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Induction of Lactation in Holstein Cows Using Progesterone Injections or Progesterone Vaginal Inserts

Research Article

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ABSTRACT

The aim of this study was to evaluate the effect of replacing progesterone injections by progesterone vaginal inserts on productive and reproductive parameters of lactating cows with reproductive problems after prolonged periods of heat stress. Fifteen Holstein cows were randomly assigned to one of three treatments: 1) treated with two progesterone vaginal inserts (P4-INS); 2) treated with progesterone and estradiol via injections (P4-INJ) and 3) control, cows with gestation and parturition normally. All cows were subjected to a Presynch-Co-Synch timed artificial insemination protocol and then inseminated at 71 d in milk. Concentration of P4 in (P4-INS) and (P4-INJ) cows during the first 7 d of induction of lactation was similar ($P>0.05$) and then increased linearly in both groups. Adjusted milk yield, total milk yield, and average daily milk yield were similar ($P>0.05$) in treated cows, but cows from (P4-INJ) group produced less milk ($P<0.05$) than control cows. Days in milk and peak milk yield were similar ($P>0.05$) among all groups. Percentage of milk fat and protein, as well as days to peak milk yield were higher ($P<0.05$) in both treated groups compared to control. Body condition score, service per conception, conception to first service, total conception, days open and culling rate at the end of lactation were similar ($P>0.05$) among treatments. Progesterone administration by injection or by vaginal insertion resulted in similar production and reproduction parameters of dairy cattle with induced lactation.

KEY WORDS heat stress, induction of lactation, milk composition, milk yield, progesterone.

INTRODUCTION

Many milk-producing regions of Mexico are located in northwestern areas of the country which are characterized as hot, arid and semiarid environments. Every summer, periods of high ambient temperature and intense solar radiation cause a drastic reduction in productivity of dairy herd operations located in these regions. Hence, low milk yield and depressed breeding efficiency are generalized problems among milk producers in these desert environments. The failure to breed results in long calving intervals and substantial non-voluntary culling losses, which leads to loss of benefits from low milk sales and increased costs of reproduction (St. Pierre *et al.* 2003). Culling rates in these dairy operations may be over 35% per year (Avendaño-Reyes *et al.* 2006) and many of these cows are culled prematurely due to reproductive failures, leading to a higher number of heifers required for replacement. The induced

lactation of cows can be an alternative to reduce the rate of culling and also to increase economic benefits in dairy operations with fertility problems during summer.

Some research confirmed the feasibility of inducing lactation in non-pregnant cows using a shortened hormonal protocol (Collier *et al.* 1975; Byatt *et al.* 1994; Magliaro *et al.* 2004; Macrina *et al.* 2011a) and most of these studies were conducted using animals with documented reproductive failures. Smith and Schanbacher (1973) showed that lactation could be induced using a 7-d treatment period of estradiol-17-ß (E2) and progesterone (P4). This treatment was designed to mimic the high levels of these steroids observed during the last month of pregnancy in cows, when significant mammary development occurs. Currently, the protocols for induction of lactation include the application of other hormones such as somatotropin (rbST), $PGF_{2\alpha}$ and dexamethasone, and after 21 d, animals initiate their lactation (Villa-Godoy *et al.* 2003). The effects of estrogen and progesterone on mammogenesis were extensively evaluated in attempts to induce lactation in non-pregnant animals. In the 1970 s, there was a flurry of activity on hormonal induction of lactation in cattle based on a variety of short-term injection schemes using estrogen and progesterone (Akers, 2006). Milk production of treated cows has been variable, ranging from 63 to 106% from the best previous lactation and it is not clear why some animals respond to the induced lactation process better than others (Magliaro *et al.* 2004). It is possible that eliminating injections from at least one hormone from the complete protocol, cows would be able to perform better. So hormone injections may be replaced by the use of controlled internal drug release (CIDR), which are devices that slowly deliver progesterone. Based on the above, the objective of the present study was to determine the effects of replacing the source of progesterone applying intravaginal devices of slow P4 delivering during the induction lactation protocols on production and reproduction parameters of dairy cattle.

MATERIALS AND METHODS

Study area, animals and treatments

The study procedures and animal care conditions performed in this study were approved by the ethic committee of the research department of the Institute Technologic de Sonora (ITSON). The experiment was carried out in the Academic Unit and Research in Milk Production of the ITSON, located in the Yaqui Valley, Sonora, northwestern México (27˚ 29' N, 109˚ 56' W). The altitude of this area is 46 m, mean annual temperature of 23 ˚C and annual precipitation of 371.6 mm. However, during summer temperatures can reach 48 ˚C and the temperature-humidity index around 85 units. The herd consisted of Holstein cows housed in open, dirty floor pens. Fifteen Holstein cows were blocked by parity and three treatments were randomly assigned within blocks, including five animals each group as follows: 1) P4 inserted (P4-INS), cows treated with two progesterone vaginal inserts; 2) P4 injected (P4-INJ), cows that received injections of progesterone and estradiol and 3) control (non-treated cows), cows that had gestation and parturition normally. Induced groups consisted of non-pregnant dairy cows which did not get pregnant during summer, so the study was performed after summer months, during fall season. Animals received daily subcutaneous injections of estradiol-17-ß 0.05 mg/kg of BW (Bioestrogen® 1.0 mg/mL; Biogenesis-Bagó, México) and progesterone in two different ways: a) one via daily subcutaneous injection 0.625 mg/kg of BW (Progesterona® 50.0 mg/mL; Zoetis Salud Animal; México) for the P4-INJ treatment group and b) via P4 vaginal inserts (Cronipres® 0.186 g Biogenesis-Bagó, México) for the P4-INS treatment group, both ways during days 1 to 7 of the experiment. Also, 0.05 mg/kg of BW of estradiol-17-ß was applied from days 8 to 14. In addition, bST (Lactotropina®, 500 mg of zinc bST, Elanco Animal Health, Guadalajara, México) was applied on days 1, 10, 20, and afterward every two weeks after initiated the lactation cycle. Lactation was triggered by administration of 0.0041 mg/kg of BW per day of flumethasone (Fluvet®, 0.50 mg/mL Zoetis Salud Animal; México) injected s.c. daily for 3 d (days 17 to 19).

Milking of induced animals was initiated on day 21 and the control group initiated their lactation at the time of calving date and these animals were handled like prepartum or dairy fresh cows. All cows were milked twice daily (05:00 and 17:00 h) and milk yield was measured using automated electronic devices (Metatron 21, Westfalia Surge, México, S.A., Aguascalientes, México) integrated to the milking system. One milk sample from each cow was collected once monthly from 2 consecutive milking, and was analyzed for fat and true protein concentrations using a milk analyzer based on ultrasonic spectrometry (lacticheck milk analyzer, page and Pedersen international, Ltd., Hopkinton, MA, USA). All cows were submitted to the same reproductive management into the herd consisted of Presynch-Co-Synch timed artificial insemination protocol (TAI) and were inseminated at 71 d after lactation initiated. Figure 1 illustrates the treatments used in the present study.

Housing and feeding

During the induction phase of the study, induced animals were separated and housed in an open dry lot pen with shades in the center, and receiving appropriate care to prevent injury due to estrous behavior associated to the estrogen-progesterone treatment. Before initiation of lactation, animals were fed a diet balanced to meet requirements of late gestation dairy cows (NRC, 2001). Three days before initiation of lactation (treatment d 21), animals received a

mixture containing equal parts of the dry cow diet and a lactating cow diet. Once milking began, animals received a lactating cow ration *ad libitum* twice daily, and forage was offered separately from commercial concentrated (60 and 40% respectively). This concentrate consisting of corn silage, alfalfa hay, steam-flaked corn, whole cottonseed, soybean meal, dried corn distillers grains, animal-marine protein blend, calcium salts of palm fatty acids, minerals and vitamins. Diet was formulated to meet nutrient requirements for lactating Holstein cows weighing 600 kg and producing 28 kg of 3.5% fat corrected milk (FCM) (NRC, 2001). Chemical composition of the lactating diet was 15.8% CP, 30.0% neutral detergent fibre (NDF) and 1.4 Mcal/kg of NE_L after adjusting for 24 kg of days in milk (DIM).

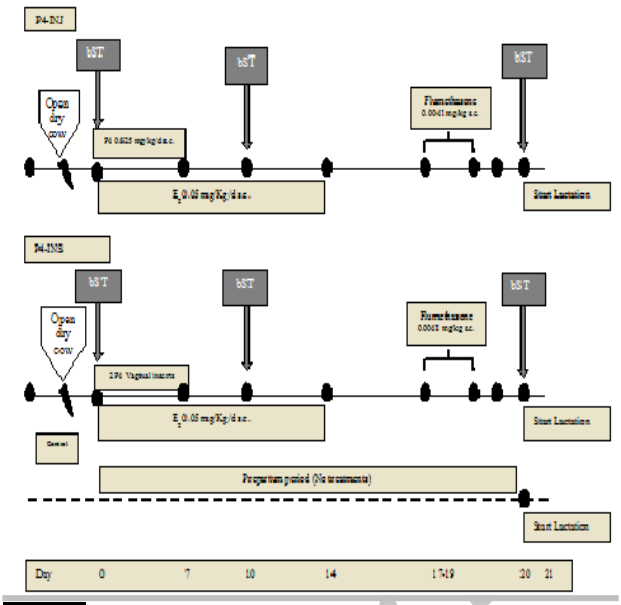

Figure 1 Diagrammatic depiction of treatments: P4-INJ, induction lactation protocol by administration of progesterone using only injections; P4-INS, induction lactation protocol by administration of P4 using two vaginal progesterone inserts (Cronipres® 0.186 g each) and C: group of cows that had gestation and parturition normally
The two induced groups received 3 bST injections of 500 mg each

Blood sampling and analyses of plasma

Blood samples of 8 mL were obtained from all cows by puncture of coccygeal vessels and placed into tubes containing K_2 EDTA (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) once daily during days 1 to 7 of the induced period. Samples were refrigerated and centrifuged at 2500 x g for 15 min and plasma was frozen at -25 ˚C for later analyses. Concentrations of progesterone were analyzed in samples from 10 animals by a validated ELISA technique (Cerri *et al.* 2004). The intra and inter-assay coefficients of variation were determined for each of 96 well plate using 2 plasma samples with known concentrations of progesterone (low=1.5 ng/mL; high=2.5 ng/mL). The sensitivity of the assay was 0.10 ng/mL and the inter-assay coefficients of variation for the low and high progesterone samples were 13.1 and 7.5% respectively. Individual samples and micro plates with coefficients of variation > 15% were re-analyzed.

Experimental design and statistical analyses

The study plan was a randomized complete block design. Animals were blocked by parity and within each block the treatments were randomly assigned. The statistical model included the effects of treatment and parity. Variables with binomial responses were analyzed by logistic regression using the logistic procedure of SAS (2004). Milk yield, milk components, and progesterone concentrations were analyzed by repeated measures using the mixed procedure of SAS (2004). The model included the effects of treatment, parity, day of measurement and interaction between treatment and day of measurement; cow nested within treatment was considered the random effect. Treatment differences with $P \leq 0.05$ were considered significant and from $0.05 < P \leq 0.10$ were designated as tendency.

RESULTS AND DISCUSSION

Progesterone concentrations

During the first seven days of the treatments, the interaction treatment x day was not significant (P=0.27). Induced cows that received progesterone by different forms (injections or vaginal insert) had similar (P=0.90) progesterone levels. Progesterone concentration during the first 7 d from cows under induced lactation protocols increase linearly in both groups, and during the seventh day they averaged 5.25 ± 0.29 ng/mL.

Productive parameters

Milk yield at 305-d was similar (P=0.12) between the two lactation protocols, and also (P=0.12) between P4-INJ group and control animals (Table 1). However, control cows produced more milk at 305 d (P<0.05) than P4-INS cows. Regarding total milk yield during the complete lactation, there was no difference (P=0.90) between P4-INJ and P4-INS groups, and similarly between P4-INJ group and control animals (P=0.90). Nevertheless, control cows produced more milk during the complete lactation (P<0.05) than P4-INS cows. On the other hand, average milk production was similar between both groups of induced lactation animals, however, control cows averaged more milk (P<0.05) than P4-INS cows (Table 1). Both groups under induced lactation protocols produced milk with higher fat and protein content (P<0.05) with respect to control cows. The number of days in milk (DIM) was similar (P>0.05) among the three groups. The induced cows, however, aver-

aged fewer DIM than natural lactation cows. Animals of all groups averaged similar milk yield (28.89±1.68 kg/d; P=0.70) at their production peak, however, cows under induction of lactation protocols reached their peak production at 118 ± 5.8 DIM (P<0.05), which was 56 days later than control cows.

Table 1 Production parameters in Holstein cows with induced lactation and with natural lactation[1]

Item	Treatments			SE
	P4-INJ	P4-INS	Control	
Milk yield to 305 d (kg)	7480^{ab}	7430^{a}	7500^{b}	20.7
Total milk yield (kg)	8976^{ab}	8916^{a}	9000^{b}	23.3
Milk yield (kg/d)	22.1^{a}	21.5^{a}	24.5^{a}	3.37
Fat (%)	3.52^{a}	3.55^{a}	3.41^{b}	0.041
Protein (%)	3.42^{a}	3.34^{a}	3.28^{b}	0.029
Days in milk	288^{a}	275^{a}	310^{a}	20.33
Peak milk yield (kg/d)	29.55^{a}	28.08^{a}	29.05^{a}	1.687
Days to peak milk yield	123^{a}	113^{a}	62^{b}	5.63

[1] The natural lactation cow was selected as a cohort for each induced cow based upon first day milking. Samples for milk composition were collected monthly for a 10 month period.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
P4-INS: progesterone vaginal inserts and P4-INJ: progesterone and estradiol via injections.
SE: standard error.

Reproductive parameters and body condition

Reproductive performance and BCS outcomes of induced and natural lactation cows are shown in Table 2. Averages of BCS at the beginning of the lactation cycle of the induced lactation and natural lactation cows were similar (P=0.80), however, at the time of artificial insemination (AI), body condition score (BCS) was lower (P<0.05) in the group of control cows. On the other hand, since all animals received the same reproduction management, all cows were inseminated at 71 DIM under the Co-Synch time AI protocol. Conception rate at first service showed no differences (P=0.80) between both groups of induced lactation animals, and also with the control group (Table 2). Services per conception in the three groups was similar (P=0.90), averaging 2.2 services. Total conception rate neither showed differences (P=0.90) between groups of cows under induction lactation protocols with respect to control cows. Days open were also similar (P=0.90) in the three groups and averaged 118 ± 5 d. No one cow from natural lactation group was culled during their lactation cycle, meanwhile 30% of the cows from the induced lactations groups were culled (P=0.80). All cows from the natural lactation group calved, while seven from ten cows in the induced lactation groups calved.

The treatment for induction lactation in dairy cows was designed to mimic high levels of the steroids hormones observed during the last month of pregnancy, when significant mammary development occurs (Smith and Schanbacher, 1973). At around day 20 of the two treatments, cows received high doses of estradiol 17-ß progesterone and other drugs via injections and finally animals began their lactation cycle. Based on the above, the induction of lactation involved more cattle handling and consequently greater stress due to the large number of injections that were applied. So trying to reduce this situation, the present study found the use of intravaginal progesterone devices during the first seven days of the protocol very practical, reducing in some way the stress to the animals. Steroids delivery using intravaginal devices has been attempted by several authors (Davis *et al.* 1983; Lucy *et al.* 2001; Lima *et al.* 2009; Rivera *et al.* 2011). It was observed in the present study that P4 concentrations in plasma increased in both groups of cows under induced lactation (P4-INS and P4-INJ), which implies that no matter the source of progesterone administration, the concentrations of this hormone in cows with induced lactation are similar to those P4 concentrations observed in late pregnancy dairy cows. Progesterone supplementation through vaginal devices has been resulted in a linear increase of this hormone (Lima *et al.* 2009). In agreement to the results found in the present study, Davis *et al.* (1983) indicated that the labor input required during treatments is reduced using progesterone vaginal devices. It was also demonstrated that no matter how progesterone is supplemented, this hormone acts synergistically with estrogen, which in turn is supplemented by injections to stimulate lobe alveolar growth in the mammary gland, thus promoting mammogenesis and finally increasing the synthesis and release of both hormones (Pelissier, 1972). It is possible that other steroid hormones perform similar to progesterone, acting on the DNA to initiate the synthesis of mRNA, and then inducing cell growth along the walls of the milk ducts, where specific progesterone receptors are located (Haslam and Shyamala, 1979). Furthermore, some days before calving, there is a decrease in progesterone secretion along the onset of milk secretion, which means that progesterone stimulates mammogenesis but inhibits lactogenesis and is required a reduction in P4 concentrations to background levels, and then initiates the synthesis of milk (Smith and Schanbacher, 1973). It is important to note that once lactation cycle is initiated and established, the administration of P4 will have no effect on milk yield (Herrenkohl, 1972), because P4 receptors are no longer present in the mammary tissue (Haslam and Shyamala, 1979). Moreover, the role of estrogens during induced lactation protocols simulate reasonable concentrations found during pregnancy, which will have a significant increase in their concentrations between days five and two prior to calving, then estrogens act synergistically with growth factors that circulate in blood mainly from liver and together stimulate the stroma of mammary epithelial cells (Rivera *et al.* 2010). In the meantime, other authors

(Baumrucker and Stemberger, 1989; Lucy, 2000; Bilby *et al.* 2004) have noted that insulin-like growth factor 1 (IGF-I) is secreted locally in the cells of the mammary gland and are considered the main promoters of growth during mammogenesis. In addition, estrogens are involved in the onset of lactogenesis proximate to calving and have basically two functions: first cause releasing of prolactin from the anterior pituitary, and second increase the number of prolactin receptors on mammary cells (Nagasawa *et al.* 1969). Other important hormones during the induction of lactation in cows are corticosteroids, which act in the mammary gland during lobe alveolar differentiation. In the present study, the corticosteroid flumethasone was applied to both treated groups. Mills and Topper (1970) argue that cortisol acts in the rough endoplasmic reticulum and the Golgi apparatus. This differentiation is necessary to allow that prolactin induces the synthesis of protein in milk, and then between days 2 and 5 before calving, blood concentration of glucocorticoids increases concurring with the expulsion of the calf (Edgerton and Hafs, 1973). However, blood glucocorticoids bind to carrier proteins (corticosteroid binding globulinin (CBG)) then inactivating the same glucocorticoids, but close to calving, this CBG decreases and there will be an increase in free glucocorticoids, then increasing lactogenic activity of glucocorticoids (Gorewit and Tucker, 1977).

Table 2 Body condition score, reproductive performance and culling rate for cows under two protocols for induction of lactation with respect a group of cows with natural lactation

Item	Treatments			SE
	P4-INJ	P4-INS	Control	
BCS at calving[1]	3.52[a]	3.56[a]	3.50[a]	0.013
BCS at timed AI[1]	3.00[a]	3.12[a]	2.75[b]	0.02
Conception 1st service (%)	40% (2/5)[a]	60% (3/5)[a]	40% (2/5)[a]	-
Services per conception	2.1[a]	2.2[a]	2.3[a]	0.22
Total conception rate (%)	80% (4/5)[a]	60% (3/5)[a]	100% (5/5)[a]	-
Days open	113[a]	116[a]	125[a]	10.0
Culling rate (%)[2]	20% (1/5)[a]	40% (2/5)[a]	0% (0/5)[a]	-

[1] Body condition of animals was scored using a 1-5 scale (Ferguson *et al.* 1994).
[2] Registered at the end of the lactation.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
P4-INS: progesterone vaginal inserts; P4-INJ: progesterone and estradiol via injections; BCS: body condition score and AI: artificial insemination.

Another important hormone that has been added in protocols to induce lactation is recombinant bovine somatotropin (bST) (Mellado *et al.* 2011; Macrina *et al.* 2011a; Macrina *et al.* 2011b), which was used in the two treated groups of present study. The bST regulates the use and absorption of nutrients, using them to increase milk yield, so that bST directly affects the receptors for endogenous bST located in hepatocytes and fat tissue. The activation of these receptors restricts systemic use of nutrients, promoting their incorporation to mammary gland (Manalu *et al.* 1991). Other effects of bST are supported by insulin-like growth factors (IGF-I and IGF-II), creating an increase in the synthesis of milk by increasing glucose assimilation (Rivera *et al.* 2010). When starting a treatment with bST, glucose production increases and decreases the oxidation process at the same, then hepatic glucose production increases and its assimilation is reduced (Bauman *et al.* 1988; Bitman *et al.* 1984). These metabolic adaptations of glucose are given just before voluntary intake feed increases, which is reduced from days before calving to the first two weeks postpartum (Bauman *et al.* 1988). Furthermore, changes in lipid metabolism vary according to the energy balance of the animal (Rivera *et al.* 2010). For example, when a cow is in early lactation, bST increases mobilization of body fat reserves, which results in high blood concentration of non-esterified fatty acids (NEFA), decreases body fat, and increases fat in milk (Bitman *et al.* 1984). In contrast, animals in middle and late lactation starting a bST treatment, exhibit a decreased in lipid synthesis and little or no changes on lipolysis and fat percentage in milk. At this stage, the use of nutrients from body reserves is redirected to other tissues for purposes of supporting the increase in milk synthesis, and simultaneously the voluntary feed intake (Bauman and Vernon, 1993). Based on the effects and mechanisms of action of the hormones used for the induction of lactation, some studies report some variation in milk yield, showing values between 60 to 70% over previous lactation (Smith and Schanbacher, 1973; Collier *et al.* 1975; Kensinger *et al.* 1979). In the present study, hormonally induced cows showed an average adjusted milk yield of approximately 7500 kg, which is very similar to that observed in control cows; in addition, total milk yield ranged from 8900 to 9000 kg in the three groups. These results show the same trend to those found by Mellado *et al.* (2011), who also indicates that production depends on the genetics of animals, herd management practices and environmental conditions. For example, bST treatment during and after induced lactation protocols in cows under these treatments may have better response because their liver tissue was fully functional and they were not in negative energy balance (Bauman *et al.* 1999). In the present study, animals under induced lactations protocols exhibited a gradual increase in milk yield and did not reach peak production until they had between 110 and 125 DIM, while those of natural lactation peaked around 60 DIM. Previous studies on induced lactation also have shown this slow increase in milk yield and longer time to reach peak milk yield (Fowler *et al.* 1991; Magliaro *et al.* 2004; Mellado *et al.* 2011).

The delayed peak during induced lactation is not fully understood. It could be that induced cows have less secre-

tory tissue at the beginning of lactation compared with non-induced cows, as mammary development occurs over 20-d period in the former and during the last month of gestation (Knight and Wilde, 1993). It is also possible that induced cows are less sensitive to cell proliferation (Finucane *et al.* 2008) and have a longer postpartum period of secretory tissue proliferation, which would be reflected in delayed peak milk yield. On the other hand, Lucy *et al.* (2009) noted that this delay to reach peak milk yield of induced cows is attributed a greater degree of uncoupling and slower recoupling of the somatotropic axis, which could delay mammary development, and in turn would prolong maximum parenchyma volume, which is necessary for peak milk yield. Milk fat and protein concentrations of animals under induction lactation protocols in the present study were within normal ranges, but were higher than cows that had natural calving; this data is consistent with the results found by Macrina *et al.* (2011a). Previous research has shown that cows and heifers induced into lactation typically have milk fat and protein percentages higher than those observed in postpartum animals (Erb *et al.* 1976; Davis *et al.* 1983; Magliaro *et al.* 2004). It appears that cows induced into lactation make the transition from the dry to lactation state easily, with little difficulty to maintain adequate feed intake. Therefore, the cows under induced lactation protocols did not undergo periods of negative energy balance, so that body condition loss are not as drastic as was found in the present study. In the present research, the induced cows conceived earlier than control cows; similar results were reported by Magliaro *et al.* (2004), which stated that the demands of a more advanced pregnancy would cause a more rapid decrease in milk production, therefore induced cows averaged fewer days in milk (DIM) than natural lactation cows. On the other hand, since a Presynch and Co-Synch TAI protocols were performed (Moreira *et al.* 2001; Chebel and Santos, 2010), all cows were inseminated at 71 DIM and a better response of cows that were induced into lactation respect to natural lactation animals was observed. However, reproduction parameters such as services per conception, conception to first service, and open days were similar in cows induced into lactation and controls. These results can be compared to those reported by Magliaro *et al.* (2004), who did not find any difference in reproductive parameters, which were even better in induced cows. Conception rate was obtained from 70% of cows under induced lactation and had a subsequent calving; however, because the induced animals had been placed under induction lactation due to bad reproductive history, 30% of them did not respond to reproductive management and were therefore culled at the end of the lactation. In the United States, a culling rate of 23.6% in dairy cows have been reported (NAHMS, 2007).

CONCLUSION

Cows hormonally induced into lactation using protocols with different sources of progesterone responded similarly compared to natural calving cows. Cows with lactation hormonally induced showed higher fat and protein in milk, and also similar milk yield than natural lactation cows. Cows with reproductive problems because of a severe heat stress as a result of hot summer months can be hormonally treated to induce a new lactation, which can be considered as an alternative for dairy farmers to increase profitability.

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