Journal of Alternative Veterinary Medicine | Autumn 2022 | Vol.5 | No.14

JOAVM
Journal of Alternative Veterinary Medicine
joavm.kazerun.iau.ir

Islamic Azad University Kazerun Branch Fars, Iran

Research Article

Effect of Human Chorionic Gonadotropin on Late Embryonic Death in Holstein Dairy Cows

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Received: 15/Oct/2022 Revised: 01/Nov/2022 Accepted: 05/Nov/2022

Abstract

Background and aim: The objective of this study was to determine whether administration of 1500 IU of hCG on Day 29±1 after artificial insemination (AI) would reduce pregnancy loss by inducing accessory corpora lutea and increasing serum progesterone concentrations in dairy cattle.
Materials and Methods: Three hundred and seventy lactating Holstein dairy cows from a farm with 1200 lactating cows were randomly assigned to a treatment and a control group. The animals of the treatment group (n=185) received hCG 1500 IU (IM), while in the control group they received the control group (n=185) received no treatment. Ovarian structures, serum progesterone levels and pregnancy status were determined on days 29±1 and 42, respectively.
Results: The Progesterone concentration on day 42 was higher (P=0.005) for cows in the hCG group (14.9±0.7 mg/ml) compared to the control group (12.1±0.7 ng/ml). On day 42, the number of accessory CLs for cows in the hCG group (1.6±0.04) were higher (P<0.0001) than cows in the control group (1.2±0.04). A Positive correlation was detected between the circulating progesterone and CL volume in the hCG group (r=0.47, P=0.001). Pregnancy losses did not differ between days 29±1 and 42 both in the control (7.6%) and in the hCG group (5.9%).
Conclusion: It was concluded that the administration of hCG (1500 IU) on day 29±1 of pregnancy in lactating dairy cows increased the number of corpora lutea and serum progesterone concentration but did not have any effect on pregnancy loss rate or pregnancy losses.

Keywords: *hCG, Accessory corpora lutea, Progesterone, Pregnancy loss, Cattle*

Cite this article as: Navid Aarabi, Amir Ashakn Mahjoor, Abbas Rowshan Ghasrodashti. Effect of human chorionic gonadotropin on late embryonic death in Holstein dairy cows. J Altern Vet Med. 2022; 5(14): 817-825.

[DOR: 20.1001.1.2717378.2022.5.14.3.7]

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Introduction

According to Committee on Bovine Reproductive Nomenclature, embryonic mortality after the maternal recognition of pregnancy, during and immediately after the placentation is a significant problem in dairy cattle (Committee on Bovine Reproductive Nomenclature, 1972). Late embryonic losses have been defined as the death of the embryo between days 25 and 45 of gestation. It was estimated that almost half (47.5%) of the total embryonic loss occurs between days 28 and 42 of gestation (Vasconcelos *et al.,* 1998). Silk et al., recorded that the pregnancy loss rate in the lactating and pluriparous cows between days 28 and 98 days of gestation were 7.2%, and in heifers was 6.1% (Silk *et al.,* 2002). Lo´pez-Gatius et al., has reported a 9.6% pregnancy loss rate occurred between days 36 and 90, which was similar to Horan et al., findings that reported a 7.5% pregnancy loss rate between days 30 and 67 of gestation in dairy cows (Lo´pez-Gatius *et al.,* 2004a; Horan *et al.,* 2004). Late embryonic death after day 27 of gestation was reported in 3.2% of dairy cows producing 6000-8000 kg of milk per year in Ireland (Silk *et al.,* 2002). Factors related to management and the cow health condition like changes in body condition score, milk production, mastitis and laminitis can influence pregnancy loss during the late embryo and early fetal period (Labe`rnia *et al.,* 1996; Lo´pez-Gatius *et al.,* 2002).

Most losses occur between days 35 and 90 of gestation (Lo´pez-Gatius *et al.,* 2002). Poor placentation has been suggested as the leading cause of late embryonic losses between days 30 and 45 of gestation. In a study, it was determined that the most pregnancy losses occurred after day 30 in cow and day 42 heifers, and losses increased as circulating concentration of progesterone around the day 30 of pregnancy decreased (Starbuck & Dailey, 2004). High-producing dairy cows may suffer from a low progesterone concentration during the luteal phase of the estrous cycle, due to an increment blood flow to the liver, which can increase the rate of steroid catabolism and lead to higher pregnancy losses (Sangsritavong *et al.,* 2002). Abnormal hormonal profiles (including progesterone and estrogen) can affect embryonic mortality (Santos *et al.,* 2008; Ayalon, 1978). In some studies, the progesterone concentration of lactating dairy cows was lower than heifers, due to: 1) Reduction of the progesterone secretion by the corpus luteum and, 2) increased the progesterone catabolism (Rhinehart *et al.,* 2008; Rabiee & Macmillan, 2001; Schwarzenberger, 2001; Vasconcelos *et al.,* 1998).

Cows in a better condition would be expected to eat more, which would increase the catabolism of progesterone and lower plasma progesterone concentrations (Rabiee *et al.,* 2001). Kastelic et al., pointed the decrement progesterone concentration usually occurred between 28 to 37 days of pregnancy. This reduction could be due to the decreased secretion of progesterone from the corpus luteum or progesterone catabolism in the liver, which causes the death of embryo/fetus between days 30 to 60 of pregnancy (Kastelic *et al.,* 1991). Lo´pez-Gatius et al., found a positive correlation between the additional CLs and the maintenance of pregnancy. They found that the animals with an additional corpus luteum (CL) were 8.3 times less likely to undergo a pregnancy loss than cows with no additional corpus luteum. They also noted that the progesterone supplementation in lactating cows on the day 30 of gestation reduced pregnancy losses. There are several reports indicating that additional CLs cause a reduction in the late embryonic death in dairy cattle (Garcı´a-Ispierto *et al.,* 2006; Lo´pez-Gatius *et al.,* 2004b).

The main source of the progesterone in pregnant cow is CL, at least until day 200 of gestation (Niswender *et al.,* 2000; Sawyer 1995). Several methods have been applied to increase the blood progesterone concentration in order to reduce the embryonic mortality (Mann & Lamming, 1999). One common method of post AI progesterone supplementation is the induction of ovulation in the dominant follicle of the follicular wave with a human chorionic gonadotropin (hCG) treatment to produce an accessory CL. As expected, an accessory CL subsequently would increase circulating progesterone concentration (Helmer & Britt 1986; Mann & Lamming, 1999; Santos *et al.,* 2001).

Human chorionic gonadotropin can increase plasma progesterone levels through two possible mechanisms: a direct effect on the primary CL and /or by developing accessory corpora lutea. The effectiveness of hCG injection to improve conception and in calf rate in lactating cattle is still equivocal. Some reports indicate that the use of hCG at AI increase progesterone levels (Santos *et al.,* 2001;

Schmitt *et al.,* 1996; Diaz *et al.,* 1998; Breuel *et al.,* 1989; Helmer & Britt 1986). Human chorionic gonadotropin treatment on days 29 and 42 after AI also induced accessory corpora luteal and increased plasma progesterone for 4 weeks, but did not reduce embryo losses (De Rensis *et al.,* 2008).

The aim of the present study was to determine whether the hCG treatment on day 30 after AI could increase P4 concentrations, and reduce the incidence of embryonic mortality on day 42 in Holstein dairy cows.

Material and Methods

This study was conducted at a Holstein dairy farm with 1200 lactating cows and 3.5% fat-corrected milk- 305-d, and the Rolling Herd Average of 9800kgs. Cows were kept in open stalls and dry lots and fed a total mixed ration (TMR) three times a day. The TMR was formulated to meet for lactating cows based on NRC, 2001 (NRC, 2001). The voluntary waiting period was around 50-60 days postpartum, the parity of the cows ranged from 2 to 4, body condition score 2.9±0.2 (Based on 0 to 5 range, 33) and the mean milk production was 37±3 kg for cows. All cows were synchronized with two PGf2α (5ml of Lutalyse, Pfizer Animal Health, NewYork, NY) injections, Treatments were given 14 days apart, starting from day 50 - 60 post-partum. All animals were inseminated at standing heat by the same technician, and with the semen from 6 proven sires.

Cows showing health disorders such as mastitis, left displacement abomasum, lameness and respiratory diseases were not included in the study and diagnosed cases during the study were excluded. Three hundred and seventy pregnant cows detected on day 29±1 post AI with a rectal ultrasonography probe (7.5 MHZ, Siui, CTs 900v, china) cows which were enrolled in this study. The presence of a normal embryo (heart beats) and the fluid-filled cavity representing the allantoic cavity in the uterus lumen as described by Pierson and Ginther were checked on day 29±1 and 42 of pregnancy with the rectal probe (Pierson & Ginther, 1984). The corpora lutea numbers and diameter were also calculated on day 29±1 and 42. Animals found to be pregnant, were randomly assigned to the control (untreated cows, n=185) or the treatment (n=185) group. Cows in the treatment group received hCG 1500 IU (chorulon, Intervet/MSD), IM on day 29±1 post AI. On day 42, pregnancy status was determined by ultrasonography examination and non-pregnant cases were considered as a case of embryonic losses. Blood samples were collected into vacuum tubes from median coccygeal vein or artery on day 29±1 and 42 of gestation. Upon after the sample collection, samples were immediately placed on ice, and serum samples were separated by centrifugation (For 10 min at 3000 ^{x} g) and stored at -22 °C until assayed. Serum P4 levels were determined by using a validated commercial Radio Immuno Assay Kit (Immunotech Kit, Marseille, France). The intra and inter-assay coefficient of variation (CVs) of progesterone were 6.5 and 9.0%, respectively. The sensitivity of the test was 0.05 ng/ml and the recovery rate of the assay ranged from 85 to 110%. Corpora lutea were assumed to be spherical by averaging the largest cross-sectional width and height. Volume was calculated as follow: $4/3 \times R^3 \times \pi$, where R (radius) = average diameter / 2.

Statistical analysis

Data were imported into SAS, version 9.2 (SAS Institute Inc, Cary, NC, USA, 2010) for statistical analysis. All quantitative data were checked to fit a normal distribution using the Shapiro-Wilk test (Shapiro & Wilk, 1965). Qualitative data from the two groups were compared by chi-square analysis (Proc FREQ, SAS). The data were analyzed using the GLM, GENMOD procedure depending on the nature of the data and comparison intended. The plasma progesterone concentration and CL volume were analyzed of variance (Proc Mixed, SAS) considering treatment, day, day * treatment and Body Condition Scoring (BCS) and milk yielded. Pearson correlation coefficient (r) was calculated for the correlation between the progesterone concentration and CL volume on day 29±1 and 42 in the treatment and control groups. P value<0.05 were considered statistically significant.

Results

There was no significant difference between the embryonic losses on day 42 in the treatment (5.9%) and control (7.6%) groups (P=0.5). No significant difference was observed in BCS and peak milk yield during current lactation among two experimental groups used in the present study. Progesterone concentration were (12.1±0.74) and (14.9±0.75)

[DOR: 20.1001.1.2717378.2022.5.14.3.7]

(P=0.001) on day 42 of pregnancy in the control and the test group, respectively (table 1). The increment of serum progesterone concentrations from Day 29±1 to 42 were higher (P=0.008) in test group (table 1).

	Day 29±1		Day 42		DAY	T	D×T
Response	**Control**	**Treatment**	**Control**	**Treatment**			
P4	11.6±0.74	11.1±0.75	12.1±0.74	14.9±0.75	0.8	0.001	0.008
CL	1.05±0.06	1.1±0.05	1.2±0.05	1.6±0.06	0.1	0.0001	0.0001
CL-vol	7829±589	7484±633	7704±527	9832±513	0.3	0.002	0.006

Table 1. Progesterone concentration, CL volume and number of cl (least square mean ± standard error). Abbreviations: D, day; T, treatment (hCG group); P4, progesterone; CL-vol, cl volume.

On day 29±1, serum progesterone concentrations were similar for cows in the control and treatment group (P=0.8). The number of accessory luteal structures was greater (P<0.0001) for cows in the treatment group (1.6±0.04) than for the ones in the control group (1.2±0.04). There was an interaction between treatment and accessory corpora lutea formation on serum progesterone concentration at Day 42 (P=0.0001).

Accessory corpora lutea formation was also higher (P<0.0001) for cows in the hCG group (59.5%) than cows in the control group (20%) (table2).

No-CL	Control on day 42	Treatment on day 42
1	80%	40.5%
1>	20% [a]	59.5% [b]

Table 2. Number of CLs on day 42 in two groups. [a,b] Data with different subscriptions in row significantly differ P≤0.05.

There was no significant correlation between the numbers of CLs and the progesterone concentration in the control group (P=0.8), whereas there was a significant positive correlation between the numbers of CL and the progesterone concentration in the treatment group (r=0.5, P=0.0001). The mean CLs volumes on day 29±1 in control (7829±589) were similar test group (7484±633) (Table 1). There were differences (P=0.006) in CLs volumes within the treated group on days 29±1 (7704±527 mm³) and 42 (9832±513 mm³) (Table 1). A significant difference was also obtained (P=0.002) between the control group (7704±527 mm³) and the treatment group (9832±513 mm³) on day 42. Any significant difference in the CLs volume of the treatment group was not found in cows with one CL on days 29±1 (8213±2539 mm³) and 42 (8865±3578 mm³) of pregnancy (P=0.4). There was a significant correlation between CLs volume and the progesterone concentrations on days 29±1 and 42 in the treated cows having one CL (r=0.4, P=0.0001). But there was no significant correlation between the serum progesterone concentration and the CL volume on days 29±1 and 42 in the control group (P=0.09).

Discussion

This study was conducted to determine the effect of hCG on the survival rate of late embryos in lactating cows. In the present study the embryonic losses on day 42 in the treatment (5.9%) and the control (7.6%) groups was not statistically different. The range of late embryonic death has been estimated about 10 to 15 percent (Inskeep & Dailey, 2005). Starbuck et al., indicated that there was a positive correlation between the progesterone concentration on day 35 of gestation and the embryo survival on day 50-60 of gestation (Starbuck *et al.,* 2004).

In the present study, treatment of cows with hCG on day 29±1 post AI increased the progesterone levels, but did not reduce the embryonic loss. It has been reported that the progesterone treatment caused the increment of embryonic growth, which resulted in a greater area for the attachment with uterine caruncles (Inskeep & Dailey, 2005; Shapiro & Wilk, 1965). Campanile et al., demonstrated that in the treated buffaloes with hCG on the day 25 after AI, the rate of embryonic death was lower than the untreated group (Campanile *et al.,* 2008). It has been reported

[DOR: 20.1001.1.2717378.2022.5.14.3.7]

that the luteotropic activity of hCG has been attributed to the newly-formed luteal tissue, which cause plasma progesterone in dairy cattle to increase (Santos *et al.,* 2001; Khan *et al.,* 2003; King *et al.,* 1982.).

Our study showed the administration of hCG on day 29±1 increased the number of copora lutea and plasma progesterone concentration on day 42 which was equivalent to Bartolome´s study who demonstrated the deslorelin implant on day 27 of gestation resulted in increased progesterone of the plasma and the number of CLs, but failed to reduce pregnancy loss (Bartolome *et al.,* 2006; Nephew *et al.,* 1994). Our findings indicated no positive correlation between additional corpora lutea and the pregnancy loss. Nevertheless, the plasma progesterone concentration had been increased, which was opposite to Lo´pez' study, who suggested that there was a positive correlation between the additional CL maintenance of pregnancy (Lo´pez-Gatius *et al.,* 2004b). The effect of hCG treatment on primary CL has been attributed to the increased size of luteal cells (Schmitt *et al.,* 1996) or the increased number of large luteal cells concomitant with a reduction in the number of small luteal cells (Breuel *et al.,* 1989; Sianangama & Rajamahendran, 1992; Diaz *et al.,* 1998; Helmer &, Britt 1987; King *et al.,* 1982), and to an increased surface area, volume and diameter of the CL (Campanile *et al.,* 2008; Santos *et al.,* 2001, Sianangama & Rajamahendran 1992; Rajamahendran & Sianangama, 1992).

Several studies indicated that the administration of hCG at the time of AI increased plasma progesterone levels from days 5 to 12 of the estrus cycle in cows with no accessory CL (De Rensis *et al.,* 2008; Bartolome *et al.,* 2006; Helmer & Britt, 1986). In the present study, treated cows with hCG having one accessory CL had a higher serum progesterone concentration on day 42 of gestation compared to untreated cows. It may be due to the increment of the number of large luteal cells (Sianangama & Rajamahendran, 1992; Diaz *et al.,* 1998; Breuel *et al.,* 1989; King *et al.,* 1982; Helmer & Britt, 1987).

Another study showed that follicle could become accessory corpora lutea after hCG in 70% of beef cows in which the primary CL was removed on day 28 of gestation (Campanile *et al.,* 2008). Sheffel et al., reported that the follicles smaller than 1 cm in diameter were able to become accessory corpora in response to hCG (Sheffel *et al.,* 1982).

Our study was in agreement with previous studies which showed that there was a significant positive correlation between the volume of CL and the serum progesterone (Khan *et al.,* 2003; Mann & Lamming, 1999; Sianangama & Rajamahendran, 1992; Bennett *et al.,* 1989; King *et al.,* 1982). Bartolome et al., reported only 45% of the cows in the deslorelin group had the accessory corpora lutea whereas in our study, 59.5% of the hCG treated group had accessory corpora lutea (Bartolome *et al.,* 2006). In our study, the occurrence of accessory corpora lutea in pregnant cows of the control group at day 42 was 8% that might be due to either a mistake in the diagnosis of accessory corpora lutrea or the incidence of estrus during the pregnancy. Thomas and Dobson reported that the incidence of estrus during the pregnancy was 5.7%, but not associated with an ovulation or metestrous bleeding (Thomas & Dobson, 1989).

Conclusion

In conclusion, our findings indicated that the treatment with hCG at the time of pregnancy diagnosis on day 29±1 in dairy cows, increases serum progesterone concentration and the number of CLs, but did not reduce embryo loss.

Acknowledgments

The authors express their appreciation to Mr. Ebrahimi for allowing access to his cattle farm and facilities, and their assistance, especially Dr. Roozitalab, Dr. Vafa, Mr. Khoshnood and Lotfi during the study.

Competing interests and disclaimer

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article and the views expressed in the submitted article are authors own.

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[DOR: 20.1001.1.2717378.2022.5.14.3.7]

مجله طب دامپزشکی جایگزین | پاییز ۱۴۰۱ | دوره ۵ | شماره ۱۴

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تاثیر گنادوتروپین کوریونی انسانی بر روی مرگ و میر اواخر دوره جنینی در گاوهای شیری هلشتاین

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تاریخ دریافت: ۱۴۰۱/۰۷/۲۳ اصلاح نهایی: ۱۴۰۱/۰۸/۱۰ تاریخ پذیرش: ۱۴۰۱/۰۸/۱۴

چکیده

زمینه و هدف: مرگ و میر رویان بعد از شناسایی آبستنی به وسیله مادر یا در حین اتصال جفت به اندومتریوم، یا بلافاصله بعد از آن یکی از بحرانی ترین مراحل جنینی در گاوهای شیری می باشد. هدف از این مطالعه بررسی تاثیر hCG بر روی کاهش مرگ و میر در اواخر دوره رویانی بود.

مواد و روش‌ها: در این مطالعه ۳۷۰ راس گاو آبستن بین روزهای ۲۸ تا ۳۰ بعد از تلقیح انتخاب و بطور تصادفی ۱۸۵ راس ۱۵۰۰ واحد hCG بصورت عضلانی دریافت کردند و ۱۸۵ راس دیگر به عنوان گروه شاهد در نظر گرفته شدند. از تمامی گاوها در هر دو گروه در روز۳۰-۲۸ و ۴۵ بعد از تلقیح نمونه خون جهت ارزیابی پروژسترون سرم خون، اخذ گردید. همچنین برای بررسی تخمدان ها و برای اندازه گیری اقطار جسم زرد و شمارش تعداد جسم زرد وآبستنی سونوگرافی انجام شد.

یافته‌ها: نتایج نشان داد، میزان پروژسترون روز ۴۵ درگروه تیمار (۰/۷± ۱۴/۹) بیشتر از گروه درمان (۰/۷±۱۲/۱) بود (P=۰/۰۰۵). همچنین تعداد جسم زرد در گروه تیمار (۰/۰۴±۱/۶) بیشتر از گروه درمان (۰/۰۴± ۱/۲) بود (P<۰/۰۰۰۱). در گروه تیمارارتباط معنی داری بین حجم جسم زرد و پروژسترون مشاهده شد (r=۰/۴۷، P=۰/۰۰۱). تحقیق حاضر نشان می دهد تجویز hCG توانسته تعداد اجسام زرد ضمیمه را درگروه تیمار زیاد کند و باعث افزایش میزان پروژسترون شود. اگر چه اختلاف معنی داری در میزان مرگ و میر جنینی در روزهای ۲۸ تا ۴۵ در گروه تیمار (٪۷/۶) و شاهد (٪۵/۹) مشاهده نشد.

نتیجه‌گیری: با توجه به نتایج حاصل از این مطالعه استفاده از hCG (1500 IU) در روز ۳۰-۲۸ بعد از تلقیح در شرایط انجام مطالعه تاثیر برروی کاهش مرگ و میر نداشته است.

واژه های کلیدی: *hCG، ضمائم جسم زرد، پروژسترون، از دست دادن بارداری، گاو*

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[DOR: 20.1001.1.2717378.2022.5.14.3.7]

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