Effects of dexamethasone and insulin alone or in combination on reproduction in dairy cows in early lactation

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SUMMARY

To evaluate the effect of administration of insulin and/or dexamethasone during early lactation on reproductive performance of dairy cows in a randomized controlled clinical trial, two hundred Holstein cows in a commercial dairy farm were enrolled in this study. The cows were randomly assigned to receive 1 of 4 treatments at day 3 or 10 of lactation (half of the cows received treatments at day 3 postpartum and the second half at day 10 postpartum): 1. i.m. injection of 20 mg of dexamethasone and 100 units of insulin , 2. s.c. injection of 100 units of insulin, 3. i.m. injection of 20 mg of dexamethasone , and 4. 10-mL i.m. injection of sterile water (control). To assess reproductive performance, the following outcomes were measured: interval from calving to first insemination, calving to conception interval, first service pregnancy risk (%), pregnancy rate at 120 days in milk , and cumulative pregnancy risk (%) till 9 month. There was no statistically significant effect of time of interaction (day 3 or 10 of lactation) on the outcomes. Survival analysis was showed no difference among groups in median days to first service. Dexamethasone treated cows had significantly lower days to pregnancy than controls. In addition, dexamethasone treated cows had a significantly greater pregnancy rate at 120 days in milk than other treatment groups and controls. The odds of the development of pregnancy at 120 days were 2 times greater in cows that received dexamethasone in comparison to controls (P=0.02). This study suggests that a single dose of dexamethasone injection in early lactation, might improve reproductive performance.

Keywords: Cattle, Glucocorticoid, Dexamethasone, Insulin, Fertility

RÉSUMÉ

Effets de la dexaméthasone et de l'insuline seule ou en association sur la reproduction chez les vaches laitières au début de la lactation

L'objectif de cette étude est d'évaluer l'effet de l'administration d'insuline et / ou de dexaméthasone en début de lactation sur la performance reproductive des vaches laitières dans un essai clinique contrôlé randomisé. Deux cents vaches Holstein d'une ferme laitière commerciale étaient inscrites dans cette étude. Les vaches ont été réparties au hasard à parts égales en 4 groupes recevant les traitements suivants au jour 3 ou 10 de la lactation : 1. Injection de 20 mg de dexaméthasone et de 100 unités d'insuline, 2. Injection de 100 unités d'insuline, 3. Injection de 20 mg de dexaméthasone et 4. Injectionde 10 ml d'eau stérile (contrôle). Les résultats suivants ont été mesurés afin d'évaluer les performances reproductives: intervalle entre le vêlage et la première insémination, intervalle vêlage insémination fécondante, taux de gestation en première lactation (%), taux de gestation à 120 jours de lactation, et taux de gestation cumulé (%) à 9 mois. Les résultats n'ont pas montré d'effet statistique sur le jour de l'injection (jour 3 ou 10 de la lactation). Aucune différence entre groupes n'a été observée sur le taux de survie. Les vaches traitées à la dexaméthasone ont montré un nombre de jour de gestation significativement inférieur aux témoins et un taux de gestation significativement plus élevé à 120 jours que les autres. Les chances de développement de la gestation à 120 jours étaient 2 fois plus élevés dans les vaches qui ont reçu dexaméthasone par comparaison aux témoins (P = 0,02). Cette étude suggère qu'une dose unique de dexaméthasone en début d'allaitement, pourrait améliorer les performances de reproduction.

Mots-clés Fr: Bovins, Glucocorticoïde, Dexaméthasone, Insuline, Fertilité

Introduction

High yielding dairy cows are typically in a state of negative energy balance (NEB) postpartum, because energy required for milk production and maintenance of body tissue functions exceeds energy ingested. Hence, metabolic and endocrine changes in early lactation allow enhanced mobilization of depot fat and skeletal muscle breakdown and favor partitioning of absorbed nutrients to the mammary gland to provide sufficient substrates for milk synthesis [10]. Although virtually all cows go through some degree of NEB post-calving [12], it is the degree and duration of NEB that most likely contributes to disease. Subclinical ketosis is the most common consequence of NEB in early lactation [6].

Glucocorticoids such as dexamethasone frequently are used in treatment protocols for ketosis. Glucocorticoids decrease blood ketone concentrations in cows with clinical ketosis [26, 30] and in healthy fresh cows [23]. On the other hand, it has been shown that elevated concentrations of beta hydroxybutyric acid (BHBA) is associated with poor fertility [4, 28].

In addition, insulin decreases fat breakdown, increases fat synthesis, and increases use of ketone bodies as energy sources, which should decrease the level and consequences of ketonemia [9].

However, there are few publications on the effects of glucocorticoids or insulin on reproduction performance of

dairy cows. The objective of this study was to evaluate the effects of a single dose of dexamethasone or insulin or a combination of dexamethason + insulin in early lactation on reproductive performance of healthy dairy cows. We hypothesized that, dexamethasone and/or insulin might be improved the reproductive performance of dairy cows. To the best of our knowledge, no studies has achieved to this hypothesis till now.

Material and Methods

ANIMALS

The full details of the study population and examination protocol have been reported previously [23]. Briefly, a total of 200 cows from a commercial dairy farm in Tehran province, Iran were enrolled in a double-blinded randomized clinical trial. A 2×4 randomized factorial design of treatments was used. Cows were blocked by parity and expected calving date. Cows were enrolled approximately 14 days before expected calving date. The cows were randomly assigned to receive the treatments at 2 different times of intervention. Half of the cows received treatments at day 3 postpartum (early treatment) and the 2nd half at day 10 postpartum (late treatment). Animals at each time of intervention, were randomly assigned to receive 1 of 4 treatments: 1) group ID, 20 mg of dexamethasone i.m. in the left semitendinosus muscle plus a 100 unit s.c. injection of isophane insulin (NPH) in the caudal left forelimb at the level of the mid-thorax; 2) group Ins, 10 mL i.m. injection of sterile water in the left semitendinosus muscle plus a 100 unit s.c. injection of isophane insulin, NPH (1 mL contains 100 IU) in the caudal left forelimb at the level of the mid-thorax; 3) group Dex, 20 mg of dexamethasone (2mg/1mL) i.m. in the left semitendinosus muscle plus a 1 mL s.c. injection of sterile water in the caudal left forelimb at the level of the mid-thorax; and 4) group control (Con), 10 mL i.m. injection of sterile water in the left semitendinosus muscle plus a 1 mL s.c. injection of sterile water in the caudal left forelimb at the level of the mid-thorax.

REPRODUCTIVE PROTOCOLS AND COVARIATES

After termination of the treatment protocol, all cows followed the normal herd reproductive management practices in the breeding period. Insemination and culling dates and pregnancy data were collected using on- farm data recordings. Reproductive performance data on all animals were collected until 9 month after the last cow was enrolled. Observations of time to pregnancy for cows that were culled during the trial before pregnancy were censored on the date of culling. For cows that were not pregnant at the termination of data collection, observations were censored on that date.

The following outcomes were measured to assess reproductive performance: interval from calving to first insemination (days to first service), calving to conception interval (days open), first service pregnancy risk (%), pregnancy rate at 120 days in milk (DIM), and cumulative pregnancy risk (%) by 9 month.

Reproductive management consisted of a voluntary waiting period of 50 days until first insemination. Thereafter, cows were inseminated following estrus detection. Pregnancy was diagnosed by transrectal sonography of the uterus at 28-35 day after artificial insemination (AI) and was confirmed at 57-63 day after AI. Cumulative pregnancy risk was the proportion of cows enrolled in the trial that eventually became pregnant till 120 DIM or 9 month after calving (Dairy cows that are pregnant after 120 days of lactation, will be more uneconomical).

Cows were scored for body condition on a scale of 1 to 5, in increments of 0.25 [7], at enrollment, the time of treatments and d 14 after intervention.

Definitions of diseases were as follows: metritis was an enlarged non-pregnant uterus and a watery red-brown fluid to viscous off white purulent cervical or vaginal discharge; retained fetal membranes (RFM) was the retention of fetal membranes for more than 24 hour after calving; clinical mastitis was a case of visually abnormal milk associated with hardness and swelling of the affected quarter; lameness was a clinical case of lameness of feet or legs requiring treatment [17]; milk fever was diagnosed as any cow that within 72 hour after parturition presented inappetence, nervous symptoms, staggering, varying degrees of unconsciousness, probable sternal recumbency and good response to intravenous calcium treatment; displacement of the abomasum was defined as decreased milk yield accompanied by an audible high-pitched tympanic resonance by simultaneous percussion and auscultation of the left or right abdominal wall between the 9th and 12th rib spaces as diagnosed by the veterinarian [19]. Subclinical ketosis (SCK) was considered as a concentration of BHBA equal or more than 1.2 mmol/L. Incidence was defined as the number of specified new cases during the 30 days of lactation divided by the total number of cows in that group. Disease diagnoses and pregnancy checks and other disease diagnoses were recorded by the veterinarian of the farm. These diseases were entered on a daily basis into a computerized database. For all diseases other than mastitis, a second occurrence of the disease had to be separated from a prior occurrence by more than 21 days to be counted as a new case.

STATISTICAL ANALYSIS

All analyses were performed using SAS software (Version 9.2; SAS Institute Inc., Cary, NC, USA). The association of treatment with parity and BCS and disease outcomes were tested with Chi-squared tests (PROC FREQ).

Reproductive performance information was collected for a minimum of 9 months after enrollment. Observations of time to pregnancy were censored for open cows on the date of

culling or at the end of study (9 months from last cow enrolled in the study). Kaplan-Meier (product limit) survival function estimates (the LIFETEST procedure in SAS) were used to calculate crude associations of treatment with median time to first breeding and pregnancy, and to generate graphs of cumulative pregnancy risk over time. The effects of treatment on time to first breeding and pregnancy were analyzed with multi- variable survival analysis using Cox's proportional hazards regression (the PHREG procedure in SAS). Both of these survival analysis procedures are nonparametric, so they do not depend on any specification of the underlying distribution of the data.

The treatment effect on the risk of proportion of cows inseminated and pregnant at 1st service, by 120 DIM, and cumulative pregnancy rate was evaluated with multivariable logistic regression models with PROC LOGISTIC of SAS. Treatment, time of intervention (day 3 or 10 of lactation), parity group (primiparous and multiparous), body condition score (BCS) class (≤3, 'thin'; 3.25–3.5, 'fair'; ≥3.75, 'fat'), occurrences of RFM, metritis, dystocia, mastitis and abomasal displacement were offered to the model for each outcome. The occurrence of SCK was considered as covariate for the risk of pregnancy by 120 DIM. Then variables were removed by manual backward stepwise elimination if the P> 0.2. Finally, interactions among variables were assessed using multivariable logistic regression (PROC LOSISTIC of SAS) modeling through a backward model-selection procedure. To determine the degree of association between the risk factors and outcome variables, odds ratio (OR) and 95% confidence intervals were calculated. For all statistical analyses differences with P<0.05 were considered significant.

Results

Characteristics of cows, variables of reproduction, and disease occurrence in treatment groups are summarized in Table I. Seven cows died or were sold after enrollment. In total, 193 cows are included in the experiment. There was no association between treatment assignment and parity group ($\chi 2$, P=0.98) or BCS category (P=0.26).

Twenty-seven cows had RFM and 24 cows had metritis. In addition, 14 cows had dystocia during parturition. All the models controlled for the effects of dystocia, RFM and metritis, but there were no significant interactions between treatment and these 3 diseases. There was no statistically significant effect of time of interaction (day 3 or 10 of lactation) on the outcomes.

Survival analysis was used to measure the time from calving to first insemination and from calving to pregnancy (Table II). There was no difference among groups in median days to first service. Dexamethasone treated cows had significantly lower days to pregnancy than controls (HR for Dex = 1.59, P = 0.04) (Table II). Fig. 1 is a survival curve demonstrating the effect of treatment on days to pregnancy.

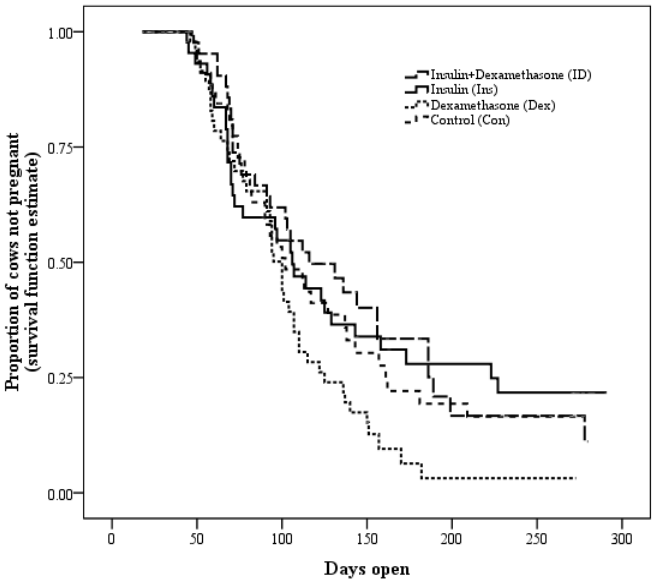

Figure 1: Kaplan-Meier survival curves of days to pregnancy up to 300 DIM in Holstein cows treated on 3rd or 10th day after parturition with a single injection of insulin plus dexamethasone (n=48; 35.4% censored), insulin only (n=47; 34.0% censored), dexamethasone only (n=49; 12.2% censored) or placebo [control] (n=48; 29.2% censored).

The raw data of relative 1st service pregnancy rate, pregnancy at 120 DIM and cumulative pregnancy rate after 9 month for Ins, Dex, ID and Con groups were shown in Table I. The relative pregnancy rates of cows that received dexamethasone treatment were better than insulin, insulin + dexamethasone received and control cows regarding all three indices. However, there was no statistically significant difference on 1st service pregnancy rate and accumulative pregnancy rate after 9 month among treatment groups. Accounting to the effects of parity, BCS, time of intervention, occurrence of dystocia, RFM, metritis and abomasal displacement, dexamethasone treated cows had a significantly greater pregnancy rate at 120 days in milk than other treatment groups and controls. A summary of the logistic regression model describing the associations of treatments and covariates with pregnancy rate at 120 DIM is reported in Table III. At 120 DIM, pregnancy rate for Ins, Dex, ID and Con groups were 51%, 67%, 44% and 51%, respectively. The odds of the development of pregnancy at 120 days were 2 times greater in cows of Dex group in comparison to controls (P=0.02). The effects of treatments on metabolic parameters and milk production were published elsewhere [23].

Discussion

The purpose of the present study was to evaluate the effect of insulin and/or dexamethasone at early lactation on reproductive performance of dairy cows. In the current study, cows that received a single dose of dexamethasone had a shorter time to pregnancy and better pregnancy rate through the lactation period. Cows in dexamethasone group showed a significant increase in the risk of pregnancy compared to controls at 120 DIM. Although, pregnancy rate at 1st service and cumulative pregnancy rate after 9 month were not

statistically affected by dexamethasone administration but increased numerically. It should be taken into account that with <50 cows per group, the power to detect fertility effects was limited in this study.

The literature on the effects of a single dose of dexamethasone at early lactation on reproductive performance in dairy cows is scarce. It was shown that administration of a single dose of isoflupredone alone or with insulin within the first 8 days of calving had no impact on reproductive performance [25]. To the best of our knowledge this is the first trial that reports an improving effect of a single injection of dexamethasone on pregnancy rate and reproductive function.

	Group ID (n = 48)	Group Ins (n = 47)	Group Dex (n = 49)	Group Con (n = 49)
Parity				
Primiparous	10	10	10	10
Multiparous	38	37	39	39
BCS				
Thin	19	21	25	26
Fair	22	22	17	19
Fat	6	4	7	4
Diseases				
Dystocia (%)	6.3	8.5	6.1	8.2
Retained Fetal Membrane (%)	12.5	12.8	14.3	16.3
Metritis (%)	10.4	12.8	12.2	14.3
Mastitis (%)	6.3	12.8	4.1	14.3
Abomasal Displacement (%)	2	0	2	0
Lameness (%)	2.1	2.1	0	2
Reproduction Indies				
Pregnancy at 1st AI (%)	25	25.5	32.6	20.4
Pregnancy at 120 days (%)	43.7	51.1	67.4	51
Accumulative Pregnancy (%)	64.6	66	87.8	71.4
Days to 1st service – mean (day)	71	66	66	70
calving to conception interval – mean (day)	111	97	96	99

Table I: Crude data of incidence of calving-related disorders, and reproductive responses in cows treated with insulin + dexamethasone (ID), insulin only (Ins), dexamethasone (Dex) and control (Con) groups.

			Time to pregnancy			
Treatment†	N	Median	Quartile 25%	Quartile 75%	Hazard Ratio	*P* value
ID	48	93	70	144	0.91	0.72
Ins	47	96	67	123	0.95	0.85
Dex	49	94	64	115	1.59	0.04
Con	49	90	69	127	Referent	
			Time to first insemination			
ID	48	62	52	76	0.88	0.56
Ins	47	61	53	71	1.05	0.84
Dex	49	60	55	72	1.01	0.95
Con	49	61	54	69	Referent	

* Cows were followed for 9 mo until pregnancy or culling and the data were analyzed using Kaplan-Meier and Cox proportional hazards survival analyses, accounting for the effects of parity, BCS at enrollment, retained placenta, and metritis.

†Treatment groups: ID, insulin plus dexamethasone; Ins, insulin; Dex, dexamethasone; Con, placebo received cows.

Table II: Summary of reproductive performance in dairy cows that received insulin, dexamethasone, insulin plus dexamethasone, or placebo once in the 3rd or 10th DIM*.

In the first publication of this experiment, it was indicated that BHBA concentrations were significantly lower for cows treated with dexamethasone with or without insulin 2 days after treatment [23]. In this respect, it was shown dexamethasone resulted in hypoketonemic effects lasting 4 to 6 days in ketotic cows [30]. Several studies revealed that there was an association between excessive NEB and decreased reproductive performance [28]. NEB and fat mobilization, which is characterized by periparturient increases in serum NEFA and BHBA concentrations, is associated with decreased reproductive performance [4]. Elevated BHBA concentrations result in impaired GnRH and/or LH secretion [2, 3] with consequent effects upon follicle growth and ovulation. On the other hand, diminished reproductive function may also be related to uterine disease [11, 22], and delayed luteal activity [29], both of which have been shown to be associated with elevated BHBA concentrations.

In addition, dexamethasone treated cows showed a better protein status and greater cholesterol concentrations than insulin and conrol groups [23]. Amino acids, along with fatty acids and glucose serve as signaling molecules to control cellular metabolic pathways and gonadotropin release [27].

There are studies on the effects of administration of dexamethasone or other glucocorticoids on reproductive outcomes in humans. It was shown that low dose (1 mg) dexamethasone injection in women patients significantly reduced the incidence of poor ovarian response to gonadotropins (2.8 versus 12.4%, $P < 0.002$) [15]. The ovarian response to gonadotropin stimulation is modulated by insulin-like growth factor 1 (IGF-1) which acts synergistically in vitro with FSH [1] through granulosa cell receptors [8]. Glucocorticoids also stimulate growth hormone (GH) [5] and IGF-1 secretion [20] and a higher IVF pregnancy rate was observed with prednisolone co-treatment compared with controls [16].

Dexamethasone may act indirectly by increasing serum growth hormone (GH) [5], serum insulin-like Growth Factor (IGF-1) [20] and consequently follicular fluid IGF-1 concentrations. Intra-ovarian regulation of IGF-1 and its binding proteins is highly complex [14]. Insulin-like Growth Factor Binding Protein 1 (IGFBP 1) gene transcription in human hepatocytes is stimulated by glucocorticoids mediated through glucocorticoid response elements and may enhance or inhibit IGF-1 action in vitro depending on culture conditions [24].

However, there is no similar report showing the same effects of dexamethasone or other glucocorticoids in dairy cows. It was shown that increases in glucocorticoid of plasma did not affect LH concentration of cows [13] and it was also reported that dexamethasone did not have any effect on LH of ewe [21]. Maciel et al. showed in their studies that dexamethasone did not affect FSH and LH concentrations. Total number of follicles (≥5 mm) and plasma estradiol concentrations were less in treatment than in control cows [18].

This study suggested that a single dose of dexametasone injection at early lactation might improve the reproductive performance. However, glucocorticoid effects on reproduction in cattle are not well defined. Future research should explore mechanisms by which either dexametasone concentrations or dexametasone signaling pathways in the reproductive system can be altered to enhance improvement of reproductive performance and potentially pregnancy rate. Further work will be needed to clarify these possibilities.

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Variable	% of pregnancy	Estimate	SE	OR	95% CP	*P* value
Intercept		-0.4077	0.34			0. 23
ID	44%	-0.4143	0.26	0.7	0.32-1.6	0.10
Ins	51%	-0.0875	0.26	1.0	0.44-2.2	0.73
Dex	67%	0.5851	0.26	1.95	0.85-4.5	0.02
Con	51%			Referent		
Dystocia	36%	-0.3904	0.29	0.46	0.14-1.45	0.18
Metritis	42%	-0.2821	0.23	0.57	0.23-1.4	0.21

*The data were analyzed using logistic regression analyses, accounting for the effects of parity, BCS at enrollment, dystocia, retained placenta, metritis and SCK.

†Treatment groups: ID, insulin plus dexamethasone treated groups; Ins, insulin; Dex, dexamethasone; Con, placebo received cows.

‡95% confidence interval around the odds ratio.

Table III: Final logistic regression model* of treatment groups† and clinical events with the subsequent pregnancy till 120 days in milk in 193 Holstein dairy cattle.

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