.72)
Days in milk	<80	4 (25)	12(75)	15 (31.15)	33 (68.75)
	80-150	8 (29.62)	19 (70.38)	9 (40.9)	13 (59.1)
	>150	7 (25.92)	20 (74.08)	7 (38.88)	11 (61.12)
No of AI	1	5 (27.77)	13 (72.33)	16 (30.09)	26 (69.91)
	2-3	11 (31.40)	24 (68.58)	8 (36.66)	22 (73.34)
	>3	4 (23.52)	13 (76.48)	5 (31.25)	11 (68.75)
Total No. of cows	158	19 (27.14)	51 (72.85)	31 (35.22)	57(64.77)

Table 2: Serum progesterone concentration 5 and 12 days after insemination in treated and control cows

Days	Treated	Control	p-value
5*	2.94±0.65 ^a	2.98±0.36 ^a	0.94
12	16.07±3.64 ^a	8.71±0.86 ^b	0.04

*Mean±SE (ng mL⁻¹), ^a, ^bMeans are different p<0.05

2.98±0.86 ng mL⁻¹; p = 0.94; Table 2). However, the progesterone concentrations of treated cows were significantly higher on day 12 (16.07±3.64 versus 8.71±0.86 ng mL⁻¹; p = 0.04; Table 2).

DISCUSSION

It has previously been suggested that low progesterone during early embryonic development may cause pregnancy failure and thereby reduce the pregnancy rate (Lucy, 2001). Increasing peripheral progesterone concentrations at an early stage (day 5) and late stage (day 15) of the estrous cycle may help reduce embryonic death in cattle. It has been hypothesized that high progesterone after insemination may enhance embryo development and may suppress luteolysis, ultimately resulting in reduced embryonic loss (Peters *et al.*, 1992; Mann and Lamming, 1999).

The objective of this research was to determine the effect of administration of exogenous hCG, 5 days after Artificial Insemination (AI) on serum progesterone concentrations and conception rate in lactating Holstein dairy cows. The results of this study demonstrated that treatment of dairy cows with 3000 IU of hCG 5 days after insemination did not improve conception rate. Cows in hCG group had greater progesterone concentrations by 12 day after AI, compare with cows in control group.

These results are in contrast with those of Sianangama and Rajamahendran (1992), who reported an increase in conception rates when lactating dairy cows were treated with an injection of 1000 IU hCG on 7 or 14 days after AI. Breuel *et al.* (1989) reported that injection of 3000 IU hCG on days 4 of the cycle preceding AI and 4 days after AI increased pregnancy rate in heifers, Similarly Santos *et al.* (2001) reported that treatment with hCG on days 5 resulted in higher conception rate on days 28, 45 and 90 after AI. Ideta *et al.* (2003) reported an improvement in conception rates when hCG is used on 5-7 days after insemination.

The results of this study are in agreement with the finding of Hanlon *et al.* (2005), they reported that an injection of 1500 IU hCG, 5 days after insemination did not improve first service conception rate in anestrous dairy cows, despite the fact that plasma progesterone concentrations were higher in treatment group. Helmer

and Britt (1986) reported that a single injection of 500 IU hCG on days 3 after AI did not improve conception rate. Similarly Breuel *et al.* (1990) reported that an i.m. injection of 3000IU hCG on days 4 after breeding did not improve conception rate in beef heifers. Howard *et al.* (2005) reported that GnRH administrated 5 days after AI increased serum progesterone concentration but did not improve conception rate in dairy cattle.

In this study injection of hCG on days 5 after insemination increased serum concentration of progesterone in treated group. This results is in agreement with the findings of Breuel *et al.* (1989), Helmer and Birtt (1987), Fricke *et al.* (1993) and Walton *et al.* (1990). Schmitt *et al.* (1996) showed increased progesterone concentrations between days 11 and 16 in Holstein heifers administrated GnRH 5 days after estrous. Similarly Willared *et al.* (2003) observed increased serum progesterone concentration between days 9 and 19 when heat-stressed dairy cows were administered GnRH 5 days after insemination. This present finding is in contrast with finding of Twagiramungu *et al.* (1995) that did not detect any increase in plasma progesterone during the 6 days after induction of an induced CL with administration of a GnRH agonist on day 7 after estrous. Martin *et al.* (1990) administered 100 µg of natural GnRH on days 2 and 8 the estrous cycle and did not detect any increase in plasma progesterone in the treated animals. The increase in serum progesterone measured in the present study probably is due to combined effects on the original and induced CL.

Many factors influence conception rate, among them is cyclicity, energy balance, heat stress, parity, milk yield production, diet, service number, days in milk and disease (Cartmil *et al.*, 2001; Gröhn and Rajala-Schultz, 2000; Hansen and Arechiga, 1999; Lucy, 2001; Moreira *et al.*, 2001; Santos *et al.*, 2004).

In this present research, parity, days in milk, number of AI and milk yield production had no effect on conception rate. These results are in agreement with the finding of Howard *et al.* (2005), Chebel *et al.* (2004), Starbuck *et al.* (2004) and Lopez-Gatius *et al.* (2004).

In conclusion, serum concentrations of progesterone may not be a limiting factor in establishment of pregnancy in dairy cows, because conception rates were not further improved by progesterone supplementation via hCG injection on day 5 after insemination in the present study. The lack of positive effect of exogenous hCG 5 days after AI in this study and others, indicates that routine hCG administration 5 days after AI may not to be beneficial. Further studies are required to investigate different methods of treating and/or managing dairy cows in order to improve their reproductive performance.

ACKNOWLEDGMENT

The authors would like to thank the Research deputy of Shahrekord University for their financial assistance, authorities and the staff of the commercial dairy farm (Goldashteh nemoneh Esfahn) for providing animals and drugs.

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