Ankara Univ Vet Fak Derg, **69**, 77-82, 2022
DOI: 10.33988/auvfd.791139

The negative effects of cold conditions on pregnancy rates in dairy cows

Mehmet CENGİZ[1,a,✉], Armağan HAYIRLI[2,b], Bülent BALLİ[2,c]

[1]Atatürk University, Faculty of Veterinary Medicine, Departments of Obstetrics and Gynecology, Erzurum, Turkey; [2]Atatürk University, Faculty of Veterinary Medicine, Animal Nutrition & Nutritional Disorders, Erzurum, Turkey.
[a]ORCID: 0000-0001-9913-3468; [b]ORCID: 0000-0002-4446-0848; [c]ORCID: 0000-0001-7542-4519

[✉]Corresponding author: mehmet.cengiz@atauni.edu.tr
Received date: 06.09.2020 - Accepted date: 19.03.2021

Abstract: This study was conducted to determine the pregnancy rate in response to two sexual synchronization protocols in 70 healthy multiparous Fleckvieh dairy cows during the cold and warm seasons. After pairing by the actual parturition date [cold seasons (-38 to 0 ºC) (Nov - Feb) *vs.* warm seasons (0 to 32 ºC) (Mar-Oct)], cows were randomly subjected to either Co-Synch (CoS) or double $PGF_{2\alpha}$ injection (dPG) on d 35 postpartum. Data were subjected to Chi-Square (χ^2) analysis to determine the pregnancy rate at the first service. Overall pregnancy rates (%) at the first service were 57.1 and 40.0 for Groups CoS and dPG, respectively (χ^2 = 2.03, P = 0.15) and 35.0 and 66.7% during cold and warm seasons, respectively (χ^2 = 6.78, P = 0.009). The protocol effect on the pregnancy rate at the first service was insignificant during cold (χ^2 = 1.17, P = 0.28) and warm (χ^2 = 1.62, P = 0.20) season. In summary, the cold conditions negatively affected the pregnancy rate at the first service. The synchronization protocol methods did not differ to improve the pregnancy rate at the first service when cows were exposed to cold conditions.
Keywords: Cold condition, dairy cow, first service, pregnancy rate, synchronization protocol.

Soğuk şartların süt ineklerinde gebelik oranları üzerindeki olumsuz etkileri

Özet: Bu çalışma, soğuk ve ılık mevsimlerde 70 adet sağlıklı Fleckvieh sütçü ineklere uygulanan iki seksüel senkronizasyon programı sonrası gebelik oranlarını değerlendirmek amacıyla gerçekleştirildi. Doğum sonrası 35. günde, inekler gerçek doğum tarihine göre eşleştirdikten sonra [soğuk mevsim (-38 to 0 ºC) (Kasım - Şubat), ılık mevsim (0 to 32 ºC) (Mart - Ekim)], Co-Synch (CoS) veya çift doz $PGF_{2\alpha}$ enjeksiyon (dPG) protokol gruplarına rastgele dağıtıldı. İlk tohumlamada gebelik oranının belirlenmesi için χ^2 analizi uygulandı. İlk tohumlamada toplam gebelik oranı, sırasıyla Grup CoS ve dPG için , 57,1 ve 40,0 (χ^2 = 2,03, P = 0,15) ve soğuk ve sıcak mevsimler için % 35,0 ve 66,7 (χ^2 = 6,78, P = 0,009) olarak bulundu. Soğuk (χ^2 = 1.17, P = 0,28) ve sıcak mevsimler (χ^2 = 1,62, P = 0,20) için ilk tohumlamada gebelik oranı üzerine protokol etkisi belirgin değildi. Özetle, soğuk şartlar ilk tohumlamada gebelik oranını olumsuz etkiledi. İnekler soğuk şartlara maruz bırakıldığında senkronizasyon protokolüne bağlı olarak gebelik oranı değişmedi.
Anahtar sözcükler: Gebelik oranı, ilk tohumlama, inek, senkronizasyon protokolü, soğuk iklim.

Introduction

Despite advancements in dairy cattle reproduction, the adverse effects of extreme ambient temperatures remain to be one of the major limitations in cattle fertility. The thermoneutral zone is an environmental temperature interval between - 5 and 25 ºC, in which lactating cows do not require additional energy for heating or cooling (19). An increase in both heat and humidity disturbs physiological processes at the cell and organ levels. The negative effect of heat stress on fertility is well documented, spanning from clinical signs of estrus, follicular development, and oocyte competence to embryonic survival in cows (15, 18, 20).

Although the cows tolerate cold stress more than heat stress (16), cold stress can be an issue of herd management during winter in cold regions depending on farm construction type. Because cows are warm-blooded animals, they need to maintain a constant core body temperature (38 ºC). The cut-off temperature (lower critical temperature) initiating cold stress depends on hair coat, wind, and rain (29, 30). The cold stress is associated with behavioral changes, which include huddling, reduced body condition, decreased feed and water consumption, and limited mobility behaviors (17, 25). Moreover, cows experiencing cold stress have lower respiration, urination, and heart rates (37), leading to dehydration, which can be accompanied by increased serum concentrations of non-esterified fatty acids, adrenalin, and cortisol as well as rates of hepatic glycogenolysis and extra-hepatic tissue lipolysis (4, 35). Blood is diverted from body extremities to protect vital organs. Decreased intake due to cold stress may further aggravate energy deficit, especially in transition dairy cows, because extra 2% energy is needed for every 1 ºC drop beyond the lower critical temperature (24). If the energy requirement is not compensated, the catabolic profile further jeopardizes reproductive physiology.

Major adaptive changes occurring in cold stress include 1) thermal insulation manifested by activity and hair coat, 2) an increase in resting metabolic rate, and 3) an increased rate of digestive passage (37). There may not be a reduction in lactation yield (18), but in lactation efficiency (38). This is because the priority of nutrients is diverted to maintenance from productive processes (39). Thus, reproductive parameters (*i.e.*, delayed resumption of cyclicity, mild estrus sign, ovulation problem, and embryonic loss) may be compromised in prolonged exposure to cold stress (40).

The synchronization of the estrus cycle and/or ovulation is one of the reproductive strategies to overcome the adverse effects of extreme ambient temperature on reproductive performance (10). This experiment was conducted to compare the pregnancy rate at first service upon two commonly used sexual synchronization protocols in Fleckvieh cows reared under below critical temperature.

Materials and Methods

Environmental conditions of the study location: The cows were housed in double-row free-stall barn with a concrete surface and covered by a plastic tent (15 m height) that protected against wind, rain, and snow, without providing insulation. The barn is located in Erzurum, the Northeastern Turkey (39º54′31″N, 41º17"E), where is a highland (altitude of 1853 m) and ruled by continental climate conditions (long and harsh winter - short and mild summer), with an average low temperature of -8.6 ºC and the average high temperature of 12 ºC. The average annual precipitation is 453 mm. Snow falls on an average of 80 days and remains for about 150 days. This study was approved by Atatürk University Animal Experiments Local Ethical Committee with decision number 2017/92.

Animals, management and experimental groups: This experiment involved 70 Fleckvieh cows that were in the second lactation without reproductive problems (*i.e.*, dystocia, retained placenta, and subinvolution) in early postpartum and that showed at least one estrus and had at least one palpable corpus luteum before the initiation of the experiment. They were milked twice daily and fed once daily *ad libitum* consumption of TMR consisting of 65% roughage (10% sainfoil hay, 50% orchard grass hay, and 40% corn silage) and 35% compound pelleted feed on a DM basis. The average milk production was 23.7 ± 5.6 (13.2 - 34.8) kg in 35 days in milk.

After pairing according to the actual parturition date [cold season (- 38 to 0 ºC) (November - February) *vs.* warm season (0 to 32 ºC) (March - October)] cows were subjected to either Co-Synch (CoS, n = 35) or double $PGF_{2\alpha}$ injection (dPG, n = 35) protocols (Table 1). The cows in Group CoS were injected with 2 mL GnRH analogue (Ovarelin®, 50 µg/mL, gonadoreline diacetate tetrahydrate, Ceva Animal Health, Istanbul, Turkey) at 06:00 a.m. on d 35 postpartum, 2 mL $PGF_{2\alpha}$ analogue (250 µg/mL cloprostenol, PGveyx Forte®, Veyx-Pharma, Schwarzenborn, Germany) at 06:00 a.m. on d 42 postpartum, and then inseminated 60 hrs later. The cows in Group dPG were injected with 2 mL cloprostenol by the 11-day interval at 06:00 a.m. on d 35 and 46 postpartum and inseminated 60 hrs later. Immediately after insemination, all cows in both groups were injected with 200 µg of GnRH.

Table 1. Synchronization protocols.

Day Relative to Calving	Co - Synch (CoS)	Double $PGF_{2\alpha}$ injection (dPG)
35	100 µg GnRH analogue	500 µg $PGF_{2\alpha}$ analogue
42	500 µg $PGF_{2\alpha}$	-
46	-	500 µg $PGF_{2\alpha}$ analogue
+ 60 hrs	Insemination with 100 µg GnRH analogue	Insemination with 100 µg GnRH analogue

Ovulatory follicles were monitored and measured by ultrasonography (Wed 3000®, Shenzen WELLD Medical Electronics Co. Ltd., Shenzen, China) before all inseminations and the cows which had follicle diameter larger than 16 mm were inseminated, whereas the cows, which had smaller follicles were not inseminated. Pregnancies were monitored on d 28, 35, and 60 relative to insemination by ultrasonography. The cows were confirmed pregnant after the detection of the amniotic vesicle, amniotic fluid, and heartbeat of the embryo. The same protocol was applied in subsequent services until all cows were confirmed to be pregnant.

Statistical analysis: To improve the pregnancy rate by 10% at an alpha error of 0.05 and beta error of 0.95, 24 cows were needed per group (PS: Power and Sample Size Calculation, Version 3.1, Nashville, TN). Cows were assigned to experimental groups prospectively within blocks of calving season. Data were subjected to the cross-tabulation to evaluate the pregnancy rate at the first service in response to the sexual synchronization protocol and the insemination season using Chi-Square analysis (Version 13.2.2; MedCalc, Ostend, Belgium). The effect of the synchronization protocol was also separately investigated during cold and warm seasons. Moreover, 2-way ANOVA (main effect of the sexual synchronization protocol and the insemination season as well as their interaction) was employed to determine the total number of insemination to achieve pregnancy. Statistical significance was declared at $P< 0.05$.

Results

The pregnancy rate was calculated according to examination results on the 60th d of gestation. The pregnancy rate at the first service in the cold season was much lower than that in the warm season (35.0%, 14/40 *vs*. 66.7%, 20/30; $\chi^2 = 6.78$, $P = 0.009$) (Table 2). The pregnancy rate at the first service in response the synchronization protocol was insignificant (57.1%, 20/35 and 40.0%, 14/35 for cows in Group CoS and dPG, respectively; $\chi^2 = 2.03$, $P = 0.15$) (Table 3). The synchronization protocol type affected the pregnancy rate at the first service during neither cold ($\chi^2 = 1.17$, $P = 0.28$) nor warm ($\chi^2 = 1.62$, $P = 0.20$) season (Table 4). No embryonic loss was detected following to first insemination.

Non-pregnant cows were subjected to the same synchronization protocol until achieving pregnancy. The total number of insemination was affected by the insemination season ($P = 0.04$), but not by the synchronization protocol ($P = 0.80$). The total number of insemination to achieve pregnancy was 1.52 ± 0.20 (1.13/1.92, 95% CI) for cows inseminated in cold and warm seasons and 1.61 ± 0.19 (1.23/1.98, 95% CI) and 2.00 ± 0.18 (1.63/2.37, 95% CI) for cows in Groups CoS and dPG and 2.09 ± 0.17 (1.74/2.43, 95% CI).

Table 2. Effect of season on the conception rate at the first service ($\chi^2 = 6.78$, $P = 0.009$).

Pregnancy status	Cold	Warm	n (%)
Non-pregnant	26	10	36 (51.4)
Pregnant	14	20	34 (48.6)
n (%)	40 (57.1)	30 (42.9)	

Table 3. Effect of synchronization protocol on the conception rate at the first service ($\chi^2 = 2.03$, $P = 0.15$).

Pregnancy Status	Cold	Warm	n (%)
Non-pregnant	15	21	36 (51.4)
Pregnant	20	14	34 (48.6)
n (%)	35 (50.0)	35 (50.0)	

Table 4. Effect of synchronization protocol on pregnancy at first insemination in the cold and warm seasons.

Season	Protocol	Pregnancy (n)	P
Cold	CoS	9	($\chi^2 = 1.17$, $P = 0.28$)
	dPG	5	
Warm	CoS	11	($\chi^2 = 1.62$, $P = 0.20$)
	dPG	9	

Discussion and Conclusion

Stress is defined as the inability of an animal to cope with its environment, a phenomenon that is often reflected in a failure to achieve genetic potential (11). Reproduction is an important physiological system that is fragile to stressors (9), including exposure to severe cold temperatures (13, 41, 42). Although cold stress is considered a subjective phenomenon, the animal evokes the sympathetic nervous system to overcome cold stress through increasing metabolic heat production, cardiac output, and mobilization of body reserves (4). Exposure to cold stress activates the hypothalamic-pituitary-adrenal axis, resulting in increased rates of lipolysis and glycogenolysis as well as elevations in hematological and metabolic parameters (14, 37). Cold stress affects cow behaviors, such as seeking shelter and shivering (5), decreased laying and feeding times (37). In agreement with previous studies (17, 25), some behavioral changes, such as immobility and crowding at standing position together and showing no signs of estrus, were observed. These were associated with the frozen and slippery floor and effort to decrease energy loss. These physiological and behavioral efforts as well as shifting priority of energy utilization to maintain body temperature can affect the pregnancy rate. Thus, the farm management decided to artificially inseminate the cows by estrus synchronization.

In actual environmental temperatures ranging from -12 to -23 ºC, the cow needs 1.2-1.4 fold energy. In a relationship with energy and protein intake at the time of the reproductive stage, the animal can gain/lose weight, which affects reproductive outcome (31). Exposure to cold stress causes a reduction in feed intake, which induces body condition loss and negative energy balance

(5). This leads to an abnormal uterine environment and decreased circulating steroidal hormones and IGF concentrations, which are associated with the suppression of LH secretion, reduced ovarian responsiveness to LH and delayed ovulation (6). Transition cows exposed to cold stress may have delayed return to estrus and prolonged days open, resulting in poor reproductive performance. Below the low critical temperature, reproduction is compromised as reflected by suppression of estradiol - 17β synthesis and pre-ovulatory LH release, follicular and oocyte development, ovulation, and embryonic survival (33). It was shown that the pregnancy rate was the lowest in February and the highest in July in Canada (22). The adverse effect of cold stress is independent of daylight length (8). In comparison with the season average of 25 ºC, there was a poor development of larger follicles in cows exposed to cold stress (27). In the presented study, pregnancy rate decreased in the cold season as compatible with previously described (8, 22). Moreover, lower circulating P_4 concentration could be one of the causes of decreased pregnancy rate and embryonic mortality during extreme winter conditions (13). The serum progesterone level was not measured in the presented study. However, embryonic loss was not observed in the pregnant cows until the 60^{th} day of gestation. This result was associated with that the cold conditions could not be harmful on embryonic survival as heat stress (16). Additionally, due to the cows, which had larger follicles than 16 mm, were inseminated, this election might support the quality of the oocytes and embryos (7).

Environmental temperature (13, 31) environment and barn infrastructure affect sexual behavior (12, 18, 26). The relationship between maximum environmental temperature and mounting activity is curvilinear fashion. At temperatures below -10 ºC, mounting activities were low (~3 hours), and increased as the temperature rose to 25 ºC (~ 8 hours), and then decreased as temperature rose above 30 ºC (~ 7 hours) (34). In cold seasons, it is reported that cows in free stall housing with a dry lot have more mounting activity and exhibit more pronounced sexual activity than hot weather (26). However, during the extremely cold season, slippery surface negatively affects mobility and estrus behaviors (17, 25). Cows on the concrete surface showed less mounting activity than those on the dirt surface in winter conditions (32, 36). Platz et al. (28) reported 19 collapsing and slipping incidences out of 23 mounting activity on the concrete surface. In the present study, none of the behavioral and metabolic response variables were measured.

Many studies showed that the pregnancy rate at the first service was reported to be high during the cool-cold seasons (1, 2, 23). Depending upon barn infrastructure inside temperature may not reach below the lower critical temperature (27). The warm season in this study lays within the thermoneutral zone, which does not interfere with metabolic status, estrus sign, ovarian activity, and managerial protocol for crew and animals. It is quite rare that research deals with cold stress in dairy cattle kept in barns equipped with curtain walls (4). This may be due to the assumption that curtain-sided barns provide suitable protection against weather conditions. The tent cover did not warm barn enough to avoid the alleys being frozen. This study was not designed to evaluate the accuracy of estrus detection, in terms of duration and intensity of estrus behaviors. Cold stress itself and frozen concrete surface could cause worsened welfare and limit sexual activity, and consequently lower pregnancy rate during the cold season.

Synchronization of estrus and ovulation become standard components in reproductive management, especially in large dairy operations (21). Under extreme cold conditions, timed-artificial insemination may help avoid injuries associated with estrus behavior on the slippery surface through separating sexually active cows. The pregnancy rate at the first service did not differ in response to the CoS and dPG protocols. Previous studies dealing with similar protocols also achieved a similar pregnancy rate at the first service (3, 23, 28).

In summary, this preliminary experiment investigating the cold stress effect on pregnancy rate has a number of limitations. These include lacking measurements of daily changes in temperature, humidity, and wind speed in the barn, data of feed intake, observations on behavioral changes, and measurements for physiological/biochemical parameters. Seasonal temperature elicited a more notable effect on the pregnancy rate than sexual synchronization protocol. Extreme cold conditions adversely affected the pregnancy rate probably by directly affecting the cow's metabolic status or indirectly affecting the cow's behavior in relation to swamped barn conditions. Employment of the CoS or dPG protocol was not advantageous to each other to improve pregnancy rate at the first service during the extreme cold conditions.

Acknowledgement

All authors declare special thanks to Nail Cinisli Dairy Farm crew for their support.

Financial Support

This research received no grant from any funding agency/sector.

Ethical Statement

This study was approved by Atatürk University Animal Experiments Local Ethical Committee with decision number 2017/92.

Conflict of interest

The authors declared that there is no conflicts of interest. The authors are responsible for the content and writing of the paper.

References

1. **Akbarabadi MA, Shabankareh HK, Abdolmohammadi A, et al** (2014): *Effect of PGF 2α and GnRH on the reproductive performance of postpartum dairy cows subjected to synchronization of ovulation and timed artificial insemination during the warm or cold periods of the year.* Theriogenology, **82**, 509-516.
2. **Alnimer M, De Rosa G, Grasso F, et al** (2002): *Effect of climate on the response to three oestrous synchronisation techniques in lactating dairy cows.* Anim Reprod Sci, **71**, 157-168.
3. **Amiridis G, Belibasaki S, Leontides L, et al** (2000): *Reproductive efficiency of three estrus synchronization schemes comprising fixed-time insemination in dairy cows.* J Vet Med B, **47**, 271-276.
4. **Brouček J, Letkovičová M, Kovalčuj K** (1991): *Estimation of cold stress effect on dairy cows.* Int J Biometeorol, **35**, 29-32.
5. **Bryant JR, Lopez-Villalobos N, Pryce JE, et al** (2007): *Quantifying the effect of thermal environment on production traits in three breeds of dairy cattle in New Zealand.* New Zeal J Agr Res, **50**, 327-338.
6. **Butler WR** (2000): *Nutritional interactions with reproductive performance in dairy cattle.* Anim Reprod Sci, **60**, 449-457.
7. **Cerri RLA, Chebel RC, Rivera F, et al** (2011): *Concentration of progesterone during the development of the ovulatory follicle: I. Ovarian and embryonic responses*. J Dairy Sci, **94**, 3342-3351.
8. **Chebel RC, Braga FA, Dalton JC** (2007): *Factors affecting reproductive performance of Holstein heifers.* Anim Reprod Sci, **101**, 208-224.
9. **Coubrough RI** (1985): *Stress and fertility. A review*. Onderstepoort J Vet Res, **52**, 153-156.
10. **De Rensis F, Garcia-Ispierto I, López-Gatius F** (2015): *Seasonal heat stress: Clinical implications and hormone treatments for the fertility of dairy cows.* Theriogenology, **84**, 659-666.
11. **Dobson H, Smith RF** (2000): *What is stress, and how does it affect reproduction?* Anim Reprod Sci, **60**, 743-752.
12. **Graunke KL, Schuster T, Lidfors LM** (2011): *Influence of weather on the behaviour of outdoor-wintered beef cattle in Scandinavia.* Livest Sci, **136**, 247-255.
13. **Gwazdauskas F, Lineweaver J, McGilliard M** (1983): *Environmental and management factors affecting estrous activity in dairy cattle*. J Dairy Sci, **66**, 1510-1514.
14. **Gwazdauskas F** (1985): *Effects of climate on reproduction in cattle* J Dairy Sci, **68**, 1568-1578.
15. **Hansen P, Arechiga C** (1999): *Strategies for managing reproduction in the heat-stressed dairy cow.* J Anim Sci, **77**, 36-50.
16. **Janský L** (1984): *Thermoregulatory responses to cold stress of various intensity.* Arch Exp Veterinarmed, **38**, 353-358.
17. **Jemal H, Lemma A** (2015): *Review on major factors affecting the successful conception Rates on biotechnological application (AI) in cattle*. Glob J Med Res, **15**, 28-37.
18. **Kadzere C, Murphy M, Silanikove N, et al** (2002): *Heat stress in lactating dairy cows.* Livest Prod Sci, **77**, 59-91.
19. **Knížková I, Kunc P, Koubková M, et al** (2002): *Evaluation of naturally ventilated dairy barn management by a thermographic method*. Livest Prod Sci, **77**, 349-353.
20. **Lee C** (1993): *Environmental stress effects on bovine reproduction*. Vet Clin North Am Small Anim Pract, **9**, 263-273.
21. **Macmillan KL** (2010): *Recent advances in the synchronization of estrus and ovulation in dairy cows*. J Reprod Develop, **56**, 42-47.
22. **Mercier E, Salisbury G** (1947): *Seasonal variations in hours of daylight associated with fertility level of cattle under natural breeding conditions*. J Dairy Sci, **30**, 747-756.
23. **Momcilovic D, Archbald L, Walters A, et al** (1998): *Reproductive performance of lactating dairy cows treated with gonadotrophin-releasing hormone (GnRH) and/or prostaglandin F2a (PGF2a) for synchronization of estrus and ovulation.* Theriogenology, **50**, 1131-1139.
24. **National Research Council** (2001): Nutrient Requirements of Dairy Cattle. 7th ed., 2-27, National Academy Press, Washington, DC, USA.
25. **Orihuela AN** (2000): *Some factors affecting the behavioural manifestation of oestrus in cattle: a review.* Appl Anim Behav Sci, **70**, 1-16.
26. **Pennington JA, Albright JL, Diekman MA et al** (1985): *Sexual activity of Holstein cows: Seasonal effects.* J Dairy Sci, **68**, 3023-3030.
27. **Peralta-Torres JA, Aké-López JR, Centurión-Castro FG, et al** (2017): *Effect of season and breed group on the follicular population and cyclicity of heifers under tropical conditions.* Trop Anim Health Prod, **49**, 207-211.
28. **Platz S, Ahrens F, Bendel J, et al** (2008): *What happens with cow behavior when replacing concrete slatted floor by rubber coating: A case study*. J Dairy Sci, **91**, 999-1004.
29. **Qi L, Bravo-Ureta B, Cabrera V** (2015): *From cold to hot: Climatic effects and productivity in Wisconsin dairy farms*. J Dairy Sci, **98**, 8664-8677.
30. **Regan W, Richardson G** (1938): *Reactions of the dairy cow to changes in environmental temperature.* J Dairy Sci, **21**, 73-79.
31. **Robinson V** (2015): Effects of cold stress on the submission and conception rates of New Zealand dairy cows. MSc, Massey University, Palmerston North, New Zealand.
32. **Rodtian P, King G, Subrod S, et al** (1996): *Oestrous behaviour of Holstein cows during cooler and hotter tropical seasons.* Anim Reprod Sci, **45**, 47-58.
33. **Roth Z** (2015): *Cellular and molecular mechanisms of heat stress related to bovine ovarian function*. Anim Sci J, **93**, 2034-2044.

34. **Thatcher WW** (1974): *Effects of season, climate, and temperature on reproduction and lactation*. J Dairy Sci, **57**, 360-368.
35. **Tucker CB, Rogers AR, Verkerk GA, et al** (2007): *Effects of shelter and body condition on the behaviour and physiology of dairy cattle in winter*. Appl Anim Behav Sci, **105**, 1-13.
36. **Vailes LD, Britt JH** (1990): *Influence of footing surface on mounting and other sexual behaviors of estrual Holstein cows*. J Dairy Sci, **68**, 2333-2339.
37. **Webster J, Stewart M, Rogers A, et al** (2008): *Assessment of welfare from physiological and behavioural responses of New Zealand dairy cows exposed to cold and wet conditions.* Anim Welf, **17**, 19-26.
38. **Young BA, Walker B, Dixon AE, et al** (1989): *Physiological adaptation to the environment.* Anim Sci J, **67**, 2426-2432.
39. **Young BA** (1981): *Cold stress as it affects animal production*. Anim Sci J, **52**, 154-163.
40. **Young BA** (1983): *Ruminant cold stress: Effect on production*. Anim Sci J, **57**, 1601-1607.
41. **Zöldag L** (1983): *Stress and reproductive disorders in cattle. 1. Effect of stress factors on the estrous cycle*. Dtsch Tierarztl Wochenschr, **90**, 152-156.
42. **Zöldag L** (1983): *Stress and reproductive disorders in cattle. 2. Effect of stressors and pregnancy*. Dtsch Tierarztl Wochenschr, **90**, 184-187.