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Repeated low doses of dexamethasone as a pretreatment for induction of parturition in Simmental cows

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Abstract: The aim herein was to evaluate the effects of the induction of parturition using repeated low doses of dexamethasone in cattle. A total of 28 Simmental cows were used for the induction of parturition (n = 20) or served as controls (n = 8). Parturition was induced by a 1.6-mg intramuscular (IM) injection of dexamethasone twice daily for 6 days, beginning on day 270, and a 40-mg IM injection of dexamethasone on day 276. The calf birth weight, incidences of dystocia, daytime parturitions, and placental retention were compared. The calf birth weights in the treatment and the control groups were 34.95 ± 1.01 and 38.25 ± 1.36 kg, respectively (P = 0.081). Calving difficulty was experienced by 2 cows (10%) in the treatment group, while calving assistance was needed for 50% of the control cows. Most calvings (90%) in the treatment group were observed during the daytime while only 1 daytime calving was observed in the control group (12.5%). The placentas were retained in 20% and 12.5% of the treatment and the control cows, respectively. In the treatment group, 11 cows calved before the completion of the induction protocol and 9 cows calved after the last dexamethasone injection (9.29 ± 2.07 h). The late calving cows had higher (P < 0.05) estrone sulfate concentrations than the early calving cows. Serum cortisol concentrations were higher in the early calving cows when compared to the late calving cows during the induction protocol (P < 0.001). In conclusion, the induction of parturition using repeated low doses of dexamethasone improved calving management.

Key words: Cow, dexamethasone, induction of parturition, pretreatment, Simmental

1. Introduction

Dystocia is a common problem that causes high economic loss on cattle farms [1,2]. Cows experiencing dystocia are at a higher risk for reproductive problems, calf loss, high cow morbidity, and mortality/culling, as well as low subsequent fertility [3]. Many risk factors for dystocia have been defined [4–6]; however, timely intervention can help to minimize the negative effects [6] and improve the overall herd health and profitability [3].

Positively skewed distributions of dystocia rates have been reported at the herd level with some herds with a high prevalence [5]. The induction of parturition may be used to better observe parturition and prevent complications that may occur in unattended parturition in herds with a high dystocia prevalence. Avoiding nighttime parturitions helps to better manage calving and provides timely colostrum to the calves. Another common indication of parturition induction is the prevention of dystocia due to fetal oversize associated with or without prolonged gestation [7,8].

High doses of glucocorticoids, with or without prostaglandins, have been conventionally used for induction of parturition in cattle [9]; however, complications, including a lack of placental and fetal maturation, decreased calf viability, high incidence of placental retention (PR) and subfertility, and low milk production, have been reported [10]. In human medicine, antenatal corticosteroid treatment has been routinely applied to ensure lung maturation in infants, especially with the risk of preterm delivery [11]. Similarly, Zerbe et al. [12] proposed a regime consisting of repeated low doses of corticosteroid therapy to mimic the physiological process for premature calving in cows. Later, a similar approach was adapted by Hartmann et al. [13] for the induction of parturition in cows. The authors proposed the protracted induction of parturition, including repeated low doses of dexamethasone for several days followed by a final high dose as an alternative protocol.

The hypothesis of this study was that using repeated low doses of dexamethasone as a pretreatment would contribute to the induction of parturition in cows with acceptable PR rates and calf birth weights. For this hypothesis, we aimed to compare the calving difficulty

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scores, calf birth weights, gestation lengths, and PR rates in a Simmental herd experiencing a high incidence of dystocia, and to better characterize the effect of the induction of parturition on the maternal concentrations of estrone sulfate and cortisol.

2. Materials and methods

2.1. Animals, housing, and management

This study was conducted at a commercial farm that housed 750 lactating Simmental cows, located in the vicinity of Ankara, Central Anatolia, Turkey. The cows were milked twice daily using an automatic milking system and were fed a total mixed ration (commercial compound, straw, alfalfa, common vetch, rolled barley, corn silage, and vitamin-mineral supplement) according to their milk yield, with ad libitum access to water. Pregnant cows were moved from the free-stall barn into individual calving pens with straw bedding to be monitored closely 3 weeks before the expected calving date (280 days). The average gestation length was about 285–290 days before the experimental period. Pregnancies were diagnosed via a B-mode ultrasound scanner (7.5-MHz linear probe, Hasvet 838, Hasvet, Turkey) at 30–40 days after insemination (AI) as a 1st check and the 2nd check was done at 60–70 days AI via rectal palpation.

2.2. Experimental design

Simmental cows (n = 28) with known artificial insemination dates were randomly divided into treatment (n = 20) and control (n = 8) groups. Parturition was induced by twice daily intramuscular (IM) injections of 1.6 mg of dexamethasone (Deksavet 0.4%, Richter Pharma, Wels, Austria) for 6 days, beginning on day 270, followed by a single 40-mg dexamethasone IM injection on the morning of day 276 of gestation. The control group received a saline injection as a placebo and calved spontaneously. Daily peripheral blood samples from the coccygeal vein were collected into potassium-EDTA tubes (BD Vacutainer) before the dexamethasone injections in the morning. All of the injections were carried out by the same staff. The experimental design was approved by the Local Ethics Committee on Animal Experiments, Ankara University, Ankara, Turkey (Approval No: 2013-14-107) and is shown in Figure 1.

2.3. Calving management and data collection

All of the cows were closely observed for signs of parturition every 6 h. When calving began, all of the cows were observed without any intervention for 2 h. At the end of 2 h, cows that had not delivered were checked transvaginally by a trained veterinarian to detect whether assistance was needed or not. If needed, assistance was immediately given and the calving difficulty was scored in accordance with the 5-point scale described by Heins et al. [14]. Scores of 1–3 were regarded as no calving difficulty, whereas scores of 4 and 5 represented calving difficulty. Newborn calves were separated from their dam, dried using towels, and weighed (kg) using an electronic scale. The calving difficulty scores, calf birth weights, incidence of daytime parturitions (parturitions between 0600 and 2000 hours), and incidence of PRs (defined as the retention of the fetal membrane for >12 h) were recorded by the technical staff. The data of the gestation lengths were collected using the herd management software.

2.4. Determination of steroid hormone levels

The blood samples were immediately centrifuged at 3000 × *g* at 4 °C using the Hettich Rotanta 460R benchtop for 10 min. The sera were separated and then stored at –20 °C in aliquots of 1.0 mL until analysis.

Estrone levels were determined using the estrone enzyme-linked immunosorbent assay (ELISA) kit (DRG International Inc., Springfield Township, NJ, USA), according to the manufacturer's instructions. Briefly, 50 µL of the standard control or plasma samples was added to a 96-well plate. Next, the wells were treated with 100 µL of enzyme conjugate solution and incubated at room temperature for 60 min. Following incubation, the wells were washed with 400 µL of wash solution 4 times, and 150 µL of substrate solution was added to the wells. The plate was then incubated at room temperature for 30 min. The enzymatic reaction was stopped by adding 50 µL of stop solution to all of the wells and the absorbance was measured using a microplate spectrophotometer (SpectraMax, Molecular Devices, San Jose, CA, USA). The estrone levels were calculated using the linear curve analysis of the SOFTmax Pro 4.0 software. The dynamic assay range for the kit was 0–2000 pg/mL and the analytical sensitivity was <6.3 pg/mL.

Measurements of cortisol levels of the serum samples were performed using the cortisol ELISA kit (Cusabio Biotech Co. Ltd., Houston, TX, USA). Briefly, all of the reagents, standard solutions, and samples were prepared according to the manual. Next, 50 µL of the standard and sample and 50 µL of the antibody (1×) were added to the wells and incubated for 40 min at 37 °C. The wells were then aspirated and washed by filling each well with a wash buffer (200 µL) 3 times. Following that, 100 µL of horseradish peroxidase-conjugate (1×) was added to each well, except the control, and incubated at 37 °C for 30 min. The aspiration/wash process was repeated 5 times and 90 µL of 3,3',5,5'-tetramethylbenzidine substrate was added to the wells and incubated for 20 min while protecting them from the light. The reaction was stopped by the addition of 50 µL of stop buffer and the absorbance values were detected at 450 nm using a spectrophotometer (SpectraMax, Molecular Devices). The cortisol levels were calculated via linear curve analysis using SOFTmax Pro

4.0 software. The analytical sensitivity was less than 0.049 ng/mL. The calculated overall intraassay and interassay coefficients of variation were <8% and <10%, respectively. The cortisol levels were expressed as ng/mL.

2.5. Statistical analysis

All of the statistical analyses were performed using SPSS 14.01 for Windows (SPSS Inc., Chicago, IL, USA). The obtained results were presented as the arithmetic mean ± standard error. The data were analyzed using the Kolmogorov–Smirnov and Levene tests to identify conformity with normal distribution and the homogeneity of the variances, respectively. The differences in the gestation lengths, calf birth weights, and estrone sulfate concentrations were calculated using Student's t-test. $P < 0.05$ and $0.05 < P < 0.10$ were considered based on significance and tendency.

3. Results

The gestation length in the treatment group was 275.60 ± 0.35 days. Spontaneous calvings were observed on days 287.38 ± 1.31 ($P < 0.05$). Calf birth weights tended to be lower after induced parturitions when compared to the control group (34.95 ± 1.01 vs. 38.25 ± 1.36 kg; $P = 0.081$).

In the treatment group, 9 cows calved at 9.29 ± 2.07 h after the IM 40-mg dexamethasone injection on day 276. Before completion of the protocol, 11 cows calved (2 cows on day 272, 1 cow on day 273, 3 cows on day 274, and 5 cows on day 275). The calf birth weights were not different between the early and late calving cows in the treatment group (34.18 ± 1.41 kg vs. 35.89 ± 1.47 kg; $P > 0.05$). Most of the calvings (18/20; 90%) in the treatment group were observed during the daytime. In contrast, only 1 daytime calving was observed in the control group (1/8; 12.5%). Of the 20 cows, two (10%) experienced calving difficulty in the treatment group, while calving assistance was needed in 4/8 (50%) of the control cows. PR was observed in 12.5% (1/8) and 20% (4/20) of the control and treatment groups, respectively. In the treatment group, 3 cases of PR were observed in cows that calved after receiving the 40-mg IM injection of dexamethasone. The descriptive results are summarized in the Table.

In order to better characterize the results, the effect of the induction and the plasma concentrations of estrone sulfate in cows that calved before and after the 40-mg IM injection of dexamethasone in the treatment group were compared. Serum estrone sulfate concentrations in the treatment group increased during the induction protocol ($P < 0.001$; Figure 2). Preinduction serum estrone sulfate concentrations were numerically higher in the early calving cows when compared to the late calving cows (150.51 ± 7.24 vs. 147.99 ± 9.52 pg/mL; $P > 0.05$). In contrast, the late calving cows had higher ($P < 0.05$) estrone sulfate concentrations (179.02 ± 3.19 pg/mL) than the early calving cows (163.92 ± 6.27 pg/mL) in the treatment group (Figure 3).

Daily changes in the plasma cortisol concentrations in the treatment group were not different during the induction protocol ($P > 0.05$; Figure 4). Serum cortisol concentrations were higher in cows that calved before the 40-mg IM injection of dexamethasone compared to the cows that calved later during the induction protocol ($P < 0.001$; Figure 5).

4. Discussion

The induction of parturition has been used to manage fetal and maternal disorders of pregnancy/parturition and

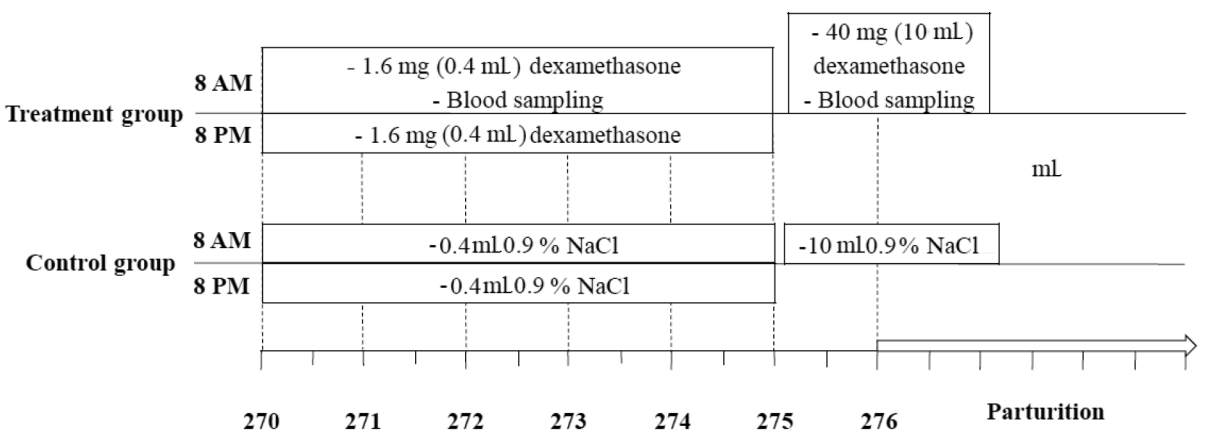

Figure 1. Schematic diagram of the experimental design.

Table. Comparison of the gestation length, calf birth weight, and frequencies of calving difficulties, daytime parturitions, and PR in the groups.

	Treatment group	Control group	P-value
Gestation length (days)	275.60 ± 0.35	287.38 ± 1.31	<0.05
Calf birth weight (kg)	34.95 ± 1.01	38.25 ± 1.36	0.081
Calving difficulty (%)	2/20 (10%)	4/8 (50%)	NA
Daytime parturition	18/20 (90%)	1/8 (12.5%)	NA
PR	4/20 (20%)	1/8 (12.5%)	NA

* NA: Not analyzed, PR: placental retention.

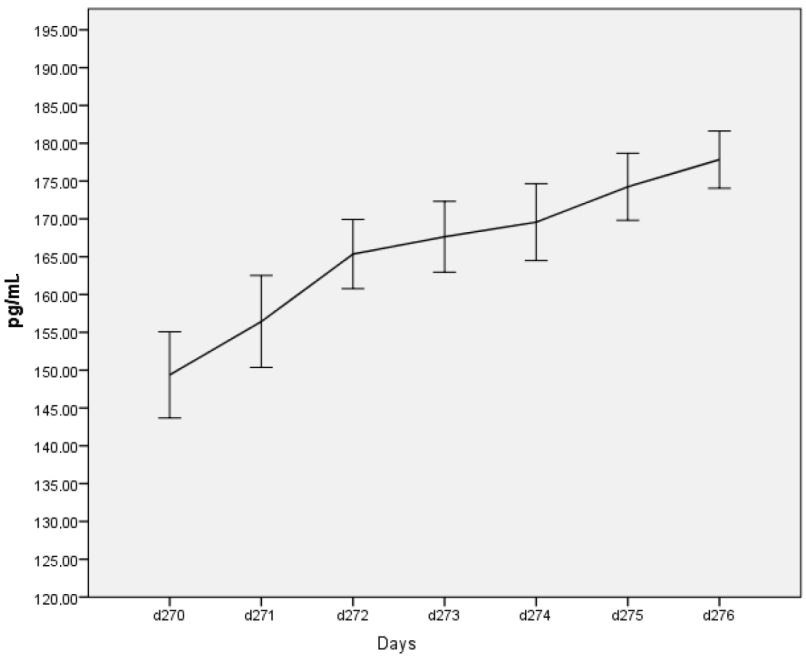

Figure 2. Serum estrone sulfate concentrations during parturition induction.

conditions that negatively affect dam and calf viability [8]. In addition, hormonal induction is a management method used to prevent dystocia due to fetuses of abnormal size or weight and prolonged pregnancy, especially in heifers [7,15]. The parturition induction protocol used in the present study successfully induced parturition in all of the cows studied and significantly shortened the gestation length. The average gestation length of 287.38 ± 1.31 days in the spontaneously calving cows was longer than that reported in previous studies. The mean gestation length for 71,461 American Simmental cattle was 284.3 ± 5.52 days. In a retrospective study that included 1775 lactation records of Simmental × South Anatolian Red F_1 × B_1 crossbred cows, the gestation length was 278.89 ± 9.63 days [16].

In the treatment group, 11 cows calved prior to induction with the final high dexamethasone dose on day 276. In a previous study, with 1.3 mg of dexamethasone given twice daily between days 268 and 273 of gestation and 40 mg of dexamethasone on day 274 of gestation, early calvings were observed in 3 out of 13 cows. The later starting time (day 270) and higher dose (1.6 mg) of dexamethasone selected in the present study were thought to be responsible for the higher incidence of early calvings.

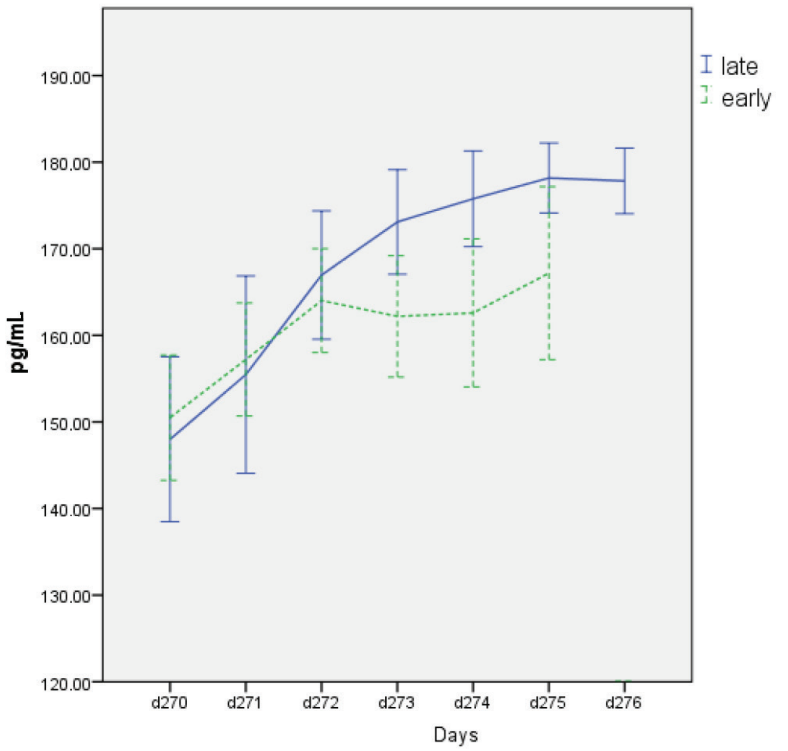

Figure 3. Serum estrone sulfate concentrations in the early and late calving cows.

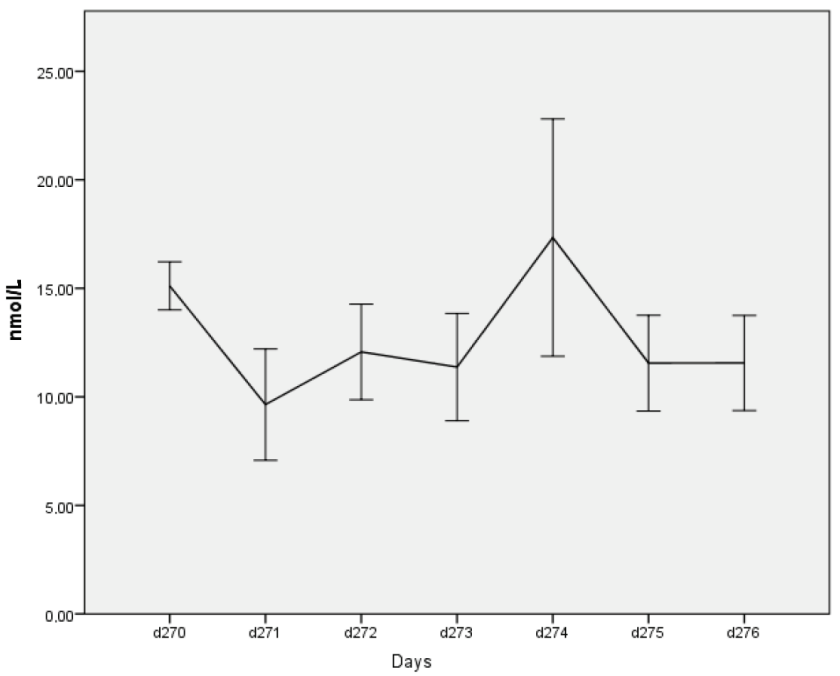

Figure 4. Serum cortisol concentrations during parturition induction.

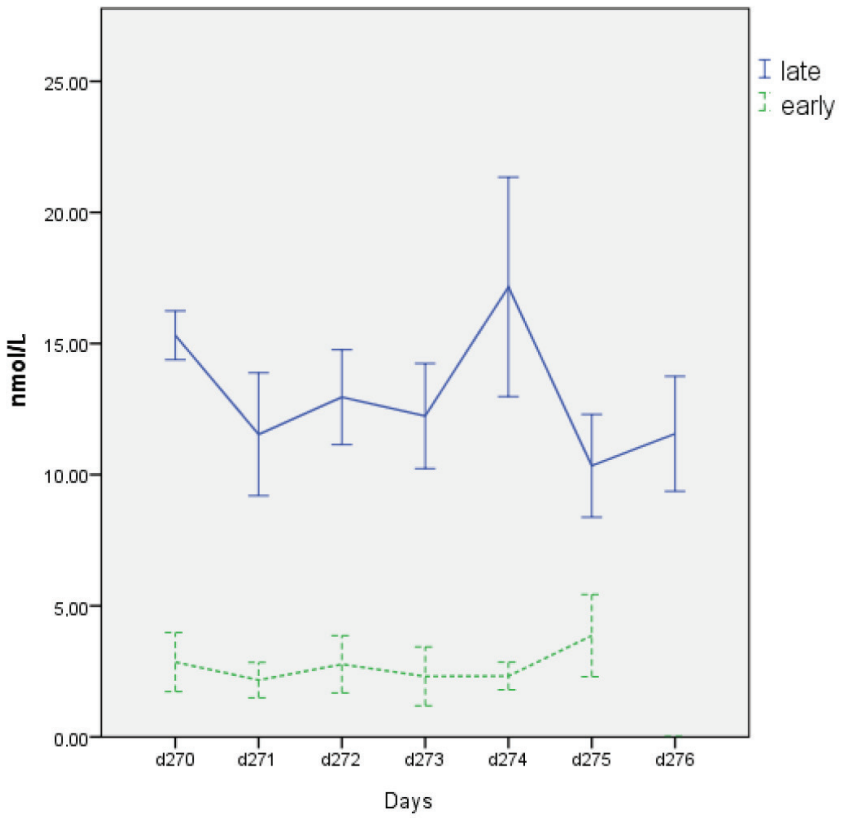

Figure 5. Serum cortisol concentrations in the early and late calving cows.

One of the interesting results of the present study was the numerical differences in the pretreatment levels of the estrone sulfate between the early and late calving cows. Increasing levels of estrone sulfate, the major estrogen in maternal circulation, are observed during late pregnancy and are significantly increased around parturition [17,18]. This increase of estrone sulfate has been associated with fetal well-being. Some researchers have reported that there is a positive association between estrone sulfate concentrations and the placental function/maturation and calf weights in beef and dairy cows [19–21]. Estrone sulfate concentrations increased gradually until day 272 in the early calving cows, in contrast to a continuous increase until day 276 in the late calving cows. Maternal estrogen concentrations at the time of the dexamethasone injection were negatively correlated with PR rates and treatment failure. Consequently, preterm estrogen levels near term were suggested to indicate placental maturity and the temporal proximity to parturition [9].

In the present study, maternal cortisol levels were not affected by parturition induction. Cortisol levels increased prior to spontaneous parturition and high levels were found at or after parturition [22]. Fetal cortisol, not maternal cortisol, plays a significant role in the initiation of parturition in ruminants. Fetal cortisol exceeds that of the mother and increases rapidly peripartum to peak at parturition [23]. A lack of maternal cortisol increase may be attributed to the timing and frequency of the samplings in the present study. Furthermore, stable cortisol levels were reported after dexamethasone-induced [24] and prostaglandin F2α-induced [25] parturitions. Interestingly, low maternal cortisol levels were observed in the early calving cows. High maternal glucocorticoids were related to the severity of physical stress and pain experienced during parturition and cattle requiring assistance at parturition had higher plasma cortisol concentrations than cattle with unassisted calvings [26].

Although they tended to be lower after the induced parturitions, the calf birth weights observed in the present study were similar to previously reported calf birth weights after spontaneous parturitions in the Simmental breed. In a Polish study [27], the birth weights of heifer and bull calves were 34.4 ± 2.9 and 35.4 ± 2.6 kg, respectively. In an American study, the mean birth weight of Simmental

calves was 37.98 ± 0.63 kg [28]. Each kilogram increment in calf birth weight resulted in a linear increase by 1.63% to 2.30% in the dystocia rate [29]. The induction of parturition is an effective method to reduce the birth weight of calves and dystocia rates. The relatively low incidence of calving difficulty after induced parturitions in the present study proved that parturition induction was helpful in herds with high dystocia rates. Nevertheless, accurate timing and records of insemination/service dates are required; otherwise, calves have poor survival rates [10]. The induction method utilized in this study reduced the gestation length and calf birth weight when compared to the control group; however, the body weights of the calves born early and late in the treatment group were similar. Similar calf viability (no calf losses; data not shown) in the treatment group was also observed. One of the limitations of the present study was that it was not possible to evaluate the daily gain of calf body weight until the weaning period. It was suggested, however, that the weaning age [30] and weaning weight [31] of calves born after parturition induction were comparable to those of calves born of normal parturition. Another limitation of the present study was the lack of individual milk yield records. In some reports, the induction of parturition resulted in the depression of milk production in the subsequent lactation [32,33].

Supervision of calving has significant importance for calf viability and postpartum disorders related to dystocia. The majority of calvings occur at night [7]. Less labor and observation would be available during the night when compared to the daytime, resulting in less supervision of the calvings and a higher risk of dystocia and related disorders [34,35]. Many researchers have aimed to minimize the rate of nighttime calvings by changing the feeding strategy [34,36,37] or medical treatment [7,9]. The interval from injection to parturition has not been predictable for a single high dose of short-acting corticosteroids and it usually takes 24–72 h, with an average of 48 h. Although corticosteroid and prostaglandin combinations have increased the predictability of calvings to some extent [32], they are also unreliable in terms of daytime calvings. The relatively short and more predictable intervals from induction to calving, and consequently the 90% daytime calvings observed in the present study, were in accordance with previous studies concluding that corticosteroid pretreatment before the induction of parturition was highly effective in avoiding nighttime calvings [7,9,38].

It was reported that the range of PR varied from 4% to 18% in cows [39]; however, this ratio could reach as high as 50%–60% [9] or more [40] when parturition is induced via hormonal treatment. Although much effort has been focused on decreasing the rate of this complication [41–43], better results were obtained by previous studies, including treatment with long-acting corticosteroids [41,44,45]. Nevertheless, there are legal restrictions for the use of long-acting corticosteroids in food-producing animals in Europe [13,38] and induction using long-acting corticosteroids has wide variability in response to treatment and lower predictability in terms of calving time [8,41]. Hartmann et al. evaluated the use of low dosages of short-acting corticosteroids for the induction of parturition and showed that this alternative approach had a positive effect on the progress of placental maturation, but it did not have a positive effect on placental separation [13]. The authors obtained a numerically lower rate of PR in cows subjected to protracted induction (54%) when compared to conventionally induced cows (70%). Normally, it is expected that this approach would result in a lower rate of PR, because Hartmann et al. determined that this protocol had a positive influence on placental maturation in vitro. In the current study, a lower and acceptable rate of PR was detected in the treatment group (20%) when compared to the control group (12.5%). It is well known that many factors are related to PR; however, the mechanisms underlying PR and the induction of parturition by corticosteroids are not well understood [46]. The differences between the current findings and the results of Hartmann et al. might be due to the timing of induction and gestation lengths [13]. Some researchers have reported that gestation length was directly associated with the risk of developing PR [39,47,48]. Cows induced with long-acting corticosteroids had a lower rate of PR (9%–22%) and higher calf mortality (7%–45%) [8]. That high mortality of calves and legislation restricts the use of long-acting corticosteroids for the induction of parturition. Lower rates of PR obtained from the present study suggest that this low dose of corticosteroid protocol may be a good alternative for the induction of parturition using long-acting corticosteroids.

In conclusion, the use of repeated low doses of dexamethasone starting on day 270 of gestation as a pretreatment for dexamethasone-induced parturition on day 276 decreased the incidence of dystocia and increased daytime calvings. The protocol resulted in an acceptable incidence of PR and no increase in calf losses. The protocol caused early calvings in some cows and the maternal estrone sulfate and cortisol concentrations differed between the early and late calving cows. The parturition induction protocol used in the present study has the potential to improve calving management in Simmental cows.

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