Praca oryginalna ***Original paper***

Effect of equine chorionic gonadotropin on pregnancy rates in anestrous dairy cows during a modified PRID-Ovsynch protocol*

CIHAN KAÇAR[1], UMUT ÇAĞIN ARI[2], SEMRA KAYA[1], MURAT CAN DEMİR[1], OĞUZ MERHAN[3], MUSHAP KURU[1], SAVAŞ YILDIZ[2], GÜVEN GÜLBAZ[4]

[1]Obstetrics and Gynecology Department, Faculty of Veterinary, Kafkas University, 36100, Kars, Turkiye
[2]Reproduction and Artificial Insemination, Faculty of Veterinary, Kafkas University, 36100, Kars, Turkiye
[3]Biochemistry Department, Faculty of Veterinary, Kafkas University, 36100, Kars, Turkiye
[4]Food Engineering Department, Faculty of Engineering, Kafkas University, 36100, Kars, Turkiye

Received 29.04.2024 *Accepted 26.07.2024*

Kaçar C., Ari U. Ç., Kaya S., Demir M. C., Merhan O., Kuru M., Yildiz S., Gülbaz G.

Effect of equine chorionic gonadotropin on pregnancy rates in anestrous dairy cows during a modified PRID-Ovsynch protocol

Summary

The objective of this study was to investigate the impact of equine chorionic gonadotropin (eCG) injections on progesterone (P4) profiles and pregnancy rates (PR) in Brown Swiss cows with unidentified estrus between days 60 and 120 after parturition. A total of 80 cows were enrolled in the study. All cows had an intravaginal progesterone-releasing device (PRID) inserted for 9 days and received a GnRH injection on day 0. On day 8, PGF2α was administered, the PRID was removed on day 9, and fixed-time artificial insemination (FTAI) was performed 60 hours after PRID removal. Half of the cows (eCG+, n = 40) received a 600 IU injection of eCG when the PRID was removed on day 9, while the other half (eCG–, n = 40) did not. Blood samples were collected on days –10, 0, 8, 9, and during FTAI to measure P4 concentrations. The PR was 37.5% in the eCG+ group and 27.5% in the eCG– group (P = 0.4). At the time of FTAI, the dominant follicle diameter was significantly larger in the eCG+ group (15.5 mm) than in the eCG– group (12.4 mm) (P < 0.001). There was no significant difference in serum P4 concentration between the two groups, except on the day of FTAI, when P4 levels were 0.72 ng/mL in the eCG+ group and 0.94 ng/mL in the eCG– group (P < 0.05). In conclusion, the administration of eCG in addition to synchronization protocols may increase pregnancy rates in cows with unidentified estrus for at least 60 days postpartum.

Keywords: anestrus, cow, eCG, progesterone, subestrus

In recent years, the increase in milk yield in cows has made the observation of estrus signs challenging, resulting in a decrease in the pregnancy rate (PR) at first insemination to 35% (1). Timely observation of estrus signs and artificial insemination are essential to improving fertility rates. However, estrus signs have become increasingly difficult to identify due to various factors, such as poor farm management and environmental conditions. Consequently, fixed-time artificial insemination (FTAI) protocols have been developed (1).

Studies have identified several factors that affect ovarian function in cows during the postpartum period. These include inadequate feed intake in the peripartum period (4, 20), a negative energy balance, and low body reserves due to high milk yield in the early postpartum period (5, 16, 20). Other influential factors include the high metabolic clearance rate of steroid hormones (35), lower circulating concentrations of progesterone (P4) and estradiol (E2) in lactating cows compared to non-lactating cows (31, 32), and reduced luteinizing hormone (LH) secretion caused by stress and malnutrition (8).

Under optimum conditions, follicular growth is a cycle comprising selection of a dominant follicle, maturation of the follicle, luteolysis and resumption of ovarian activity. Failure of any of these processes prolongs postpartum anestrus. Failure of cows to express estrus and poor estrus detection can increase the incidence of anestrus in a herd (21). Different

* This study was supported by Kafkas University Scientific Research Projects Office (KAÜ-BAP) under project number 2017-TS-44.

hormone combinations have been used in cows during the postpartum period, including gonadotropin-releasing hormone (GnRH) with the Ovsynch protocol (3, 14, 15), and P4-E2-prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)-equine chorionic gonadotropin (eCG) (2, 19, 30). After injection of eCG in FTAI in cows, follicle development (12, 23), ovulation (19, 23), and pregnancy rates (PR) have been reported to increase (13, 29).

We hypothesized that eCG injection during the PRID Delta synchronization protocol in cows that do not show estrus behavior between days 60 and 120 might have an effect on P4 concentration and on PR. Therefore, the aim of the study was to investigate the effects of eCG injection during the PRID Delta synchronization protocol on P4 levels and on PR in cows without estrus signs between days 60 and 120 postpartum.

Material and methods

Ethical statement. This study was conducted with the approval of the Local Ethics Board for Animal Experiments of Kafkas University (KAÜ-HADYEK-2017/32), Kars, Türkiye.

Location, animals, and feeds. The present study was conducted at the Ali Rıza AKSOY Research and Application Farm of the Kafkas University Veterinary Faculty in Kars, Türkiye, on a sample of 80 Brown Swiss cows that did not exhibit any signs of estrus (such as standing to be mounted, mounting other cows, mucus discharge, swelling, and reddening of the vulva) between days 60 and 120 postpartum. Estrus was observed three times daily for at least 30 minutes. The study included only clinically healthy cows that were housed in a closed barn system and fed a mixed ration consisting of meadow grass, sainfoin, concentrated feed, and molasses, with an average daily milk yield of 13-17 L during lactation. The body condition scores (BCS) were assessed using a 5-point grading system.

Synchronization protocol. The cows selected for the study were aged 3-7 years, did not exhibit any pathological discharge, and were deemed clinically healthy. They were in the lactation period, and their estrus had not been detected 2 to 4 months after calving. The cows were randomly allocated into two groups. The first group (eCG–) (n = 40) received an intramuscular injection of 2 mL GnRH (Ovarelin®, Ceva, Türkiye) on day (d) 0, and an intravaginal P4-releasing device (PRID Delta®, 1.55 g progesterone, Ceva, Türkiye) was inserted using an applicator after cleaning the vulva with a cotton ball soaked in Baticonol antiseptic solution. Lubricating gel was applied, and the PRID was placed in the vagina. On d 8, one day before the removal of the PRID, 5 mL of PGF2α (Estrumate® RTM, Ceva, Türkiye) was injected intramuscularly. The PRID was removed from the vagina on d 9, and the cows were inseminated 60 hours later. The eCG+ group of cows (n = 40) received 600 IU of eCG intramuscularly (Oviser®, Hipra, Türkiye) upon removal of the PRID, and FTAI was performed 60 hours later (Fig. 1). The follicle diameter was measured by ultrasonography (7.5 MHz, Draminski Iscan®, Draminski, Poland) just before insemination in both groups. To determine the P4 concentration produced by the PRID, 5 mL blood samples were taken with vacuum injectors from the tail vein into EDTA-free tubes on d –10, d 0 (the day when the PRID was inserted), d 8 (just before the PGF2α injection), and at FTAI. Pregnancy diagnosis was performed by ultrasound 30 ± 2 days after FTAI.

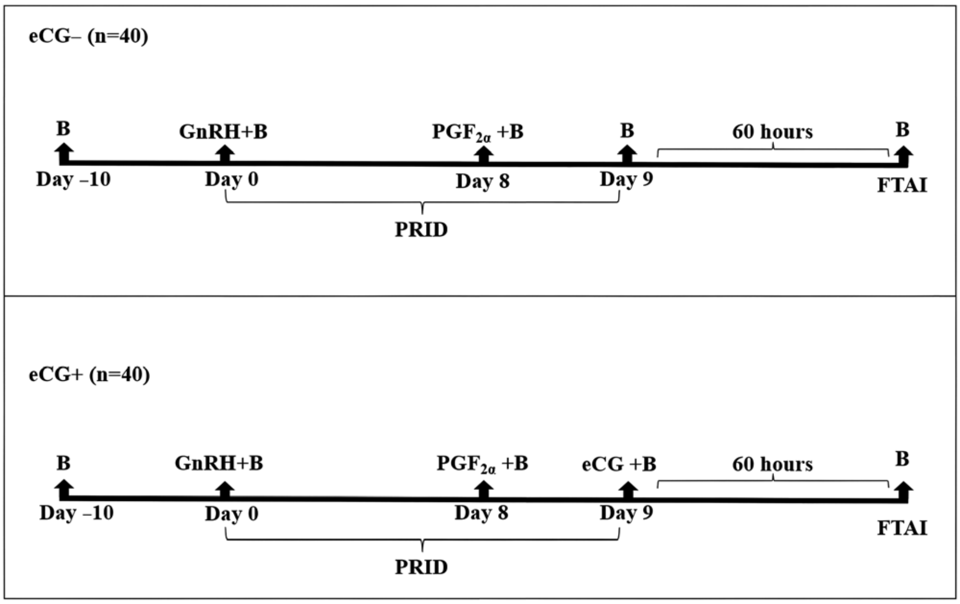

Fig. 1. Hormone treatments and blood sample collection days prior to fixed-time artificial insemination (FTAI)

Explanations: The eCG– group of cows were given a GnRH injection on day 0, followed by the insertion of progesterone-releasing intravaginal devices (PRID). On day 8, PGF2α was administered one day before the removal of PRID. On day 9, the PRID was removed, and the cows were inseminated 60 hours later. In contrast, the eCG+ group of cows received eCG treatment upon removal of PRID. Blood samples were taken on several days for hormone analysis. B – blood sample, eCG – equine chorionic gonadotropin

Blood sampling and P4 analysis. The blood samples were centrifuged at 3000 rpm for 10 min, and the sera obtained were stored at –20°C until analysis. Serum P4 concentrations were measured using commercial ELISA (MyBioSource®, San Diego, USA) kits.

Statistical analysis. The data were analyzed statistically with the SPSS® software (SPSS 20, IL, USA). Normality of distribution was assessed by the Kolmogorov-Smirnov test. The Mann-Whitney U test was used to compare follicle diameters between the groups. A chi-square analysis was used to compare PR. The Student's t-test was used to compare P4 concentration between the groups if the data met the assumption of normality, and the Mann-Whitney U test was used if the data was non-parametric. The results are presented as mean ± standard error of the mean (SEM). Statistical significance was set at $P < 0.05$.

Results and discussion

In cows without estrus signs, the PR was 37.5% in the eCG+ group compared to 27.5% in the eCG– group. Although there was a 10% difference between the groups, it was not statistically significant ($P = 0.4$, Tab. 1). The dominant follicle diameter was 15.5 mm in eCG+ cows injected during fixed-time artificial insemination (FTAI) and 12.4 mm in eCG– cows. Therefore, the dominant follicle diameter was larger in cows treated with eCG, and a statistically significant difference was found between the two groups in terms of follicle size ($P < 0.001$, Tab. 1).

Administration of PRID caused an increase in serum P4 concentrations. The serum P4 concentration at d 8 was 4.02 ± 0.13 ng/mL in the eCG– group and 4.05 ± 0.18 ng/mL in the eCG+ group, whereas on d 9, it was 5.64 ± 0.15 ng/mL in the eCG– group and 5.86 ± 0.11 ng/mL in the eCG+ group. However, no significant differences in serum P4 concentrations were observed in blood samples collected prior to FTAI. At the time of FTAI, the serum P4 concentration was 0.94 ± 0.06 ng/mL in the eCG– group and 0.72 ± 0.05 ng/mL in the eCG+ group ($P < 0.05$, Fig. 2). There were no significant differences in PR between cows with BCS ≥ 2.75 and those with BCS < 2.75 in either group. Similar results were obtained when comparing the eCG+ and eCG– groups (Tab. 2).

The use of eCG injections has been shown to have positive effects on fertility in cows undergoing FTAI in P4/E2-based protocols (7, 12, 19, 29). This treatment increases the level of gonadotropin support for follicle development, thus increasing the size of the dominant follicle and the likelihood of ovulation, which improves the fertility of grazing purebred or crossbred beef and dairy cattle (23). In anestrous cows, the addition of eCG to P4 implant-based treatments supports serum progesterone concentration. The greatest improvement in the pregnancy rate following eCG addition was observed in cows that calved more than 60 days before breeding. This may be more pronounced with the trophic effect of eCG on follicular and luteal development and hormone production, leading to a reduced incidence of delayed ovulation, luteal insufficiency, and/or premature luteal regression (33).

Tab. 1. Pregnancy parameters with and without eCG treatment in cows without estrus symptoms

Parameters	eCG–	eCG+	P value
Number of animals	40	40	> 0.05
Body condition score	2.76	2.72	> 0.05
Dominant follicle diameter	12.4 ± 0.32	15.5 ± 0.35	0.001
Pregnancy rate/Artificial insemination	27.5	37.5	0.4

Tab. 2. Pregnancy rates in groups with and without eCG according to body condition scores

Groups	Body Condition Score		P value
	> 2.75	< 2.75	
eCG–	33.33	18.75	> 0.05
eCG+	41.66	31.25	> 0.05
P value	> 0.05	> 0.05	–

eCG treatment in cattle enhances the development of small, medium, and large follicles in the ovary, as well as the ovulation rate of the dominant follicle and P4 secretion during the luteal phase (26). In a similar study, Nelore heifers that were treated with eCG had a higher pregnancy rate than heifers that were not treated with eCG (29). In a study conducted on Nelore cows with appropriate body condition undergoing P4-based FTAI in the early postpartum period, eCG injection did not have a significant effect on the pregnancy rate (22). Another study showed that eCG treatment in the first synchronized estrus improved reproductive performance in the short term, but did not have a significant effect on subsequent inseminations (28). Another study found no significant differences in follicular dynamics between cyclic and anestrus groups treated with eCG treatment or calves were separated from the cows for 56 h, despite differences in the cyclicity of the cows. The follicular diameter and P4 concentrations of anestrous cows treated with eCG or 56 h – calf removal were also found to be similar (2).

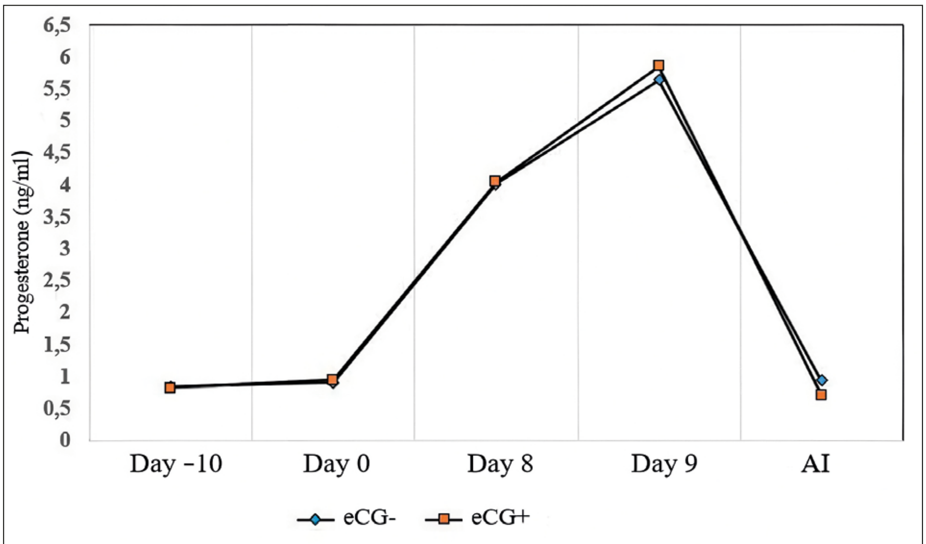

Fig. 2. Serum progesterone (P4) concentration in groups according to blood collection days

Explanations: Cows were synchronized using a progesterone-releasing intravaginal devices (PRID)-based fixed-time artificial insemination (FTAI) protocol and divided into 2 groups according to an injection of equine chorionic gonadotropin (eCG). The eCG+ group was treated with eCG on day 9, while the eCG– group was untreated. Blood samples for serum P4 analysis were taken on days –10, 0, 8, 9, and at the time of FTAI

The administration of eCG during the removal of the vaginal device in anestrus cows has been demonstrated to enhance ovulation rates and improve luteal function (19). Moreover, eCG treatment can reduce differences in ovulation timing (11). However, one study reported no increase in ovulation rates following eCG administration. Treatment with eCG has been shown to result in larger corpus luteum and higher P4 concentrations in cows that ovulate after synchronization (19). In this study, intravaginal devices containing P4 increased serum P4 concentrations in cows without estrus signs. For healthy cyclic activity and continued follicular activity in the ovary, the genital organs must be exposed to P4 for a certain period. However, the concentration of P4 was found to be lower in cows treated with eCG during FTAI, which may have contributed to the increased likelihood of ovulation due to the resulting peak in LH release. This lower concentration of P4 could also potentially explain the difference in PR between cows treated with eCG and those without.

Long-term treatment with progesterone (21 days) did not increase PR in cows with high BCS, but the combination of long-term progesterone and eCG treatment increased PR per preovulatory follicle and artificial insemination success in cows with low BCS (7). The effect of eCG injections on fertility in lactating Holstein cows was limited to cows with low BCS. The eCG treatment improved ovulation rates or corpus luteum development, especially in cows with low BCS, which may lead to increased fertility in these cows. Cows with a BCS below 2.75 have shown increased follicle growth and fertility with eCG treatment in cases of insufficient LH support (34). However, in the present study, there were no differences in the PR of anestrus cows with or without eCG injection, regardless of whether their BCS was ≥ 2.75 or < 2.75. It is likely that factors other than BCS affect the anestrus mechanism in cows.

The eCG treatment showed no significant effect on follicular diameter during artificial insemination and subsequent P4 concentration. eCG treatment during Ovsynch+P4 did not improve reproductive outcomes in dairy cows (25). Although some studies have suggested an improvement in pregnancy rates in timed artificial insemination protocols for specific subgroups of cows (older cows, cows diagnosed with anovulatory anestrus), these findings have not been consistently replicated (6).

In lactating dairy cows undergoing the Presynch-Ovsynch program, eCG treatment at a dose of 400 IU three days before artificial insemination at the first service did not increase the size of the largest follicle, follicle growth rate, or PR 48 h after application (24). The dose and timing of eCG treatment could be delayed to affect the maturation period of the preovulatory follicle. In one study, it was found that follicle size, corpus luteum volume, serum P4 concentrations, and PR were higher when eCG was applied two days before the removal of the intravaginal P4 device rather than during the removal of the device in cows undergoing FTAI (12). During the FTAI, the diameter of the largest follicle found in the ovaries was larger in heifers treated with eCG than in untreated heifers (29). The follicle diameters of anestrous and cyclic cows treated with eCG were similar. Cows with eCG treatment or anestrus induced by removing calves from suckling for 56 hours showed follicular diameter and growth rate similar to cyclic cows (2). The addition of eCG to P4 and E2-based treatments increased the ovulation rate of the dominant follicle in anestrous cows due to the stimulating effect of eCG on the growth rate (19). The most important finding of the present study was that the follicle diameter at FTAI was larger in the cows that did not show signs of estrus with eCG injection compared to those that were not injected with eCG. Although this difference in follicle diameter did not affect PR, eCG injection increased the growth rate of the follicle in anestrous cows. The larger diameter of the largest follicle in the eCG group can be explained by the fact that eCG increases FSH and LH release for follicle development. The increase in follicular diameter induced by eCG may be related to the positive effect on steroidogenic cells in the ovulatory follicle and the formation of a more competent corpus luteum observed in treated cows (19).

eCG treatment increases pregnancy rates and plasma P4 concentrations in timed insemination protocols for lactating dairy cows under severe heat stress conditions. The increased P4 concentrations in eCG-treated cows may be due to the stimulation of the dominant follicle and the developing CL (13). In addition, eCG enhances various enzymes involved in steroid biosynthesis (28), increases mitochondrial activity of the CL, and regulates mRNA coding proteins involved in LH receptor intracellular signaling in granulosa cells (10), thereby promoting progesterone secretion. In our study, progesterone values were measured 10 days before hormone application and on the day hormone application started to determine whether cows were in anestrus.

Applications of eCG include treatment of anestrus, induction of puberty, stimulation of fertility, and superovulation (18). Since the only way to produce this hormone is to extract it from the blood of pregnant mares, concerns have been raised about the welfare implications of eCG production methods (17). The use of other hormones alone or in combination has not been successful in achieving the desired results (9). In this study, the treatment was applied in accordance with the procedure recommended by the pharmaceutical company from which eCG was obtained. No adverse effects were observed in the treated cows.

In conclusion, eCG injections have a positive impact on follicle development and subsequently increase pregnancy rates in cows exhibiting anestrus between days 60 and 120 postpartum. Incorporating eCG into synchronization protocols for cows without estrus signs holds potential for enhancing fertility outcomes. Nevertheless, further comprehensive investigations are warranted to elucidate the precise mechanisms underlying the effect of eCG on fertility.

References

1. *Aungier S. P. M., Roche J. F., Sheehy M., Crowe M. A.*: Effects of management and health on the use of activity monitoring for estrus detection in dairy cows. J. Dairy. Sci. 2012, 95, 2452-2466.
2. *Barreiros T. R. R., Blaschi W., Santos G. M. G., Morotti F., Andrade E. R., Baruselli P. S., Seneda M. M.*: Dynamics of follicular growth and progesterone concentrations in cyclic and anestrous suckling Nelore cows (Bos indicus) treated with progesterone, equine chorionic gonadotropin, or temporary calf removal. Theriogenology 2014, 81, 651-656.
3. *Bartolome J. A., Sozzi A., McHale J., Melendez P., Arteche A. C. M., Silvestre F. T., Kelbert D., Swift K., Archbald L. F., Thatcher W. W.*: Resynchronization of ovulation and timed insemination in lactating dairy cows, II: Assigning protocols according to stages of the estrous cycle, or presence of ovarian cysts or anestrus. Theriogenology 2005, 63, 1628-1642.
4. *Baruselli P. S., Reis E. L., Marques M. O., Nasser L. F., Bó G. A.*: The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates, [in:] Animal Reproduction Science. Elsevier 2004, 479-486.
5. *Beam S. W., Butler W. R.*: Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. J. Reprod. Fertil. Suppl. 1999, 54, 411-424.
6. *Beasley L., Cogger N., Compton C.*: Use of equine chorionic gonadotropin in lactating dairy cattle: a rapid review. N. Z. Vet. J. 2023, 71, 53-64.
7. *Bilbao M. G., Massara N., Ramos S., Zapata L. O., Farcey M. F., Pesoa J., Turic E., Vázquez M. I., Bartolome J. A.*: Extending the duration of treatment with progesterone and equine chorionic gonadotropin improves fertility in suckled beef cows with low body condition score subjected to timed artificial insemination. Theriogenology 2015, 86, 536-544.
8. *Butler W. R., Smith R. D.*: Interrelationships between energy balance and postpartum reproductive function in dairy cattle. J. Dairy. Sci. 1989, 72, 767-783.
9. *Cardoso Neto B. M., Barbosa L. P., Aguiar C. da S., Souza R. S., Santana A. L. A., Mendes C. S., Dutra P. A., Leite M. C. de P.*: Follicle-stimulating hormone to substitute equine chorionic gonadotropin in the synchronization of ovulation in Santa Inês ewes. R. Bras. Zootec. 2012, 41, 603-606.
10. *Castilho A. C. S., Nogueira M. F. G., Fontes P. K., Machado M. F., Satrapa R. A., Razza E. M., Barros C. M.*: Ovarian superstimulation using FSH combined with equine chorionic gonadotropin (eCG) upregulates mRNA-encoding proteins involved with LH receptor intracellular signaling in granulosa cells from Nelore cows. Theriogenology 2014, 82, 1199-1205.
11. *Cavalieri J., Rubio I., Kinder J. E., Entwistle K. W., Fitzpatrick L. A.*: Synchronization of estrus and ovulation and associated endocrine changes in Bos indicus cows. Theriogenology 1997, 47, 801-814.
12. *Dorneles Tortorella R., Ferreira R., Tonellotto dos Santos J., Silveira de Andrade Neto O., Barreta M. H., Oliveira J. F., Gonçalves P. B., Pereira Neves J.*: The effect of equine chorionic gonadotropin on follicular size, luteal volume, circulating progesterone concentrations, and pregnancy rates in anestrous beef cows treated with a novel fixed-time artificial insemination protocol. Theriogenology 2013, 79, 1204-1209.
13. *Funakoshi D., Shiotani H., Seki M.*: Equine chorionic gonadotropin treatment and timed artificial insemination for dairy cow production under heat stress. J. Reprod. Dev. 2024, 70, 30-34.
14. *Kacar C., Yildiz S., Pancarci S. M., Kaya M., Oral H., Gurbulak K., Gungor O.*: Administration of GnRH treatment prior to ovsynch protocol to stimulate ovarian cycle in cows with functional anoestrus. Bull. Vet. Inst. Pulawy 2006, 50, 497-501.
15. *Kuru M., Kacar C., Oral H., Kaya S., Cetin N., Kaya D., Demir M. C.*: Effect of two prostaglandin F2α injections administered 24 hours apart on the pregnancy rate of Simmental cows subjected to the Ovsynch or Ovsynch + Controlled internal drug release (CIDR) protocols. Med. Weter. 2020, 76, 6451-2020.
16. *Leroy J. L. M. R., Vanholder T., Van Knegsel A. T. M., Garcia-Ispierto I., Bols P. E. J.*: Nutrient prioritization in dairy cows early postpartum: mismatch between metabolism and fertility? Reprod. Domest. Anim. 2008, 43, 96-103.
17. *Manteca Vilanova X., De Briyne N., Beaver B., Turner P. V.*: Horse welfare during equine chorionic gonadotropin (eCG) production. Animals 2019, 9, 1053.
18. *Murphy B. D.*: Equine chorionic gonadotropin: an enigmatic but essential tool, [in:] Anim. Rep. 2012, p. 223-230.
19. *Núñez-Olivera R., De Castro T., García-Pintos C., Bó G., Piaggio J., Menchaca A.*: Ovulatory response and luteal function after eCG administration at the end of a progesterone and estradiol' based treatment in postpartum anestrous beef cattle. Anim. Reprod. Sci. 2014, 146, 111-116.
20. *Opsomer G., Gröhn Y. T., Hertl J., Coryn M., Deluyker H., De Kruif A.*: Risk factors for postpartum ovarian dysfunction in high producing dairy cows in Belgium: A field study. Theriogenology 2000, 53, 841-857.
21. *Peter A. T., Vos P. L. A. M., Ambrose D. J.*: Postpartum anestrus in dairy cattle. Theriogenology 2009, 71, 1333-1342.
22. *Pinheiro V. G., Souza A. F., Pegorer M. F., Satrapa R. A., Ereno R. L., Trinca L. A., Barros C. M.*: Effects of temporary calf removal and eCG on pregnancy rates to timed-insemination in progesterone-treated postpartum Nellore cows. Theriogenology 2009, 71, 519-524.
23. *Prata A. B., Pontes G. C. S., Monteiro P. L. J., Drum J. N., Wiltbank M. C., Sartori R.*: Equine chorionic gonadotropin increases fertility of grazing dairy cows that receive fixed-time artificial insemination in the early but not later postpartum period. Theriogenology 2017, 98, 36-40.
24. *Pulley S. L., Wallace L. D., Mellieon H. I., Stevenson J. S.*: Ovarian characteristics, serum concentrations of progesterone and estradiol, and fertility in lactating dairy cows in response to equine chorionic gonadotropin. Theriogenology 2013, 79, 127-134.
25. *Randi F., Sánchez J. M., Herlihy M. M., Valenza A., Kenny D. A., Butler S. T., Lonergan P.*: Effect of equine chorionic gonadotropin treatment during a progesterone-based timed artificial insemination program on reproductive performance in seasonal-calving lactating dairy cows. J. Dairy. Sci. 2018, 101, 10526-10535.
26. *Rensis F. de, López-Gatius F.*: Use of equine chorionic gonadotropin to control reproduction of the dairy cow: a review. Reproduction in Domestic Animals 2014, 49, 177-182.
27. *Rigoglio N. N., Fátima L. A., Hanassaka J. Y., Pinto G. L., Machado A. S. D., Gimenes L. U., Baruselli P. S., Rennó F. P., Moura C. E. B., Watanabe I.-S., Papa P. C.*: Equine chorionic gonadotropin alters luteal cell morphologic features related to progesterone synthesis. Theriogenology 2013, 79, 673-679.
28. *Rowe S. M., Pryor L., Tranter W. P., Hosie J., Cavalieri J.*: Effect of equine chorionic gonadotropin on reproductive performance in a dairy herd in Northern Queensland, Australia. Theriogenology 2019, 125, 30-36.
29. *Sá Filho M. F., Ayres H., Ferreira R. M., Marques M. O., Reis E. L., Silva R. C. P., Rodrigues C. A., Madureira E. H., Bó G. A., Baruselli P. S.*: Equine chorionic gonadotropin and gonadotropin-releasing hormone enhance fertility in a norgestomet-based, timed artificial insemination protocol in suckled Nelore (Bos indicus) cows. Theriogenology 2010, 73, 651-658.
30. *Sales J. N. S., Crepaldi G. A., Girotto R. W., Souza A. H., Baruselli P. S.*: Fixed-time AI protocols replacing eCG with a single dose of FSH were less effective in stimulating follicular growth, ovulation, and fertility in suckled-anestrus Nelore beef cows. Anim. Reprod. Sci. 2011, 124, 12-18.
31. *Sangsritavong S., Combs D. K., Sartori R., Armentano L. E., Wiltbank M. C.*: High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17β in dairy cattle. J. Dairy. Sci. 2002, 85, 2831-2842.
32. *Sartori R., Haughian J. M., Shaver R. D., Rosa G. J. M., Wiltbank M. C.*: Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows. J. Dairy. Sci. 2004, 87, 905-920.
33. *Shephard R.*: Efficacy of inclusion of equine chorionic gonadotrophin into a treatment protocol for anoestrous dairy cows. N. Z. Vet. J. 2013, 61, 330-336.
34. *Souza A. H., Viechnieski S., Lima F. A., Silva F. F., Araújo R., Bó G. A., Wiltbank M. C., Baruselli P. S.*: Effects of equine chorionic gonadotropin and type of ovulatory stimulus in a timed-AI protocol on reproductive responses in dairy cows. Theriogenology 2009, 72, 10-21.
35. *Wiltbank M., Lopez H., Sartori R., Sangsritavong S., Gümen A.*: Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology 2006, 65, 17-29.

Corresponding author: Murat Can Demir, Kafkas University, Faculty of Veterinary, Obstetrics and Gynecology Department, 36100, Kars, Turkiye; e-mail: murat.can.demir@hotmail.com