



Effect of Human Chorionic Gonadotropin and Flunixin Meglumine on Pregnancy Rate in Dairy Cows

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Abstract

Background and aim: Growing infertility and a reduction in pregnancy rates are two of the most challenging issues facing modern dairy cows. Our objectives were to determine pregnancy rates, plasma progesterone concentrations, and corpus luteum (CL) numbers in high-producing dairy cows during two distinct periods (cool and warm seasons). This evaluation followed the administration of: 1) human chorionic gonadotropin (hCG) on day 5 after AI, 2) a combined treatment with hCG on day 5 and flunixin meglumine between days 14 and 16 after AI, and 3) flunixin meglumine alone between days 14 and 16 after AI.

Materials and Methods: Cows were subjected to pre-synch programming in two experiments (cool period, $n = 396$) and warm period, $n = 181$), respectively. Environmental data in the warm period indicated that cows experienced high heat stress during the trial in the warm period (THI = 85-92). Cows in both experiments were randomly divided into four groups: Group 1 received hCG, and Group 2 was given hCG on day 5 and FM on days 14-16, Group 3 was treated with FM on days 14-16, and the untreated group was the control group. Ovarian structures and serum progesterone levels were determined on days 5 and 12 after AI, respectively. Ultrasonography was used to evaluate the pregnant status of cows on days 28-30.

Results: There was a significant difference in p4 concentrations 12 days after IA between groups in the cool period, in both the hCG and hCG+FM groups ($p = 0.001$). But the discrepancy in p4 was not observed between groups in the warm period ($p = 0.7$). The increase in CL for cows treated with hCG compared with control cows was greater during the cool (1.7 vs. 1.1) period compared to the warm (1.1 vs. 1.3) period ($p = 0.001$). In the warm season, no significant difference in pregnancy rate on day 30 between the four groups was obtained.

Conclusion: Our results indicated that administration of flunixin meglumine did not improve pregnancy rates in dairy cows during either the warm or cool seasons. Conversely, treatment with hCG on day 5 after artificial insemination induced accessory corpora lutea, increased plasma progesterone concentration, and improved pregnancy rates in dairy cows during the cool period only. None of these effects were observed during the warm period.

Keywords: hCG, Flunixin meglumine, Progesterone, Pregnancy, Heat stress

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Introduction

Embryonic death reduces bovine fertility and causes significant economic loss in the dairy industry (Kidder *et al.*, 1954; Diskin & Sree Nan, 1980), which has been attributed to a variety of factors such as herd size, management, metabolic, and disease states, as well as changes in physiology associated with increased milk production (Lopez *et al.*, 2004; Wiltbank *et al.*, 2006; Caraviello, *et al.*, 2006). One of the most critical variables limiting dairy cow reproductive success is estrus detection effectiveness. Controlling follicular dynamics and luteal life span is one strategy for managing the reduction in reproductive performance of dairy cattle (Lucy, 2001). Synchronized cows usually have a small ovulatory follicle at the time of insemination; furthermore, it would result in the production of smaller corpus luteum that may produce lower progesterone concentrations that are optimal for fertility (Vasconcelos *et al.*, 2001; Perry *et al.*, 2005; Gaja *et al.*, 2008). This problem deteriorates due to the high metabolism of P4 that is characteristic of high-producing dairy cows (Sangritavong *et al.*, 2002; Wiltbank *et al.*, 2006). Progesterone plays a vital role in maintaining pregnancy and triggers the production of an extensive range of endometrial compounds, growth factors, immunosuppressive agents, enzymes, ions, and steroids (Geisert *et al.*, 1992; Forde *et al.*, 2011). One study suggests that combined injections of human chorionic gonadotropin (hCG) and flunixin meglumine could potentially improve pregnancy rates in cows experiencing repeat breeding (Jawad *et al.*, 2021).

A study showed that the use of progesterone supplementation from days 5–9 following insemination can increase both embryonic development and the secretion of interferon τ (Mann *et al.*, 2006). Findings by Satterfield showed that administration of progesterone 36 hours after mating results in increasing the blastocyst diameter on day 9 in sheep (Satterfield *et al.*, 2006). Several studies have shown that low progesterone concentrations after post-insemination reduce fertility in dairy cattle (Stevenson *et al.*, 1993, Gumen & Wiltbank, 2005; Diskin *et al.*, 2006). To tackle this problem, numerous therapies are available to increase peripheral progesterone concentration following AI, encompassing inducing accessory CL or supplementing progesterone directly (Inskip, 2004;

Stevenson *et al.*, 2007). hCG is one of the most often used methods to induce CL; administration of hCG during the early phase results in new CL, which leads to an increase in progesterone concentration (Stevenson *et al.*, 2007; Santos *et al.*, 2001). Maternal recognition of pregnancy initiates approximately 14 days after breeding (Bazer *et al.*, 1991; Roberts *et al.*, 1996; Thatcher *et al.*, 1994). If interferon τ is not secreted sufficiently, the uterus produces PGF2 α , which causes the corpus lutea to regress, decrease progesterone secretion, and reduce the pregnancy rate (Roberts *et al.*, 1996; Roberts *et al.*, 1992; Thatcher *et al.*, 2001). Flunixin meglumine (FM) is an anti-inflammatory agent that inhibits cyclooxygenase, preventing the conversion of arachidonic acid to PGF2 α (Anderson *et al.*, 1990; Odens *et al.*, 1995).

One study found that giving FM on day 14 after AI results in a 10% increase in pregnancy rates in beef cattle due to a decrease in serum PGFM (Merrill *et al.*, 2005). FM treatment has been shown to inhibit PGF2 secretion in beef and dairy cattle for at least 24 hours (Guilbault *et al.*, 1987). Heat stress reduces pregnancy rates due to its detrimental effects on follicle development, corpus lutea function, and hormone profiles, leading to early embryo death (Hansen & Arechiga, 1999; Badinga *et al.*, 1985; Barker *et al.*, 1994).

According to one research, fertilization rates are normal, but mortality occurs between conception and maternal pregnancy identification (Thatcher & Collier, 1986; Wise *et al.*, 1988). It has been observed that heat stress has an effect on the oocyte by destroying it, affecting protein synthesis throughout the morula and blastocyst stages, and perhaps decreasing endometrial function (Hansen *et al.*, 2007). Many different methods including as the administration of HCG, GnRH, CIDR, and NSAIDs, have been used to minimize the effect of heat stress on reproductive performance. The use of hCG to increase pregnancy rates under heat stress is still controversial. Some studies found a favorable impact on pregnancy rates, while others did not (Dunlap & Vincent, 1971). The current study intended to determine if administering hCG and FM on days 5 and 13, respectively, following AI would enhance conception rates in warm and cool seasons. In addition, the effect of hCG on CL number and plasma progesterone concentration was evaluated.

Materials and Methods

The study was conducted on 2500 lactating Holstein-Friesian cows from a single, well-managed dairy herd. The cows were kept in open stalls and fed a total mixed ration (TMR) three times a day. The TMR was formulated to meet or exceed requirements for lactating cows milked three times in a day, according to the NRC. Voluntary waiting Period was around 50–60 days postpartum, the parity of the cows ranged from 2 to 4, and the body condition score was determined on the basis of scales 1–5 (NRC, 2001). Cows undergoing an abnormal puerperium, such as dystocia, twinning, retained placenta, or metritis, were excluded. Cows with clinical conditions detected during the course of the study, such as mastitis, lameness, digestive disorders, abnormal genital discharges, and pathological abnormalities of the reproductive tract detectable on palpation per rectum, were also withdrawn from the program. All the animals in cool ($n = 396$) and warm periods ($n = 181$) were pre-synchronized with two cloprostenol (lutalyz, Pfizer, USA) IMs. Treatments were given 14 days apart, starting on day 30–35 postpartum. Twelve days following the second PGF2a treatment, the Ovsynch protocol, consisting of an injection of GnRH (Vetaroline; Aburaihan Co.), followed by administration of PGF2a 7 days later, and a final injection of GnRH given 24 hours after the third PGF2a treatment, began. Cows were subjected to timed AI 16–22 hours after the last injection of GnRH. All cows were inseminated by the same technician, and semen from seven proven sires was used. The cows were randomly divided into four groups. Group-1 (cool period, $n=100$), (warm period, $n=50$) received 1500 IU hCG (IM) on day 5 after the initial AI; group-2 was treated with 100 μg FM (Flunixin meglumine, flunex, Razak) for three days (IM) between days 14 and 16 post-insemination (cool period, $n=98$) and (warm period, $n=50$); group 3 received 1500 IU hCG on day 5 and 100 μg FM for 3 days on days 14–16 after AI (cool period, $n=101$); (warm period, $n=41$); and group 4 was selected as the control with no treatments (cool period, $n=97$) and (warm period $n=40$).

Blood samples were collected via venipuncture of the median caudal vein or artery into evacuated tubes on days 5, 12 after TAI for later analysis of serum P4. The first sample was collected immediately before

administration of treatment. Upon collection, samples were immediately placed on ice, and serum was separated by centrifugation (for 10 min at $3000 \times g$) and stored at -22°C until assayed. Serum P4 concentrations on days 5, 12 after AI were determined by using a validated commercial radio immunoassay kit (Immunotech kit, Marseille, France). The intra- and inter-assay coefficients 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%. Ovarian structures on d 5 and 12 were scanned and mapped using transrectal ultrasonography (7.5 MHz transrectal probe, SIUI, CT 900V, China). Ultrasound measurements of CL dimensions (length (L) and width (W)) were used to calculate luteal tissue volume. First, the radius (R) was calculated by the formula $R = (L/2 + W/2) / 2$, and then the volume (V) by using the formula $V = 4/3 * \pi * R^3$ (Vasconcelos *et al.*, 2001). The pregnancy status of cows was estimated for four groups at days 28–30 and confirmed on days 40–45 by ultrasonography.

Temperature-humidity index (THI) was calculated as per following equation:

$$\text{T-H index} = 0.81 T + 0.01 \text{RH} (0.99 T - 14.3) + 46.3$$

Where: T: mean air temperature in $^\circ\text{C}$.

RH: mean relative humidity%.

Statistical analysis

Data were imported into SAS version 9.2 (SAS Institute Inc., Cary, NC, USA, 2010) for statistical analysis. Descriptive statistics were done using PROC MEANS in SAS. All dependent data were checked to fit a normal distribution using the Shapiro-Wilk test (Shapiro & Wilk, 1965). Qualitative data from the 8 groups was compared by chi-square analysis (Proc FREQ, SAS). Differences in corpus luteal volume and progesterone concentrations between subjects treated with hCG, hCG+FM, FM, and untreated were tested by the ANOVA procedure. To confirm the present hypothesis that hCG administration on day 5 after AI increases the number of CLs and progesterone concentration, two sample t-tests were used to compare the numbers of CL and p4 values on day 5. Compared with day 12 after AI in the control group as well as in the treatment group. Pearson correlation was used to assess the relationship

between CL volume and plasma p4 concentration on day 12 after AI in groups separately. Regression analyses were conducted according to the method of Hosmer and Lemeshow (Hosmer & Lemeshow, 1989). The selected potential risk factors were then subjected to stepwise logistic regression approaches with hierarchical backward elimination and swapping. The P-values for data inclusion and exclusion were set at 0.05 and 0.10, respectively. All variables that had been selected or retained in those stepping approaches entered the final likelihood ratio (LR), from which the final odds ratio estimates with 95% confidence intervals were derived. P-values <0.05 were considered statistically significant.

Results

The mean (\pm SD) milk yields of the cows during the study were 39.4 ± 3.4 kg/day in the cool season and 35.4 ± 3.2 kg/day in the warm season.

In the cool season, there was a significant difference between the pregnancy rate on the day 28–30 in the treated (hCG, $p = 0.03$) (56%), (hCG+FM, $p = 0.05$) (55.1%), control (41.2%), and treated FM (40.6%) groups. Whereas, there was no significant difference between the treated (FM) and control groups. In the warm season, no significant differences in pregnancy rate on day 30 between the four groups were obtained (Figure 1 and 2).

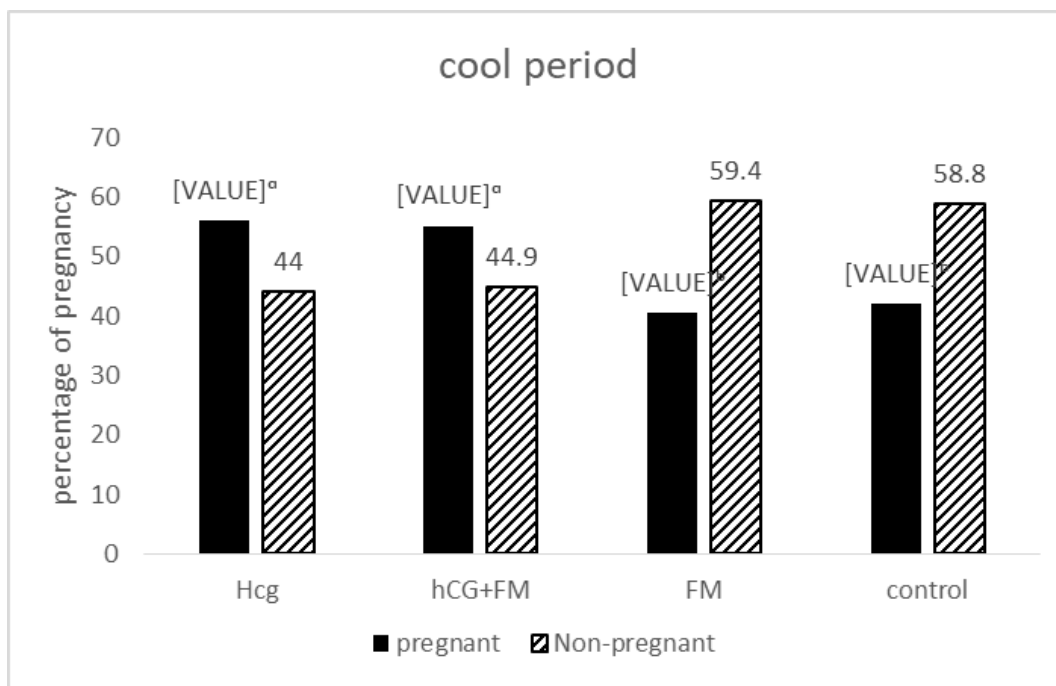


Figure 1. Pregnancy rate between four groups on d 30 of gestation in cool period.

The formation of the accessory luteal structure was greater (hCG, $p = 0.001$) and (hCG+FM, $p = 0.001$) for cows in the treatment groups (68%, 70.4%), respectively, than for the ones in the control group (26.5%). But in the warm season, no significant difference in the number of CLs between groups was observed. The increase in CL for cows treated with hCG compared with control cows was greater during the cool (1.7 vs. 1.1) period than during the warm (1.1 vs. 1.3) period ($p = 0.001$). There was a positive correlation between BSC and pregnancy, as well as

the number of CL in hCG and hCG+FM - treated cows, so that the rate of pregnancy and CL number increased in cows with a BSC greater than 2.75 compared with cows with a BSC lower than 2.75. (Table 1).

The discrepancy was not obtained in p4 concentration on day 5 after IA between groups in the cool ($p = 0.8$) and warm periods ($p = 0.6$). Whereas there was a significant difference in p4 concentrations on the 12th day after IA between groups in a cool period. In both the hCG and hCG+FM groups, the amount of p4 was greater than the other groups ($p = 0.001$). But the

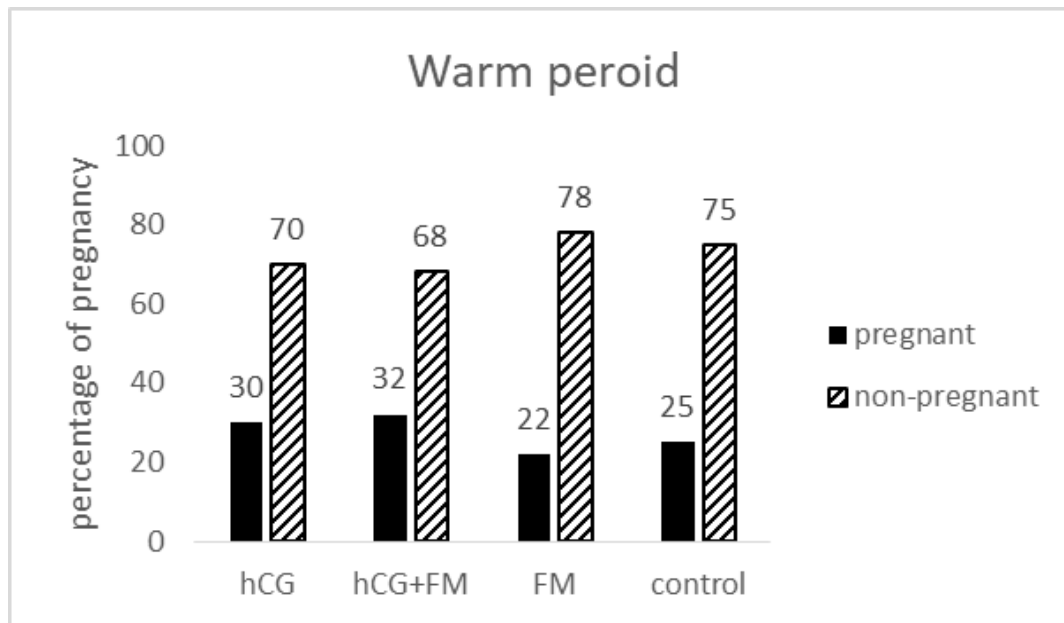


Figure 2. Pregnancy rate between groups on d 30 of gestation in warm period.

discrepancy in p4 was not observed between groups in the warm period ($p = 0.7$) (Table 2).

On Days 5 and 12, there were no differences in CL volumes between the third and control groups ($p = 0.7$). The discrepancy was observed in the CLs volume in the treatment hCG and hCG +FM groups from Days 5 to 12. The average volumes of CL were greater on Day 12 in the treated groups than the other groups ($p = 0.001$) (Table 2).

The significant association between CLs volume and progesterone concentrations on days 5 and 12 in both hCG and hCG +FM treated was obtained in the cool period ($r = 0.4$; $p = 0.0001$). But there was no significant correlation between serum progesterone and CL volume on days 5 and 12 in both the control and FM-treated groups ($p = 0.09$).

	Pregnancy status				The number of CL			
	open		Pregnant		1		1>	
BCS	hCG	hCG+FM	hCG	hCG+FM	hCG	hCG+FM	hCG	hCG+FM
2.75<	87%	79.50%	13% ^a	20.50% ^a	52.20%	59.10%	26% ^a	22.70% ^a
2.75 ≥	31.20%	34.40%	68.80% ^b	65.60% ^b	47.80%	40.90%	74% ^b	77.30% ^b

Table 1. Effect of body condition score at AI on pregnancy rate and the number of CL. ^{a,b} Data with different subscription in column significantly differ $p \leq 0.05$.

On Day 5, plasma progesterone concentrations were similar in the cold period for cows in the control, hCG, hCG+FM, and FM groups ($P = 0.8$). On Day 12, plasma progesterone concentrations in cows in the treatment groups (hCG, hCG+FM) were greater ($P = 0.001$) (13 ± 3.7 ng/mL) (12.7 ± 3.9), respectively, than in cows in the control and FM groups (9.7 ± 2.9), (10 ± 2).

The rising number of CLs and treatment during the cool period improved the odds of the pregnancy rate by 2.5 and 1.8 times, respectively. The rise in concentration of progesterone following hCG treatment increased the odds of a pregnancy by 1.1 times (Table 3).

Item	Cool period				Warm period			
	Treatment				Treatment			
	hCG	hCG+FM ²	FM	Control	hCG	hCG+FM	FM	Control
Progesterone: ng/mL								
on day 5	6.1	5.7	4.9	5.2	4.8	5.5	5.8	6.1
on day 12	13 ^a	12.7 ^a	10 ^b	9.7 ^b	11.3	10.4	9.8	9.6
CL ¹ number	1.68 ^a	1.70 ^a	1.19 ^b	1.21 ^b	1.21	1.16	0.90	1.11
CL volume mm ³	14253±8850 ^a	14331±12390 ^a	9672±8680 ^b	9210±1781 ^b	9113±8343	10012±12426	9272±2212	9865±8645

Table 2. Effect of treatment on plasma progesterone concentration at days 5 and 12 and corpora lutea number at days 12 after AI. ^{a,b} means with different superscript within rows are different; $p < 0.05$, 1-corpora lutea, 2-flunixin meglumine.

Variable	Class	Odds ratio	95% Confidence interval	P- value
Number of cl	CL > 1	2.5	1.33 - 4.92	0.01
	CL = 1	Reference		
Season	cool period	1.8	1.11 - 3.2	0.005
	warm period	Reference		
Progesterone	continuous	1.1	1.045 - 1.2	0.002

Table 3. Logistic regression predicting likelihood of pregnant cows Backward likelihood ratio test = 77.8, 4 df, $p = 0.0001$. Hosmer and Lemeshow goodness – of – fit, test=3.17, 8 df, $p = 0.9$ the model fits.

Discussion

Progesterone is essential for the formation and maintenance of pregnancy. Between days 8 and 16 of pregnancy, the mortality rate is estimated to be over 40% (Diskin & Morris, 2008; Sreenan & Diskin, 1986). Several studies have found that administering hCG on day 5 after AI increases the concentration of progesterone in dairy cows, heifers, and beef cows (Stevenson *et al.*, 2007; Santos *et al.*, 2001; Chagas *et al.*, 2005; Nishigai *et al.*, 2005). The administration of hCG on the fifth day following estrus results in an increase in progesterone due to the development of accessory CLs (Schmitt *et al.*, 1996b). Fricke and a colleague observed that a 1500 IU hCG injection on day 6 following estrus triggered ovulation and the formation of a new CL (Fricke *et al.*, 1993). A study found that administering flunixin meglumine during embryo transfer increases pregnancy rates in both Angus cows and Holstein heifer (Tahir *et al.*, 2021). Another study suggests that combined injections of human chorionic gonadotropin (hCG) and flunixin meglumine could potentially improve pregnancy rates in cows experiencing repeat breeding (Jawad *et al.*, 2021).

Our data showed that administration of hCG on day 5 after AI increased the number of CL and progesterone concentrations on day 12 after AI in the

cool season, but administration of hCG on day 5 after AI did not increase the number of corpora lutea and plasma progesterone concentrations on day 12 after AI in the warm season, which is consistent with Santos's (Santos *et al.*, 2001) study, which demonstrated that injection of hCG on day 5 after AI did not increase the number of CL and progesterone concentration in the warm period (Santos *et al.*, 2001). Schmitt and colleagues found that inducing an accessory CL with hCG on days 5 or 6 after insemination did not increase pregnancy rates in fertile heifers and lactating dairy cows during summer heat stress (Schmitt *et al.*, 1996b). Another study found that giving hCG to cows on day 5 following AI during the warm season had no effect on pregnancy rates (Hansen & Arechiga, 1999).

Two studies suggest that injecting flunixin meglumine into recipient beef cows undergoing embryo transfer could potentially increase their pregnancy rates (Kasimanickam *et al.*, 2018; Kasimanickam *et al.*, 2019). Administration of human chorionic gonadotropin (hCG) on days 4–7 after insemination has been shown to increase progesterone levels and improve pregnancy rates in dairy cows, as evidenced by studies demonstrating both effects (Santos *et al.*, 2001; walker *et al.*, 2005; Rensis *et al.*, 2008). Additionally, a study published reported that

administering hCG on day 5 after insemination elevated progesterone concentrations and improved pregnancy rates in both the hCG and ketoprofen groups (Singh, 2020).

Cattle embryos are susceptible to high temperatures during the first 2 to 3 days after fertilization, and they will die if exposed to high temperatures (Hansen *et al.*, 1999). Therefore, it is unlikely that inducing an accessory CL by treatment with hCG on day 5 after AI would improve the conception rate of heat - stressed dairy cows. Wolfenson and coworkers (1995) observed that cows had a 2 to 3 days earlier emergence of the second-wave dominant follicle and decreased the number of medium-sized follicles in heat stress (Wolfenson *et al.*, 1995). Perhaps, by d 5 after AI, the first-wave dominant follicle of some cows might have lost dominance, and that would decrease the response to hCG during the warm period. We determined a significant correlation between plasma progesterone concentration and CL volume, which agreed with earlier research that found a strong positive relationship between CL volume and plasma progesterone (Santos *et al.*, 2001; Stevenson *et al.*, 2007; Raja Mahendran & Sianangama, 1992). The effect of hCG on early embryo death is debatable. Some studies found that giving hCG on day 5 following AI improved the pregnancy rate (Breuel *et al.*, 1990; Sianangama & Mahendran 1992; Singh, 2020), whereas others found no change in the pregnancy rate in lactating cows (Eduvic & Seguin, 1982, Hansel *et al.*, 1976). Our findings show that treated cows with hCG on day 5 after AI increased pregnancy rate on day 30 after AI compared to the control group during the cool season but did not improve pregnancy rate in the warm season, which is consistent with the findings of Santos, who found that administration of hCG on day 5 after AI increased pregnancy rate on day 28 after AI during the cool season but had similar conception rates during the warm season (Santos *et al.*, 2001). Some studies found that administering FM after AI enhanced the likelihood of pregnancy (Merrill *et al.*, 2005) or at the time of embryo transfer (Purcell *et al.*, 2005; Scenna *et al.*, 2005). Treatment with FM on day 14 after AI may reduce PGf2 secretion and allow for more time for maternal recognition.

A similar result as ours was obtained in one study, which indicated that administration of GnRH on day

5 and another study showed that injections of hCG on day 6 after AI and ketoprofen on day 11 following AI improved pregnancy rates (Kraevskiy *et al.*, 2020; Rossetti *et al.*, 2011). However, it has been reported that administration of hCG on the 7th day and flunixin meglumine on the 16th day after insemination could not increase the pregnancy rate compared with the untreated group in Nelor cows (Eyyüp & Cevdet, 2023).

Research conducted found that giving FM to cows on days 13-15 following AI did not boost the conception rate when compared to the control group in both cool and warm weather. FM administration on day 13 following AI failed to improve pregnancy establishment in beef heifers and cows, according to Greay and colleagues (Geary *et al.*, 2009). One study showed that FM-treated cows and heifers had higher pregnancy rates than untreated cows, which contradicted our findings (Merrill *et al.*, 2005). Another study found that giving FM to cows on day 13 following AI did not boost conception rates in the warm season (Kaveh *et al.*, 2007). Cows with low BCS are more likely to be in anestrus and have poorer fertility, according to Ferguson (Ferguson, 1991). Our findings confirmed the Santos study, which found that cows with a BCS greater than 2.75 at the time of artificial insemination had a higher pregnancy rate and number of CL than those with a BCS equal to or lower than 2.75 (Santos *et al.*, 2001).

Conclusion

Our data show that hCG therapy on day 5 following AI in dairy cows significantly increased serum progesterone concentrations, the number of CLs, and the pregnancy rate compared to the control group in the cold period. The administration of flunixin, meglumine in both cool and warm periods, and hCG to heat-stressed cows had no effect on plasma progesterone levels, the number of CLs, or the pregnancy rate.

Conflict of Interest

None declared.

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تأثیر گنادوتروپین کوریونی انسانی و فلونکسین مگلو مین بر روی میزان آبستنی در گاوهای شیری

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چکیده

زمینه و هدف: افزایش ناباروری و کاهش میزان آبستنی دو مورد از چالش برانگیزترین مسائل در گاوهای شیری مدرن است. هدف از انجام این مطالعه بررسی تأثیر گنادوتروپین کوریونی انسانی روز ۵ و تزریق فلونکسین مگلو مین در روزهای ۱۴-۱۶ بعد از تلقیح بروی آبستنی در گاوهای شیری پرتولید در دو فصل سرد و گرم بود.

مواد و روش‌ها: در این مطالعه تعداد ۵۵۷ راس گاو شیری انتخاب به طور تصادفی به چهار گروه در هر دو فصل سرد (تعداد ۳۹۶ گاو) و گرم (تعداد ۱۸۱ گاو) تقسیم شدند. شاخص گرما - رطوبت (۸۵-۹۲) نشان داد که گاو ها در فصل گرم تحت استرس گرمایی بودند. گروه یک: تزریق هورمون گنادوتروپین کوریونی انسانی روز ۵ بعد از تلقیح، گروه دو: تزریق هورمون گنادوتروپین کوریونی انسانی روز پنجم و فلونکسین مگلو مین بین روزهای ۱۴-۱۶ بعد از تلقیح، گروه سوم: تزریق فلونکسین روزهای بین ۱۴-۱۶ و گروه چهارم: گروه کنترل. جهت بررسی میزان پروژسترون در روزهای ۵ و ۱۲ بعد از تلقیح نمونه خون اخذ گردید، همچنین جهت بررسی تعداد جسم زرد، اندازه گیری اقطار جسم زرد در روزهای ۵ و ۱۲ و آبستنی در روز ۲۸-۳۰ بعد از تلقیح سونوگرافی انجام شد.

یافته‌ها: نتایج نشان داد که میزان پروژسترون در گروه های یک و دو بیشتر از دو گروه دیگر در فصل سرد بود ($p=0/001$; $12/7$ و 13) در صورتیکه تفاوت معنی داری در بین گروه ها در فصل گرم مشاهده نشد ($p=0/7$; $10/4$ و 11). اجسام زرد تشکیل شده بعد از تزریق کوریونیک انسانی در دو گروه ۲ ($1/68$) و ۳ ($1/70$) بیشتر از دو گروه دیگر ($1/3$ و $1/1$) به ترتیب در فصل سرد بود ($p=0/0001$)، این اختلاف در بین گروه ها در فصل گرم مشاهده نشد. همچنین اختلاف معنی داری در بین گروه ها در فصل گرم از لحاظ آبستنی مشاهده نشد ولی در فصل سرد تغییرات معنی دار بود.

نتیجه گیری: با توجه به نتایج حاصل از این مطالعه استفاده از فلونکسین مگلو مین تأثیری در بهبود باروری در دو فصل سرد و گرم نداشت ولی تزریق گنادوتروپین کوریونی انسانی روز ۵ بعد از تلقیح توانست تعداد جسم زرد و میزان پروژسترون و همچنین میزان آبستنی را در فصل سرد افزایش دهد ولی در فصل گرم هیچ تأثیری مشاهده نشد.

واژه‌های کلیدی: کوریونیک انسانی، فلونکسین مگلو مین، پروژسترون، آبستنی، استرس گرمایی

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