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Evaluation of the Effect of Muscular Injection of Progesterone on Days 2-5 Following Insemination on Pregnancy Rate in Dairy Cows

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Abstract: It was hypothesized that suboptimal progesterone concentrations during the late embryo and early fetal period may act to compromise conceptus development in dairy cattle. The importance of progesterone during early pregnancy in cows is well established. Adequate secretion of progesterone is clearly the major requirement for success of early pregnancy. The aim of this study was to evaluate the efficacy of exogenous progesterone injection from day 2 to 5 following AI on conception rate in dairy cattle. At 48 h after the AI the cows were alternately assigned to treatment group (n = 40), received 4 consecutive day, from Day 2 to 5, i.m. injection of 100 mg progesterone (Vetagesterone, Aburaihan, Iran, Each mL contains: 2.5 mg progesterone) and control group (n = 68), received no treatment. The study population was stratified by parity, milk yield production, number of AI and days in milk. Pregnancy proportion in treatment and control groups were 30.88 and 35%, respectively. The results demonstrated that there was no significant differences between two groups ($p < 0.05$).

Key words: Muscular injection, progesterone, insemination conception rate, dairy cows

INTRODUCTION

Conception rate in modern dairy cow are at an all time low and continue to decline at an alarming rate. First service conception rate to artificial insemination decreased from approximately 65% in 1951 to 40% in 1996 in New York (Butler, 1998; Starbuck *et al.*, 2004). The reasons for this are numerous and vary but perhaps the main cause is early embryo mortality during the first 3 weeks of pregnancy. During early pregnancy, embryo must inhibit the development of the luteolytic mechanism to maintain the secretion of progesterone necessary for continuing development. Thus, embryos must secrete a protein, interferon tau, (IFN- τ), which acts locally within the uterus to inhibit luteolytic PGF2 α secretion by inhibiting the development of oxytocin receptors on the luminal epithelium (Robinson *et al.*, 1999; Mann and Lamming, 2001).

It has been known for many years that progesterone is a critical hormone during early pregnancy. Progesterone play a major role in stimulating the production of variety of endometrial secretions necessary for the successful development of embryo (Geisert *et al.*, 1992). As early as 1950s studies were being carried out to investigate progesterone supplementation as a means to improving

conception rates (Mann and Lamming, 1999). In these studies, a range of different cows has been treated with different progesterone therapies over a range of different time periods. While many of these studies have demonstrated improvements in pregnancy rate, others have not. Further analysis revealed that the timing of progesterone supplementation, initial fertility of the herd, targeting progesterone treatment to cows with a particular problem, is the critical factors (Mann and Lamming, 1999; Starbuck *et al.*, 2001).

A slower than normal rise in progesterone concentration and a lower total progesterone concentration, in the first 6 days after estrus, have been measured in low-fertility cows (Villarreal *et al.*, 2004; Shelton *et al.*, 1990; Bage *et al.*, 2002).

Studies on the relationship between the pattern of maternal progesterone secretion and development of the embryo have demonstrated that in cows that had exhibited a late post ovulatory rise in progesterone, on day 16 following insemination, the embryo poorly developed and producing little interferon tau. In contrast in cows with an earlier post ovulatory rise in progesterone, embryos were well elongated and were producing large quantities of interferon tau (Mann and Lamming, 2001). Furthermore, in cattle, treatment with

progesterone from day 2-5 has been shown to result in a 10 fold increase in coceptus elongation on day 14 (Garrett *et al.*, 1988). Later increases in progesterone have failed to cause any marked increase in embryo development (Kerbler *et al.*, 1997).

In the present study we have investigated the effect of i.m. injection of progesterone from day 2-5 following AI on conception rate in dairy cattle.

MATERIALS AND METHODS

The experiment was carried out in a large commercial dairy herd, 600 Holstein in milk, located in suburbs of Char-Mahal and Bachtiary province during a period from February to March 2005. Cows were milked three times a day at 0600, 1400 and 2100. 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 animals were kept in a open shed barn with concrete floor. The cows, on the basis of their milk yield production, were fed a total mixed ration. The herd was under veterinary health care throughout the year. All cows were routinely checked and treated for health disorders, 4 week after calving. Two person observed animals 24 h per day, for estrous detection, with an emphasis on two time of each day, at evening (just before dusk) and at morning (just after sun rise). Cows were inseminated at estrous by on farm technician. At 48 h after the AI the cows were alternately assigned to treatment group (n = 40), received 4 consecutive day, from Day 2 to 5, i.m. injection of 100 mg progesterone (Vetagesterone, Aburaihan, Iran, Each mL contains: 2.5 mg progesterone) the doses of progesterone used in this study were the doses recommended by the above-mentioned company and

control group (n = 68), received no treatment. Cows entered the study matched by parity, milk yield production, days in milk and number of AI. Cows that were detected in estrous after day 18 were re-inseminated and recorded as non pregnant (open) to the prior AI. The uteri of cows not observed in estrus 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 tests.

RESULTS

In total 108 cows entered in the study. Of 68 cows in treatment group, 21 cows and of 40 cows in control group 14 cows were pregnant at rectal examination. Unstratified analysis showed no effect of i.m. injection of 100 mg progesterone on Days 2-5 following AI on pregnancy proportion (p<0.05). The effect of this treatment on pregnancy outcome was statistically indifferent among lactation, milk yield production, days in milk and number of AI (Table 1).

DISCUSSION

The numerous studies have examined the effects of progesterone supplementation on pregnancy rate in cattle (Mann and Lamming, 1999; Mann and Laming, 2001; Mann *et al.*, 2006). In these studies, progesterone supplementation initiated at the time of onset of the post ovulatory rise (between days 4 and 5) has resulted in consistent increases in pregnancy rate. However, when supplementation has been initiated later than this, consistent improvements have not been reported (Mann and Lamming, 1999; Strong *et al.*, 2005).

Table 1: The effect of i.m. injections of 100 mg progesterone during 2-5 days following AI on conception rate in dairy cattle, matched by parity, milk yield production, days in milk and number of AI

Variable	Level	Control group (n = 40)		Treatment group (n = 68)	
		No. of pregnant (%)	No. of open (%)	No. of pregnant (%)	No. of open (%)
Parity	1	8 (72.72)	3 (27.27)	9 (34.61)	17 (65.38)
	2	3 (30)	7 (70)	5 (31.25)	11 (68.75)
	≥3	3 (15.78)	16 (84.21)	7 (26.92)	19 (73.07)
Milk production (kg)	<30	7 (43.75)	9 (56.25)	11 (47.82)	12 (52.77)
	>30	7 (29.16)	17 (70.83)	10 (22.22)	35 (77.77)
Days in milk	50-120	10 (41.66)	14 (58.33)	19 (33.92)	37 (66.07)
	≥121	4 (25)	12 (75)	2 (16.66)	10 (83.33)
No. of AI	1	8 (38.09)	13 (61.90)	9 (25)	27 (75)
	2-3	3 (37.50)	5 (62.5)	5 (27.77)	13 (72.22)
	>3	3 (27.27)	8 (72.72)	7 (50)	7 (50)
Total No. of cows	108	14 (35)	26 (65)	21 (30.88)	47 (69.11)

Low progesterone has been linked to early pregnancy failure and poor embryo development (Mann and Lamming, 1999; Walton *et al.*, 1990), while supplementing cows with progesterone has been shown to enhance conceptus development (Garrett *et al.*, 1988).

The result of this study demonstrate that progesterone injection early in the luteal phase, during the period of 2 to 5 days following insemination can not enhance pregnancy rate in dairy cattle. The results of this study are agreement with the finding of Santos *et al.* (2004a). They pointed in their review that treatment with exogenous progesterone before day 4 would be expected to advance uterine secretion of PGF 2α and cause premature luteolysis. The results of this study are contrast to the finding of Garret *et al.* (1988). Their study showed that treatment with progesterone from day 2-5 following of AI, result in a 10 fold increase in conceptus elongation on day 14 and can cause increase in pregnancy rate.

Many factors influence conception rate, among them is cyclicity, energy balance, heat stress, parity, milk yield production, diet, service number, days in milk and diseases (Cartmill *et al.*, 2001; Gröhn and Rajala-Schultz, 2000; Hansen and Arechiga, 1999; Lucy, 2001; Moreira *et al.*, 2001; Santos *et al.*, 2004b). In the present study days in milk, number of AI, milk yield production and parity did not affect the pregnancy rate in two groups. These results are agreement with the finding of Chebel *et al.* (2004), Starbuck *et al.* (2004) and Lopez-Gatius *et al.* (2004).

In conclusion a full understanding of the biology of progesterone action during pregnancy recognition, maintenance of pregnancy, the control of embryo development and IFN- τ production is of paramount importance in determining strategies to reduce the high early embryo mortality that occurs in dairy cattle, while along-term solution to this problem should concentrate on prevention, in the shorter term progesterone therapies are available to treat this problem. Clearly if progesterone supplementation was to be effectively used to increase embryo survival, it would have to be in a targeted fashion rather than blanket treatment of all cows.

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