Journal of Animal and Veterinary Advances 10 (16): 2176-2180, 2011
ISSN: 1680-5593

Effects of Post Insemination Flunixin Meglumine Injection on Corpus Luteum Maintenance, Plasma Progesterone Concentrations and Pregnancy Rate in Heat Stressed Holstein Dairy Cows

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Abstract: Aim of the current study was to evaluate the effect of Flunixin Meglumine (FM) post insemination injection on Corpus Luteum (CL) function, its Progesterone (P_4) secretion and pregnancy rate of dairy cows in heat stress condition. Estrus cycles of 120 Holstein cows were synchronized utilizing ovsynch protocol and cows were artificially inseminated. Environmental data indicated that the cows experienced medium heat stress during trials (mean daily temperature-humidity index = 79-84). They were randomly divided into three equal groups of 40. Two groups received FM injection between days 2-5 and 10-13 post inseminations once daily and the third group was selected as control. Blood samples were collected on days 7 and 14 post insemination for analysis of plasma P_4 concentrations. Ultrasonography scanning was performed on these days for CL detection following which its volume was calculated. The pregnancy status of the cows was estimated at days 27-30 by ultrasonography and confirmed on day 42 by palpation per rectum. Plasma P_4 concentrations showed no significant difference in groups receiving treatment compared by the control ($p>0.05$). However, CL volume was different between the treated groups on day 14 ($p = 0.03$). The pregnancy rate in group FM 10-13 (20%) was higher than that of group FM 2-5 (17.5%) and control (15%) but this improvement was not statistically significant ($p>0.05$). Treatment of heat stressed dairy cows with FM post insemination between days 2-5 or 10-13 led to CL maintenance but this treatment could neither increase the serum P_4 concentrations nor improve the pregnancy rate.

Key words: Flunixin meglumine, heat stress, corpus luteum, progesterone, holstein dairy cow, Iran

INTRODUCTION

Summer heat stress is a major factor contributing to lower fertility rate in lactating dairy cows. This global problem inflicts heavy economic losses and affects about 60% of the world dairy cow population (De Rensis and Scaramuzzi, 2003). During periods of heat stress, milk production, feed intake and physiological activity are diminished also, fertility is depressed during months of high temperature as well there is a carry-over into the cooler autumn period (Roth *et al*., 2004). The immediate and delayed effects of heat stress on fertility are multi-factorial. Heat stress disrupts follicular development, dynamics of follicular waves, steroidogenic capacity of theca and granulosa cells, Corpus Luteum (CL) development and function, oocyte quality and embryonic survival. Thermal stress before insemination has been associated with decreased fertility (Howell *et al*., 1994; Wolfenson *et al*., 1993). These changes have been linked to early embryonic loss. An early fetal loss at rate of 10%

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is commonly accepted and there is a significant correlation between season of insemination and early fetal loss (Garcia-Ispierto *et al*., 2006).

Maternal Recognition of Pregnancy (MRP) in cows begins around day 4 and Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is an important hormone involved in luteolysis. High concentrations of $PGF_{2\alpha}$ have a detrimental effect on developing embryos because $PGF_{2\alpha}$ stimulates endometrial vascularity, blastocyst hatching and embryo implantation (Charpigny *et al*., 1997; Dinchuk *et al*., 1995; Lim *et al*., 1997; Psychoyos *et al*., 1995; Reese *et al*., 1999; Van der Weiden *et al*., 1993). Losses around time of MRP is due to inability of certain embryos to inhibit secretion of $PGF_{2\alpha}$ (Thatcher *et al*., 1994). There are some evidences that $PGF_{2\alpha}$ may act directly on pre-compacted and compacted embryos by interfering with sub-sequent development and viability (Scenna *et al*., 2004). Heat stress can affect endometrial $PGF_{2\alpha}$ secretion, leading to premature luteolysis and embryo loss. Most embryonic loss occurs before day 42 in heat stressed cows. It was documented that early stages of embryonic development (8-16 cell) are more susceptible to heat stress but there are also high risks of embryonic loss at days 13 and 14 of pregnancy. As a consequence of these phenomena, it is expected that ambience with high temperatures significantly influence pregnancy rates in dairy herds.

Flunixin Meglumine (FM) is a non-steroidal anti-inflammatory medication inhibits the cyclooxygenase-2 enzyme, preventing conversion of arachidonic acid to $PGF_{2\alpha}$. The suppressive effects of FM on $PGF_{2\alpha}$ begin within 30 min of administration and persist for 6-24 h (Aiumlamai *et al*., 1990; Anderson *et al*., 1990). Guilbault *et al*. (1987) reported that $PGF_{2\alpha}$ secretion is markedly suppressed by FM treatment in dairy beef and dairy cow for at least 24 h. The objective of the present study was to evaluate whether the injection of flunixin meglumine post insemination would improve corpus luteum function and its progesterone secretion as well as pregnancy rate of dairy cows in heat stress condition.

MATERIALS AND METHODS

Animals and experimental location: The field study was conducted from 1st July to 20th September 2008 on 120 registered Holstein cows at the Mahdasht dairy farm in Sari (latitude of 36°34'N and longitude 53° 5'E, 16 m above sea level). The cows of the study were non-seasonal with year-round calves. They were housed in a free stall lot and fed a Total Mixed Ration (TMR) that included mainly alfalfa, corn silage, beet pulp, cotton seed, soybean, corn and barley thrice daily and fed according to the NRC. The age of the cows ranged from 3-7 years and they were machine-milked 3 times daily. The mean (±SD) milk yields of the cows during study was 28.45±9.62.

Daily maximum and mean temperatures as well as minimum and mean relative humidity were obtained from a meteorological station located 4 km apart of dairy farm. Temperature-humidity Index (THI) was calculated as per following equation:

$$\text{THI} = \text{Dry bulb temperature } (^\circ\text{C}) + (0.36\times\text{dew point temperature } (^\circ\text{C}) + 41.2$$

Treatments: The 60 days after calving was chosen as the voluntary waiting period. All the cows were examined by a real-time B-mode ultrasound scanner equipped with a 6-8 MHz liner array transducer (BCF, England) to determine condition of the ovaries. Treatment was only performed when the presence of a dominant follicle and a functional CL were detected. All cows were subjected to Timed Artificial Insemination (TAI) based on an ovsynch protocol consisting of 100 μg intramuscular injection of GnRH (CinnaRelin®, ScioGen Ltd, Iran) 7 days before and 48 h after a 25 mg intramuscular injection of $PGF_{2\alpha}$ (Lutalyse®, Pfizer, USA). Cows received one TAI with frozen thawed proofed semen, 16-22 h after the second GnRH injection.

The cows were randomly assigned into three equal groups (n = 40). Group 1 was treated with 100 μg FM (Flunixin meglumine, Flunex®, Razak, Iran) between days 2-5 post insemination once daily regularly. Second group received same dose of FM between days 10-13 post insemination once a day regularly. The third group was selected as control group and was not given any treatments.

Blood collection and transrectal ultrasonography: Blood was collected from all animals on days 7 and 14 post insemination from the coccygeal vein into heparinized tubes. Plasma was separated by centrifugation (for 10 min at 3,000 g) and stored at -20°C until assayed. Plasma Progesterone (P_4) concentrations were determined using a validated commercial Radioimmunoassay (RIA) kit (Immunotech kit, France). Average sensitivity of the assay is 0.2 ng mL^{-1} and average inter-assay and intra-assay coefficients of variation are 10 and 7%, respectively.

Ultrasonographic examination was performed on days 7 and 14 post insemination for detection of CL. The CL Volume (V) was estimated using the following equation for a modified prolate ellipsoid:

$$V = 0.523\times A\times B\times B$$

Where:
A = Maximum length
B = Transverse diameter

The pregnancy status of cows was estimated for the three groups at day 27-30 by ultrasonography and confirmed at day 42 by palpation per rectum.

Statistical analysis: Differences of plasma P_4 concentrations and CL volume in the three groups between days 7 and 14 post insemination were compared using one way ANOVA with commercial software (SPSS Version 11.5 for Windows, SPSS Inc., Chicago, IL). Fisher's exact test was used to analyze different pregnancy rates and the presence of CL in the three treatment groups. Pearson correlation was used to assess the relationship of CL volume and plasma P_4 concentrations in day 7 and day 14 post insemination in the groups separately. Values of $p \le 0.05$ were considered as significantly different.

RESULTS

Based on environmental data, the magnitude of heat stress experience in Mahdasht region, Mazandaran province was classified as medium heat stress. THI between 79 and 84 for mean daily temperature, the range achieved during this trial would constitute heat stress at an alert level. Individual cows exhibit signs of heat stress such as panting, hyperthermia, hypersalivation and high rectal temperature (39.2-40.8°C).

There was no significant difference in plasma P_4 concentrations among the treatment and control group (Table 1). Data also showed that there was not any significant difference in plasma P_4 concentrations on day 14 between the two treatment groups ($p = 0.40$). Effect of FM 2-5 on plasma P_4 concentrations was not significantly different in comparison with the control group on days 7 ($p = 0.44$) and 14 post insemination ($p = 0.25$). The effect of FM 10-13 on plasma P_4 concentrations was not significantly different in comparison with the control group on day 14 post insemination ($p = 0.76$).

The effect of FM on CL maintenance in the three groups between days 7 and 14 post insemination is shown in Table 1. In the control group, 14.8% (7/38) of cows showed decreases of CL volume during days 7-14 post insemination while in groups FM 2-5 and FM 10-13, it was 42 (13/31) and 33.3% (12/36), respectively. Table 2 shows the effect of FM on CL volume in the three groups between days 7 and 14 post insemination. The CL volume was only different between the treatment groups on day 14 ($p = 0.03$).

Table 1: Effect of injection of Flunixin Meglumine (FM) in different times (days 2-5 and 10-13 of post insemination) on Plasma progesterone (P_4) concentration and presence of functional Corpus Luteum (CL) in days 7 and 14 post insemination (n = 40)

	Plasma P_4 concentrations ($ng\ mL^{-1}$) (days)		Number of cows with CL (days)	
Groups	7	14	7	14
FM (2-5)	0.79±0.91	0.99±0.92	31	31
FM (10-13)	ND	1.21±1.97	36	36
Control	0.95±1.01	1.35±2.13	38	38

Table 2: Effect of injection of flunixin meglumine in different times (days 2-5 and days 10-13 of post insemination) on volume of Corpus Luteum (CL) in days 7 and 14 post insemination (double or triple ovulation or CL formation was omitted)

		CL volume (cm^3) (days)	
Groups	Cow	7	14
Flunixin meglumine (2-5)	25	6.00±4.59	5.57±4.21[a]
Flunixin meglumine (10-13)	21	7.70±6.15	9.05±6.77[b]
Control	22	6.10±2.93	8.31±4.08

[a, b]Different superscript in the same columns show significant differences ($p \le 0.05$)

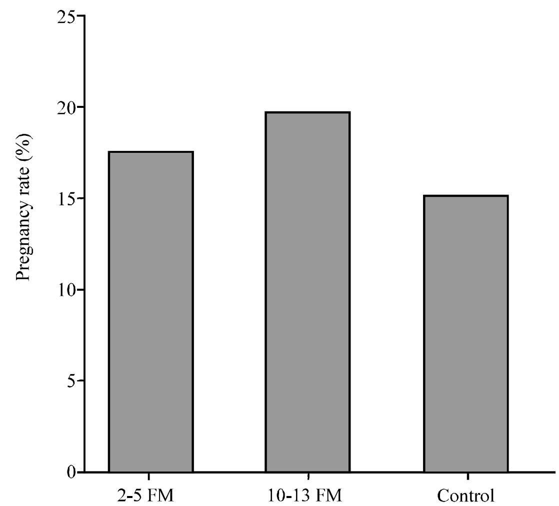

Fig. 1: Effect of 4 days intramuscular injection of Flunixin Meglumine (FM) in different time of post insemination (2-5 and days 10-13) on pregnancy rate (%) of Holstein dairy cows (n = 40) in heat stress condition

There was no statistically significant correlation between plasma P_4 concentrations and CL volume in day 7 and day 14 post insemination among three groups ($p > 0.05$). The first service pregnancy rate of the treatment groups (2-5 and 10-13) and the control group were 17.5, 20 and 15%, respectively which were not significantly different ($p > 0.05$, Fig. 1).

DISCUSSION

Results of the current study manifested that injection of FM could not improve the relationship between plasma

P_4 concentrations and CL volume in day 7 and day 14 post insemination in the treated groups compared by control group. Wolfenson *et al.* (2002) in an *in vitro* study showed that thermal stress caused a negative effect on the function of CL. This indicates that chronic effect of heat stress is possibly carried out from an impaired follicle to an impaired CL. It was also illustrated that luteonzied theca cells are more susceptible to heat stress than granulosa cells hence, P_4 secretion decreases even if CL formation to be completed (Wolfenson *et al.*, 2002). Consistent with the findings of Wolfenson *et al.* (2002), the present study documented same findings in an *in vivo* condition in heat stressed dairy cows. Moreover, in the present study plasma P_4 concentrations among the groups were not significantly different on days 14. Environmental conditions that implicate heat stress as a causative factor in reducing reproductive performance in dairy cows only begin to manifest the signs whilst THI is >72. Also, THI vigilance might be subjected as THI is of 70-78 (Wolfenson *et al.*, 2002). Plasma P_4 concentrations in chronically heat stressed cows in summer decreased (or tend to decrease) compared to those cooled in summer by various means (Badinga *et al.*, 1993; Guzeloglu *et al.*, 2001; Hendriksen *et al.*, 2000; Roth *et al.*, 2000).

There is scarce information on effect of FM administration on either early or late point after breeding. Merrill *et al.* (2003) reported that FM appears to decrease the stressed induced embryonic loss since, it suppresses the $PGF_{2\alpha}$ side effect and prevents luteolysis. In the present study administration of FM post insemination, slightly improved the pregnancy rate in heat stressed cows of two treated groups in contrary to the control.

Administration of FM between days 10-13 shows a better pregnancy rate (20%) than days 2-5 (17.5%). Merrill *et al.* (2003) reported no differences in concentrations of the metabolite of PGF in serum collected from cows before or after a 4 h trucking stress (±FM or control). In a subsequent study Merrill *et al.* (2004) elucidated heifers and cows that received FM 14 day after AI (±trucking stress) had higher AI pregnancy rates than heifers and cows receiving no FM (±trucking stress) (Merrill *et al.*, 2004). In the present study, administration of FM in days 10-13 could lead to late regression of CL (suppressive effect) however, there was no divergence in plasma P_4 concentration despite CL maintenance. This fact demonstrate that under chronic summer heat stress conditions, P_4 production is markedly reduced in luteinized theca cells as well as in luteinized granulosa cells. This finding is completely in agreement with that of Wolfenson (Wolfenson *et al.*, 2002).

Flunixin meglumine affects on CL volume but the correlation between plasma P_4 concentrations and CL volume was not significant. A study that was performed on cows supported these findings and revealed that even when cows were supplemented with exogenous P_4 and sub-sequently exposed to $PGF_{2\alpha}$, embryo development was inhibited (Hockett *et al.*, 2004). Kafi *et al.* (2006) demonstrated that administration of clinically recommended dose of ketoprofen during the pre and periovulatory period in dairy cows could be capable of delayed luteal regression and impair final growth of ovulatory follicles leading to a disturbed normal process of ovulation and corpus luteum development in dairy cows. Elli *et al.* (2001) showed that ibuprofen can improve pregnancy rate after embryonic transfer in normal temperature condition by preventing formation of $PGF_{2\alpha}$ via inhibiting cyclooxygenase activity.

CONCLUSION

Treatment of heat stressed dairy cows with flunixin meglumine post insemination between days 2-5 or 10-13 led to corpus luteum maintenance however, this treatment could neither increase the serum P_4 concentrations nor improve the pregnancy rates.

ACKNOWLEDGEMENT

The researchers wish to thank Mahdasht milk and meat complex, especially Mr. Farsad Mohagheg Dolatabadi for his skillful assistance and financial support of the project.

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