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Endocrinological and Metabolic Profile in Relation to Pregnancy at the First Insemination in Cows Housed Under Cold Conditions

Soğuk Çevre Koşullarında Barındırılan İneklerde İlk Tohumlamada Gebelikle İlgili Endokrinolojik ve Metabolik Profil

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ABSTRACT

This study was performed to investigate the relationship between endocrinological and metabolic profiles and the pregnancy rate at the first insemination in peripartum dairy cows housed under cold conditions. Temperature inside the barn was recorded hourly during the periparturient period. Blood samples were collected before (last 14 day), on the day (0 day), and after parturition (3, 4, 6, 8, 15, 22, and 29 days) from 26 peripartum Simmental cows and analyzed for anti-Müllerian hormone, β-hydroxybutyric acid, insulin-like growth factor 1, insulin, cortisol, malondialdehyde, progesterone, thyroid-stimulating hormone, tri-iodothyronine, and thyroxine concentrations. The cows were divided into 2 groups: pregnant and non-pregnant based on results at the first insemination. The average ambient temperature ranged from −7°C to +11°C in the tent barn. Serum anti-Müllerian hormone (2.00 ± 0.04 vs. 1.89 ± 0.04 mU/L; $P < .006$), insulin (2.10 ± 0.03 vs. 2.51 ± 0.05 ng/mL; $P < .0001$), malondialdehyde (49.0 ± 1.30 vs. 44.0 ± 1.2 ng/mL; $P < .001$), and progesterone (44.1 ± 2.2 vs. 41.7 ± 2.1 pg/mL; $P < .002$) concentrations were different between pregnant and non-pregnant cows. In conclusion, anti-Müllerian hormone and insulin have a determinative role on pregnancy rate in peripartum cows housed under cold condition.

Keywords: Cold stress, cow, fertility, metabolism, pregnancy

ÖZ

Bu çalışma, soğuk koşullarda barındırılan peripartum ineklerde ilk tohumlamada endokrinolojik ve metabolik profil ile gebelik oranları arasındaki ilişkiyi araştırmak amacıyla yapılmıştır. Ortam sıcaklığı, periparturient dönem boyunca saatlik olarak kaydedilmiştir. Anti-Müllerian hormon (AMH), β-hidroksibutirik asit (BHBA), insulin benzeri büyüme faktörü 1 (ILGF-1), insülin, kortizol, malondialdehit (MDA), progesteron (P4), tiroid uyarıcı hormon (TSH), tri-iyodotironin (T3) ve tiroksin (T4) konsantrasyonlarının analizi için 26 adet Simmental inekten doğum öncesi (-14 gün), doğum günü (0 gün) ve doğum sonrası (3, 4, 6, 8, 15, 22 ve 29 gün) farklı aralıklarla kan örnekleri alınmıştır. İnekler, ilk tohumlama sonuçlarına göre geriye dönük olarak gebe (PG) ve gebe olmayan (NPG) olmak üzere iki gruba ayrılmıştır. Çadır ahırda ortalama ortam sıcaklığı −7°C ile +4°C arasında değişmiştir. İneklerin (PG ve NPG) serum AMH (2,00 ± 0,04 vs. 1,89 ± 0,04 mU/L; $P < ,006$), insulin (2,10 ± 0,03 vs. 2,51 ± 0,05 ng/mL; $P < ,0001$), MDA (49,0 ± 1,30 vs. 44,0 ± 1,2 ng/mL; $P < ,001$) ve P4 (44,1 ± 2,2 vs. 41,7 ± 2,1 pg/mL; $P < ,002$) konsantrasyonları farklıydı. Sonuç olarak, AMH ve insulin soğuk şartlarda barındırılan ineklerde gebelik oranları üzerine belirleyici bir rol oynar.

Anahtar kelimeler: Soğuk stresi, inek, fertilite, metabolizma, gebelik

INTRODUCTION

Infrastructure and suprastructure of housing systems are designated to be ecostructural addressing animal welfare and compatible with environmental conditions. Tent-covered barns become common which provide a microclimate inside while removing harmful gases such as ammonia and methane and also odors.[1] However, the tent-type barns' suitability for geographical regions with severe winter conditions is controversial due to the feature that the tent can only protect against rain and wind. Thus, the effect of cold conditions in tent barns on fertility is worth investigating.

Generally, herds' fertility rate varies depending on genetic, managerial, and environmental factors and their interactions.[2] Stress is one of the most important factors that negatively affect the health and productivity of cows and other farm animals.[3] Stress is the reaction of an organism to external forces that disrupt physiological order by affecting homeostasis[4] and homeorhesis.[5] The extreme heat or cold environments cause stress in cows.[6,7] Both intrinsic (e.g., rumen movements, milk production) and extrinsic factors (e.g., high temperature, high humidity, solar radiation, low wind speed) are involved in the initiation and intensification of heat or cold stress in cows.[7,8]

This experiment was conducted to evaluate the hormonal, metabolic, and reproductive changes during the peripartum period and their effects on pregnancy rate at the first insemination in cows kept in a tent-type barn during the cold season.

MATERIALS AND METHODS

Experimental Design, Housing, and Grouping of Cows

The research was carried out on 26 pregnant (PG) Simmental cows at the end of the third lactation in a private farm (TR250001027418; Nail Cinisli Agriculture Livestock Food Industry and Trade Inc., location: 39°54′N, 40°51′E) in Turkey. Etik kurul tarih: 03.04.2018 Protokol no: 95. Cows were housed in a 2 × 2 free-stall nylon-covered tent barn. In this barn model, the cows were protected only from wind and rain but exposed to outdoor temperature and humidity changes. Cows had *ad libitum* feed and water access to meet the NRC recommendation.[9] Staff observed the cows and noted the changes such as estrus signs, abnormal vaginal discharge, foul odor after calving, and anorexia in the barn. Cows experiencing dystocia, retention of fetal membranes, mastitis, metritis, endometritis, and ovarian pathologies such as cysts were excluded from the study. A computer-assisted automated milking system recorded all milking data. The average milk yield of the cows was measured at 5756 kg for 305-days lactation in the previous lactation.

Pregnant cows were moved into the close-up paddocks and maternity pens, which were in a concrete barn, 14 days before expected parturition. In this area, the temperature was kept in the thermoneutral zone by utilizing the barn's furnace heating system. Three days after parturition, healthy cows were transferred to the main nylon tent barn and kept there for the rest of the lactation cycle.

Ambient Temperature Recording

Two digital thermometer and hygrometer data loggers were placed into the tent and concrete barns to measure and record temperature and humidity on an hourly basis during the entire study period.

Blood Sampling and Serum Analysis

Blood samples were collected from the coccygeal vein (*Vena caudalis*) on the 14th day (before expected parturition), on the day of parturition, and then sampling was extended to 3 , 4, 6, 8, 15, 22, and 29 days after parturition. Blood samples were centrifuged at 4000 rpm for 10 minutes, and serum was stored at −20°C until analyses. Measurements were performed according to the manufacturer's guidelines for β-hydroxybutyric acid (BHBA), insulin-like growth factor 1 (IGF-1), insulin, cortisol, malondialdehyde (MDA), anti-Müllerian hormone (AMH), progesterone (P4), thyroid-stimulating hormone (TSH), tri-iodothyronine (T3), and thyroxine (T4) using the enzyme-linked immunosorbent assay technique.

Reproductive Examination and Artificial Insemination

On the 15th , 22nd, and 29th day postpartum, uterine involution and restart of ovarian activity were monitored using transrectal ultrasonography. Uterine involution was monitored by measuring cervical and uterine horn diameters. The presence of large follicles and corpus luteum (CL) was accepted as the restart of postpartum ovarian activity. All of the cows, which had a healthy postpartum period, were inseminated at first estrus after the voluntary waiting period (>42th day). The same technical staff performed artificial insemination using the same semen throughout the study. The pregnancy checks were made on the 35th day after insemination, and the embryonic and fetal survival was monitored until 120 days of gestation by ultrasonographic examination and rectal palpation.

Statistical Analysis

The temperature was presented as minimum-maximum daily using the UNIVARIATE procedure.[10] The cows were divided into 2 groups as PG and non-pregnant (NPG) according to the first insemination results. Metabolic, hormonal, and reproductive parameters were subjected to a one-way analysis of variance according to the General Linear Model procedure. The linear model was used to analyze the pregnancy status, time, and pregnancy-time effect. Changes over time were determined by the repeated measurement approach. Statistics significance was accepted at the level of $P < .05$.

RESULTS

Ambient Temperature Changes in the Tent and Concrete Barn

At the farm level, the average ambient temperature ranged between −7 °C and +11 °C during the whole study period. It ranged from −7°C to +4°C and from +6°C to +11°C in the tent (postpartum) and concrete (prepartum) barns, respectively (Figure 1).

Evaluation of Fertility Parameters

The cows' fertility status was evaluated with 4 main criteria; pregnancy status at the end of the study, conception at first insemination, number of inseminations per eventual gestation, and days open till eventual gestation (Table 1). A total of 24 out of 26 cows were PG at the end of the study. Twelve of these cows were conceived at first insemination, and none of them showed embryonic and fetal death later on. Days open in the PG cows was 57.9 ± 10.1, whereas it was 193.5 ± 30.4 in the NPG cows (Table 1). For all PG cows, the average number of inseminations per pregnancy was 1.92 ± 1.35, and days open was 94.3 ± 54.0.

Metabolic and Hormonal Profiles

Serum concentrations of AMH (2.00 ± 0.04 vs. 1.89 ± 0.04 ng/mL; $P < .05$), insulin (2.10 ± 0.033 vs. 2.51 ± 0.058 mU/L, $P < .0001$), MDA (44.0 ± 1.2 vs. 49.0 ± 1.3 ng/mL, $P < .001$), and P4 (44.1 ± 2.2 vs. 41.7 ± 2.1 pg/mL, $P < .01$) were significantly different between the PG and NPG cows (Table 2). Serum concentrations of BHBA, IGF-1, cortisol, P4, TSH, T3, and T4 were similar for the PG and NPG cows. Expectedly, these parameters changed during the experimental period ($P < .0001$). Change patterns during the experimental period between the PG and NPG cows tended to

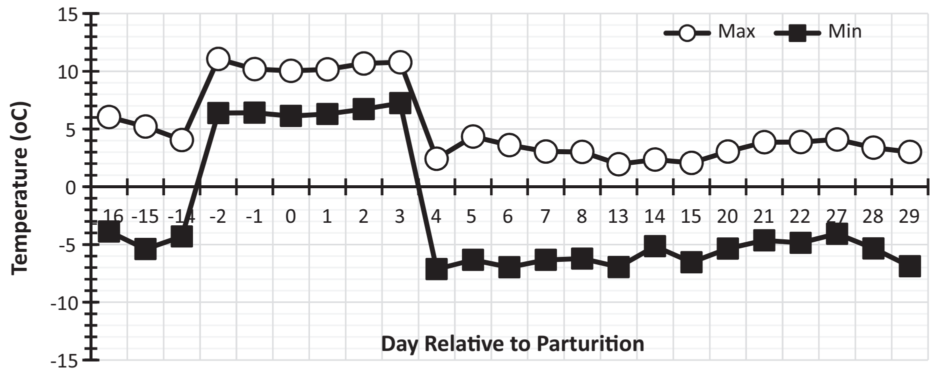

Figure 1. Temperature inside the barn during the experiment process (°C). Max, maximum; Min, minimum.

Table 1. Fertility Status of Cows Upon Insemination During the Cold Season

Pregnancy Status	n	Insemination Number	Days Open
Non-pregnant	2	5.00 ± 1.41	193.5 ± 30.4
Pregnant	24	1.92 ± 1.35	94.3 ± 54.0
	12	1	57.9 ± 10.1
	8	2	98.4 ± 9.2
	1	3	144.0 ± NA
	1	4	171.0 ± NA
	1	5	204.0 ± NA
	1	6	263.0 ± NA

NA, not applicable.

Table 2. Metabolic and Hormonal Profiles of Cows Upon Insemination During the Cold Season

	Group		Statistical Effect		
Parameter	Pregnant	Non-pregnant	Group	DRC	Group × DRC
AMH (ng/mL)	2.00 ± 0.04	1.89 ± 0.04	0.005	0.0001	0.0552
IGF-1 (ng/mL)	2.15 ± 0.032	2.11 ± 0.04	0.5388	0.0001	0.9989
Insulin (mU/L)	2.10 ± 0.033	2.51 ± 0.06	0.0001	0.0001	0.9910
Cortisol (ng/mL)	37.2 ± 1.1	39.6 ± 1.0	0.0898	0.0001	0.3860
P4 (pg/mL)	44.1 ± 2.2	41.7 ± 2.1	0.0024	0.0001	0.9556
TSH (pg/mL)	493 ± 17	532 ± 16	0.1030	0.0001	0.9858
T3 (pg/mL)	372 ± 12	368 ± 9	0.4231	0.0001	0.8203
T4 (ng/mL)	8.85 ± 0.12	8.68 ± 0.14	0.4637	0.0001	0.3540
BHBA (ng/mL)	5.12 ± 0.12	5.19 ± 0.12	0.6353	0.0001	0.9702
MDA (ng/mL)	44.0 ± 1.2	49.0 ± 1.3	0.0011	0.0001	0.5243

DRC, days relative to calving; AMH, anti-Müllerian hormone; IGF-1, insulin-like growth factor 1; P4, progesterone; TSH, thyroid-stimulating hormone; T3, tri-iodothyronine; T4, thyroxine; BHBA, β-hydroxybutyric acid; MDA, malondialdehyde.
DRC, Doğuma kıyasla gün sayısı; AMH, Anti-Müllerian hormon; IGF-1, İnsülin benzeri büyüme faktörü 1; P4, Progesteron; TSH, Tiroid situmile edici hormon; T3, Triiodotironin; T4, Tiroksin; BHBA, Beta-hidroksibütirik asit; MDA, Malondialdehit.

be different for AMH levels (Figure 1; group by time interaction, $P = .0552$). AMH concentrations for the PG cows were higher in the late prepartum period and the first month of early postpartum than that for the NPG cows (Figure 2).

Ultrasonographic Measurements

Diameters of the cervix (41.5 ± 1.29 vs. 42.2 ± 1.50 mm, $P > .05$) and the right (28.6 ± 1.12 vs. 30.6 ± 1.27 mm) and left (25.3 ± 0.97 vs. 27.0 ± 1.15 mm) uterine horns were not different between the PG and NPG cows ($P > .05$; Table 3). No difference was observed clinically in terms of ovarian activities of the cows. All cows showed estrus at least once in their first 42 days of parturition, and at least 1 CL or at least 1 Graafian follicle was present at early 22 days of calving.

Behavioral Changes

Gathering, trembling, and decreased mobility were notable in the first 3-10 days after releasing from maternity pen in the concrete barn to lactating group in tent farm. Thereafter, the intensity of these behaviors gradually diminished. The significant signs of estrus, such as standing for mounting or attempting to mount,

Table 3. Uterine Measurements of Cows Upon Insemination During the Cold Season

	Group		Statistical Effect		
Parameter	Pregnant	Non-pregnant	Group	DRC	Group×DRC
Cervix diameter (mm)	41.5 ± 1.3	42.2 ± 1.5	0.7018	0.0001	0.3645
Right horn diameter (mm)	28.6 ± 1.1	30.6 ± 1.3	0.2010	0.0018	0.8443
Left horn diameter (mm)	25.3 ± 1.0	27.0 ±1.2	0.2339	0.0964	0.9105

DRC, days relative to calving.

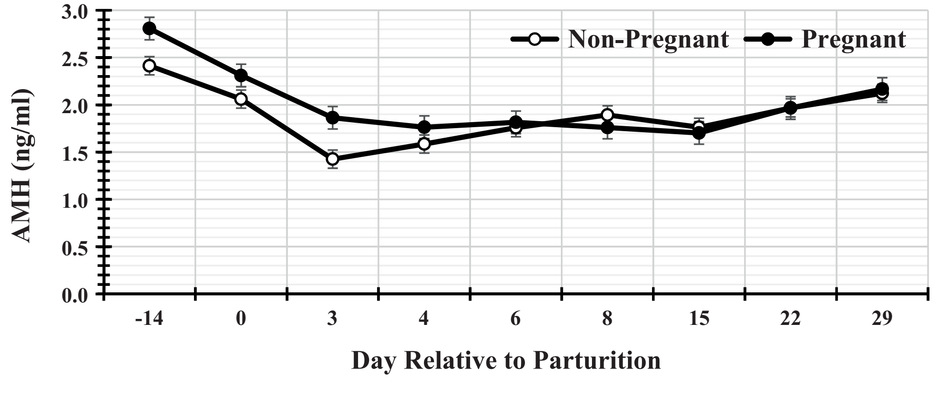

Figure 2. Alterations in AMH concentrations of cows upon insemination during the cold season. AMH, anti-Müllerian hormone.

were not observed in the tent barn. The most considerable sign was sweat and steam on the skin surface of the cows in estrus.

DISCUSSION

Behavioral changes reported in previous studies[11,12] due to acute cold stress exposure were also observed in this study when the cows were transferred from the concrete barn (from the last 2 weeks of gestation to 3-day early postpartum follow-up) to the tent barn. These included gathering, trembling, and reluctant mobility for 3-10 days, which diminished thereafter, probably adapting to a new environment.

The AMH is synthesized by developing antral follicles (3-5 mm follicles) from the ovaries, and it prevents excessive consumption of primordial follicles from the follicular pool. Therefore, it is considered a reliable biomarker of ovarian reserves for cyclicity.[13] The PG cows tended to have a higher serum AMH concentration than the NPG cows at the first insemination (Table 2). This difference was especially significant during the prepartum period and in the first week postpartum (Figure 2). The positive correlation between fertility and AMH concentration was mentioned by Ribeiro et al.[14] However, the difference between the PG and NPG cows decreased in the first week postpartum. This equalization could be due to the failure to achieve extreme cold conditions.

Endocrine IGF-1, which has a positive effect on follicular and embryonic development, is used to monitor the interactions between nutrition and reproduction.[15,16] Previous researchers stated that the cows, which showed physiological ovarian activity and ovulation, has a greater IGF-1 level in the postpartum first week than the cows with inactive ovaries, cystic follicles, and persistent corpus luteum.[17] In the present study, IGF-1 showed the typical pattern as compatible with previous studies.[18,19] It started to increase during the late postpartum, reached the basal level in the first week postpartum, and then gradually increased (data not shown). This could be also due to exclusion of the cows with postpartum abnormal ovarian activity and uterine problems from the study.[20]

Serum insulin was for the PG cows even before calving, which remained high during postpartum. Insulin affects glucose metabolism and steroidogenesis, supports estradiol and P4 production from granulosa cells, and stimulates androgen synthesis from theca cells.[21] The cows with high blood insulin concentration have greater chances of ovulation in the first early 50 days of postpartum and have a lesser interval between calving to the first insemination.[22,23] All these positive effects were interrelated with the positive effects of insulin on follicular development and the production of steroid hormones.[24] However, in the present study, all cows showed estrus at least once in their first 42 days of parturition, and at least 1 CL or at least 1 Graafian follicle was present in the first 22 days of calving.

Stressors can suppress P4 production[25] and may affect reproductive performance, indirectly. P4 is a primary hormone necessary for pregnancy continuation.[26] In this study, serum P4 concentration was consistently higher for the PG cows than that for the NPG cows during the peripartum period. Previous studies have shown a positive correlation between the P4 concentration and ovulation success and pregnancy.[27]

Cortisol is a glucocorticoid derivate, which is an essential regulator of energy metabolism, especially under acute and prolonged stress such as parturition and the postpartum period.[28] Lucy[29] stated that stress negatively impacted fertility and acute and chronic cold conditions were a stressor, leading to an increase in glucocorticoid concentration and the development of secondary changes in productivity. Increased plasma cortisol level was associated with stressful management and environmental factors such as nutrition, housing, and cold stress (acute and chronic) in previous reports.[30]

Thyroid hormones play an essential role in steroidogenesis in granulosa cells,[21] resumption of ovarian activity in post-parturient cows, and the cows with higher plasma T3 and T4 levels have shorter calving to conception interval according to Reist et al.[31] However, a difference was not detected between the groups. The cold exposure did not cause a significant decrease in T3 and T4 levels in the PG and NPG cows.

The BHBA, an indicator of subclinical ketosis, tends to increase in energy-deficient cows.[32] Higher concentrations of BHBA than 1.2 mmol/L in the first postpartum signifies subclinical ketosis.[32] The cold condition might induce lipolysis to elevate the BHBA concentration, especially in the NPG cows.

Upon exposure to the cold environment, development of oxidative stress was attained by the MDA measurement, an endpoint degradation product of lipid peroxidation[33,34] and impacts cell membranes as Castillo et al[33] reported that MDA levels significantly increased in the cows during the transition period, 1 week before and after parturition, Turk et al[34] suggested higher MDA level in prepartum than early and late puerperium. Although higher MDA was expected in the early lactation period due to increased susceptibility to production diseases,[35] prepartum oxidative stress was almost double higher in this study. This result was associated with increased oxidative status, in both advanced pregnancies.

A healthy postpartum process in the uterus and ovarium is essential for reproductive performance. The delayed uterine involution causes delayed bacterial elimination, postpartum uterine infections, profound damage to the endometrium, and infertility.[36,37] In the present study, regular postpartum gynecological examinations were administered, and the cows with postpartum metritis were excluded, purposefully. Thus, the possible adverse effects of delayed involution and metritis on the pregnancy rate could be eliminated, and the potential adverse effects of environmental conditions and endocrinological changes could be evaluated. As a result, a similar involution pattern was observed in both groups. This could be attributed to cows' well-being or failure to achieve the cold stress.

In conclusion, in cold exposure down to −7°C, there were continuous endocrinological and metabolic changes related to stress. High serum AMH, insulin, and P4 concentrations were associated higher rate of conception at the first insemination in the presented conditions. In addition to the reduction in infrastructure cost, the tent-covered barns are suitable for mild/moderate cold environments without adversely affecting welfare and productivity.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Atatürk University (Date: 03.04.2018, Decision No: 2018/95).

Peer-review: Externally peer-reviewed.

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