

## EFFECT OF TREATMENT WITH GnRH OR HCG ON DAY 5 AFTER ARTIFICIAL INSEMINATION ON LUTEAL ACTIVITY OF DAIRY COWS

Amine Abdelli<sup>1,a</sup>, Mohamed Besbaci<sup>2,b\*</sup>, Aldjia Benabdelaziz<sup>3</sup>, Rachid Khelili<sup>3</sup>,  
Ibrahim Belabdi<sup>2</sup>, Mounir Mebarki<sup>4</sup>, Rachid Kaidi<sup>2</sup>

<sup>1</sup>Department of Agricultural Sciences, University of Bouira, Bouira, Algeria

<sup>2</sup>Laboratory of Biotechnology in Animal Reproduction (LBRA), Institute of Veterinary Sciences,  
University of Blida 1, Blida, Algeria








<sup>3</sup>Nuclear Research Center of Draria (CRND), Algiers, Algeria

<sup>4</sup>Department of Veterinary Science, University of Batna, Batna, Algeria.

\*Corresponding Author:

E-mail: [besbaci@univ-blida.dz](mailto:besbaci@univ-blida.dz)

(Received 26<sup>th</sup> May 2020; accepted 10<sup>th</sup> August 2020)

a:  ORCID 0000-0001-9977-1232, b:  ORCID 0000-0002-7934-6359, c:  ORCID 0000-0002-0349-6625,  
d:  ORCID 0000-0002-0938-6208, e:  ORCID 0000-0001-7220-3809, f:  ORCID 0000-0002-7744-4094,  
g:  ORCID 0000-0002-7895-2103

**ABSTRACT.** The objective of this study was to compare the effect of human chorionic gonadotrophin (hCG) and gonadotropin releasing hormone (GnRH) administration on day 5 after artificial insemination (AI) on luteal function. Thirty-three dairy cows were synchronized by the Ovsynch protocol and assigned randomly into 3 equal groups to receive on day 5 after AI 1500UI hCG, 100µg GnRH, and 2mL saline solution (Control), respectively. progesterone concentration (P4) in blood was measured every 3 days from day 5 to 23 after timed AI. Ultrasound examination of ovaries was performed on days -10, 0 (day of AI), 2, 5, 8, 11 and 14. The results revealed a development of an accessory corpus luteum (CL), 100% in the group received GnRH and 90.9% in that received hCG. No accessory corpus luteum in control group was observed. Total luteal tissue area on the ovaries was increased in hCG and GnRH group compared to control. Plasma P4 concentration was significantly ( $P < 0.05$ ) higher in hCG and GnRH groups than control after day 11 AI. The comparison treatments showed that total luteum tissue area and P4 concentration were lower in GnRH group than in hCG one.

**Key words:** *Accessory corpus luteum; Follicle; Progesterone (P4); Gonadotropin.*

### INTRODUCTION

Numerous studies [1,2] have reported that reproductive performance of dairy cows has declined as milk yields have increased. Some of these reports suggest that, at least in high-yielding cows, the fall in fertility may be due to an increment of both early [3] and late [4] embryonic loss. Many factors can induce the loss of embryo, but the most important component seems to be the insufficiency of maternal luteal function, expressed by a decrease in blood P4 [5]. In addition, the enhancement of P4 catabolism in the liver, particularly in dairy cows, is also responsible of decreasing of P4 concentration [6]. Subsequent studies have, indeed, emphasized the important role of P4 in the first week after insemination in establishing an optimum uterine environment to support conceptus elongation around the time of maternal recognition of pregnancy [7]. Consequently, several approaches have been used to increase the concentration of P4 in blood in order

to reduce the occurrence of embryo death [8]. Increased plasma P4 concentration has been carried out to improve cow fertility either by using administering exogenous P4 supplements, using a controlled internal drug-release (CIDR) [9], P4 releasing intravaginal device (PRID) [10] and feeding the animal P4 [9] or by increasing its endogenous secretion [8]. Thus, studies have shown that administration of GnRH [11] or hCG [12,13] after AI can induce accessory corpus luteum formation which in turn enhances P4 secretion. Using a large number of animals, Stevenson et al. [14] assessed the effects of a variety of interventions between day 4 and day 9 after AI on fertility, including administration of GnRH and hCG. Interestingly, in this comparative study, the conception rate in GnRH-treated cows was lower by compared to hCG and control groups. This difference was attributed by the authors to the dose of GnRH and its timing of administration that can alter luteal composition and subsequent serum concentrations of P4. Howard et al. [11] found that administration of GnRH during the early luteal phase (i.e. day 5 of the estrous cycle) induces ovulation of the first wave dominant follicle and formation of a functional accessory CL, which increases circulating P4 in blood. Based on the the findings of the above studies [11,14,15], the objective of this work was to compare the effects of early administration (day 5 of the estrous cycle) of GnRH and hCG on CL development and P4 secretion, in dairy cows.

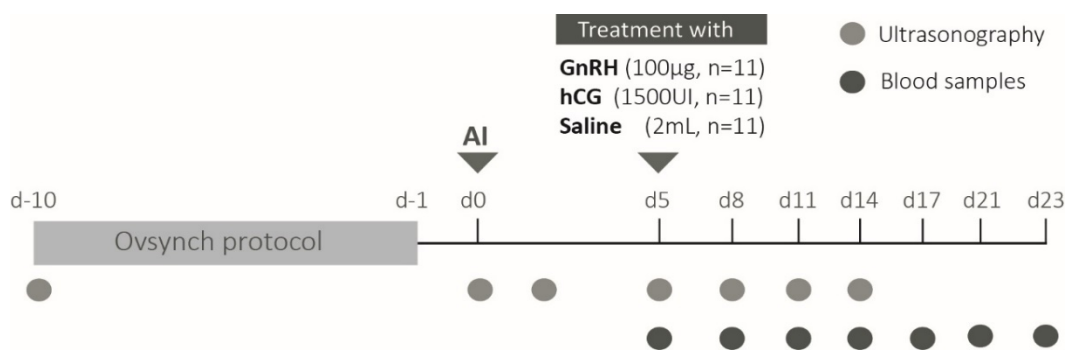
## MATERIAL AND METHODS

The protocols and procedures applied in this study were according to the ethical principles for use of experimental animals as established by the Institutional Animal Care Committee of the National Administration of the Algerian Higher Education and Scientific Research (Ethical approval number: 98-11, Law of August 22, 1998).

### *Animals and treatments*

Thirty-three primiparous lactating Holstein cows (2-3 years old; 45 to 80 day in milk; an average milk yield of  $27.65 \pm 1.6$  kg/day) were used in this study, from of April to August. Animals were housed in free-stall barns at a commercial dairy farm in Blida, Algeria ( $36.4736^\circ$  N,  $2.8323^\circ$  E, and altitude 63 m). Cows were selected by clinical examination (rectal palpation) of both the ovaries to confirm the cyclicity and the reproductive tract to find any abnormalities, such as vaginal discharge, before AI and only those in a healthy reproductive status were included in the study.

Cows were fed twice daily a diet consisting of grass and clover supplemented with a commercial concentrate (18% of crude protein), as well as roughly crushed maize grains, soybean meal, barley and vitamin–mineral mixture. All cows were synchronized by Ovsynch protocol initiated with Gonadorelin (Cystoreline<sup>®</sup> CEVA, France) followed, seven days later, by the administration of PGF2 $\alpha$  analogue (Enzaprost<sup>®</sup> CEVA, France) and two days after this, by a second Gonadorelin injection. Animals were inseminated 12–16 h after the second injection of Gonadorelin (day 0). Five days after AI (day 5), cows were assigned randomly to one of the three treatment groups (eleven cows per treatment group): 1) injection of 1500UI hCG (Chorulon<sup>®</sup> Intevet given i.v), 2) 100 $\mu$ g GnRH (Cystoreline<sup>®</sup> CEVA given i.m) and 2mL of 0.9% saline as control group “Fig. 1”.



**Fig. 1.** Schematic diagram of treatment protocol in which GnRH, hCG or saline was given to lactating dairy cows 5 days after AI. All cows received the Ovsynch protocol and were sham AI 12–16 h after the second injection of GnRH. Blood samples were collected every three days (from d 5 to d 23), and ultrasonography was used on days - 10, 0, 1, 5, 8, 11, and 14 (AI: artificial insemination; d: day).

### Blood sampling and P4 measurement

For determination progesterone concentration, five milliliters of blood of each animal were collected, immediately before administration of treatments and every 3 days from day 5 to day 23 after timed AI. Blood samples were recovery from the caudal vein into 5mL evacuated tubes (Vacuette® blood collection tubes) every 3 days from day 5 to day 23 after timed AI (TAI) for analysis of serum P4. Samples were chilled on ice packs immediately after collection and centrifuged at  $1,935\times g$  for 15 min within 4 h of collection, and the serum was stored at  $-20^{\circ}\text{C}$  until analysis. Serum P4 concentrations were measured using a solid-phase, no-extraction radioimmunoassay (Coat-a-Count P4 kit, Diagnostic Products Corp., Los Angeles, CA) in Nuclear Research Center, Algiers, Algeria. The sensitivity of the assay was  $0.1\text{ ng mL}^{-1}$ . The inter- and intra-assay CV were 7.3 and 7.4%, respectively.

### Ultrasonography

All cows were scanned by transrectal ultrasonography (5 mHz transrectal probe, Chison 600 VET, Tokyo, Japan). Ovarian structures were mapped and sized on day 10 before AI and 0, 1, 5, 8, 11 and 14 after AI “Fig. 1”. Cows were considered to have synchronized ovulation when 1 or more follicles  $\geq 10\text{ mm}$  were observed at the time of AI by ultrasound examination and then disappeared at 1 or 2 days later. Principal CL was the result of the first ovulation, accessory CL was the result of the second and the total CL area was the addition of principal CL and accessory CL area. Pregnancy rate was realized 45 d after AI by transrectal ultrasonography. All ultrasound examinations and measurements were performed by the same operator.

### Statistical analysis

All statistical analyses were carried out using SAS 9.1 (SAS Institute, Cary, NC, USA). A repeated measures approach using ANOVA with mixed linear models (fixed effects of treatment, day and their interaction, random effect of cow) was used for P4 concentrations and CL measurements. All the resulting data was checked for normality using the PROC UNIVARIATE of SAS. If the variable did not fit the normal distribution, it was transformed by rising to the power of lambda. The appropriate lambda value was obtained by conducting a Box–Cox transformation analysis using the TRANSREG procedure of

SAS. Conception rate and diameter of follicles were analyzed by using ANOVA (procedure GLM; SAS). For clarity, corresponding means  $\pm$  SEM of the non-transformed data are presented in the results. Stacked line plots of the studied variables were generated using Prism 6.07 (GraphPad Software, Inc. La Jolla, CA USA).

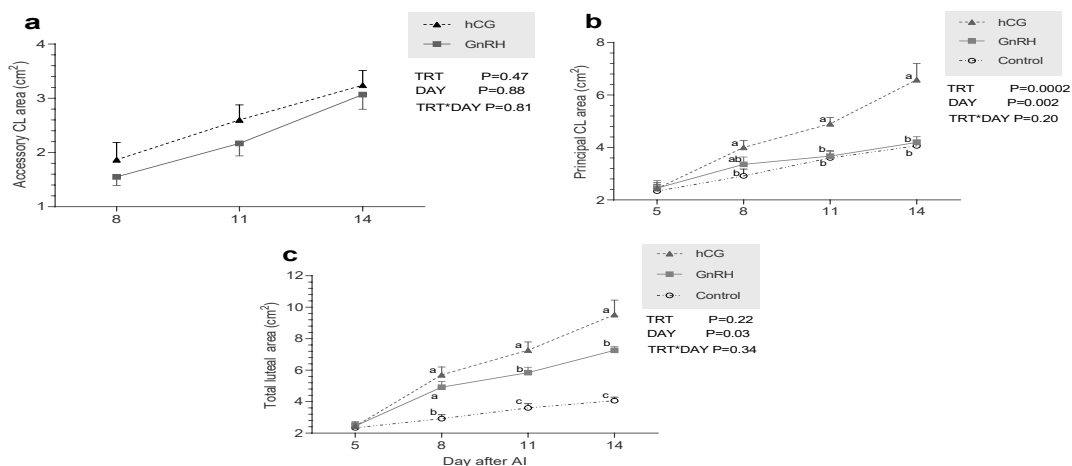
## RESULTS

### *Ovarian status of cows at day 5 after AI*

The ultrasound examination performed on day 5 after AI, just before treatment, revealed the presence of CL and dominant follicle. Mean diameter of dominant follicles did not differ significantly ( $p>0.05$ ) between GnRH, hCG and control groups ( $11.91\pm 1.9\text{mm}$ ;  $12.84\pm 1.34\text{ mm}$  and  $12.37\pm 1.6\text{mm}$  respectively).

### *Corpora Lutea*

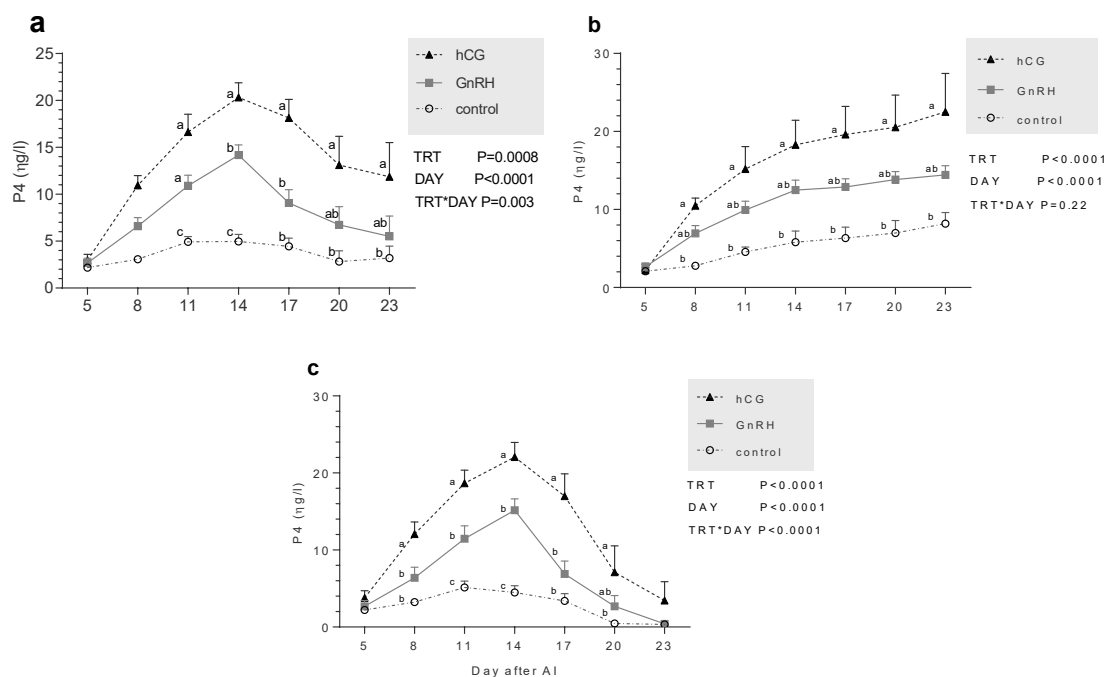
Treatment at day 5 after AI induced ovulation of the dominant follicle and formation of an accessory CL in all cows treated with GnRH, 90.9% (10/11) in cows treated with hCG and there are no ovulation and accessory CL formation in the control group. The ultrasonography detection of accessory CL area was very difficult, since the accessory CL was in growth; the clearest ultrasound images and most accurate measurements of accessory CL area were obtained at day 14 after AI. Accessory CL area induced by GnRH and hCG treatment showed no significant change neither over the time or between treatment groups, nor a group-by time interaction “Fig. 2.a”. Principal CL areas were not significantly influenced by GnRH treatment but they tended to be greater for cows treated with hCG comparing with control and GnRH-group after day 8 and day 11 relative to insemination, respectively “Fig. 2.b”. Further, the repeated measures ANOVA revealed, here too, a significant effect of hCG and GnRH treatments on total CL areas after day 5. Total CL areas tended to be greater for hCG-treated cows than for GnRH-treated cows after day 8 after AI “Fig. 2.c”. There were significant effects of measurement time on principal CL areas ( $p=0.0002$ ) and total CL areas ( $p=0.03$ ) but there was no effect of treatment or a group-by time interaction.



**Fig. 2.** Mean ( $\pm$  SEM) area of luteal tissue ( $\text{cm}^2$ ) of control cows, hCG (1500 IU) treated cows and GnRH (100 $\mu\text{g}$ ) treated cows: (a) accessory corpus luteum area; (b) principal corpus luteum area; (c) total area of luteal tissue (accessory corpus luteum+ principal corpus luteum).

**P4 concentration**

All cows (pregnant and non-pregnant) treated with hCG tended to have increased circulating P4 concentrations from day 11 to day 23 after AI, relative to control cows and on day 14 and day 17, relative to GnRH-treated cows “Fig. 3.a”. Cows treated with GnRH tended to have increased circulating P4 concentrations on day 11 and day 14, relative to control cows. Among pregnant animals, hCG-treated cows had higher concentrations of plasma P4 from day 8 to day 23 after AI, relative to controls; plasma P4 concentrations of GnRH- treated pregnant cows from day 8 to day 23 after AI did not differ significantly compared to control and hCG-treated groups “Fig. 3.b”. Among non-pregnant animals, GnRH-treated cows “Fig. 3.c” tended to have decreased circulating P4 concentrations at day 8 and day 17 relative to hCG-treated group. An effect of group-by-time interaction was detected in all cows ( $p < 0.01$ ) and in non-pregnant cows ( $p < 0.0001$ ), but not in pregnant cows ( $p = 0.22$ ).



**Fig. 3.** Mean ( $\pm$  SEM) progesterone concentration in blood serum (ng/mL) of (a) all cows (b) pregnant cows and (c) not pregnant cows.

**Pregnancy rate**

The pregnancy rate was 45.45% (5/11) in either control or GnRH-treated cows. However, this rate was 54.54% (6/11) in hCG-treated cows. There was no significant difference ( $p > 0.05$ ) between groups.

**DISCUSSION**

The present study, investigated whether treatment with GnRH on day 5 after AI could be as effective as hCG treatment by increasing P4 concentrations, in Holstein cows.

Increasing P4 concentrations is attributable to stimulating the steroidogenesis from the existing corpus luteum and/or by induced ovulation of the dominant follicle present at the time of treatment. Early treatment with hCG [16] and with GnRH [11] has proved to be more effective compared with late treatment; both hCG and GnRH injections given on day 5 of the estrous cycle consistently induced ovulation of the dominant follicle giving accessory CL. In the present study all cows treated with GnRH at day 5 post-AI had an accessory CL (100%) which confirms the previous observation of Schmitt et al. [17] and is different to that of Stevenson et al. [14], which found that 60% of the cows had formed accessory CL after treatment with GnRH between day 4 and day 9 after AI. Similarly, in the present study, 90.9% cows treated with hCG at day 5 post-AI formed an accessory CL.

The type of hormone (hCG or GnRH) injected 5 days after AI did not influence the size of the accessory CL ( $p=0.48$ ). However, principal CL area was significantly larger in hCG group compared to GnRH and control groups. No significant difference between GnRH group and control was observed. The results of the current study were similar to those reported Stevenson et al. [14] and Rizos et al. [13]. This finding may be, also, explained by the reduced effectiveness of hCG given during the late luteal stage of the estrous cycle [18]. The increase of luteal tissue after treatment with hCG arises from the LH-like action of hCG on luteal cells [17]. In the present study, administration of hCG not only resulted in ovulation of the dominant follicle present on day 5 and the formation of an accessory CL, but also stimulated the increase of principal CL area which was larger than in GnRH-treated animals. The area of principal CL in cows treated with GnRH on day 5 did not differ significantly from that of control cows ( $p>0.05$ ). Regardless of treatment period, the LH-like effect of hCG on the ovarian cells may last for 30 h after treatment [17]; in contrast, the LH-like effect of GnRH on the ovarian cells may last only for 5 h after treatment [19]. Therefore, the longer half-life of hCG in the blood and slower turnover of the hCG-LH receptor complex on the surface of granulosa cells is possibly responsible for an increased gonadotropic stimulation on the day 5 ovulatory follicle (for review see De Rensis et al. [18]; Lonergan. [20]) and its subsequent differentiation into a principal CL.

In the current study, injection of hCG on day 5 increased plasma concentration of P4 compared with that of control or GnRH treated cows. Several authors [11,12,14] detected higher (than control) P4 concentration in dairy cows after induction of an accessory CL with GnRH or hCG, administered between day 4 and 7 after AI. It is worth mentioning that the stimulation of P4 secretion by the principal CL after treatment with hCG was higher compared to GnRH treatment. This latter finding is supported by the observation that principal CL area in hCG-treated cows was larger compared with GnRH-treated cows and a strong positive and close correlation was observed between the total luteal area and the P4 level in the current study and in previous studies [21,22]. Indeed, Mann. [23] showed that P4 level is related to the diameter of the CL that secretes P4 until it reaches its final size.

The main objective of administration of GnRH or hCG is to improve the fertility of cows by increasing P4 concentration [12–14]. The results from the present study showed no significant difference between P4 concentration of hCG-treated pregnant cows and GnRH-treated pregnant cows. However, in non-pregnant cows, there was significant

difference between P4 concentration of hCG-treated cows and GnRH-treated cows and between GnRH-treated cows and control cows. A lack of effect of treatment to improve pregnancy rate, despite the increase of P4 production in treated cows, may be due to other factors than P4 concentrations. Thus, fertility after GnRH or hCG administration has been reported to be related to many factors [18,24].

## CONCLUSION

Treatment of lactating dairy cows with GnRH or hCG on day 5 after AI effectively induced accessory CL formation and increased total CL area. However, treatment with GnRH on day 5 post-insemination failed to increase principal CL area. hCG treatment at day 5 after AI tended to be more effective than GnRH for increasing P4 production.

## DECLARATION OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this article.

**Acknowledgements.** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## REFERENCES

- [1] Lucy, M.C. (2001): Reproductive Loss in High-Producing Dairy Cattle: Where Will It End? *Journal of Dairy Science*. 84 (6): 1277–1293.
- [2] Bello, N.M., Stevenson, J.S., and Tempelman, R.J. (2012) Invited review: Milk production and reproductive performance: Modern interdisciplinary insights into an enduring axiom. *Journal of Dairy Science*. 95 (10): 5461–5475.
- [3] Van Hoeck, V., Sturmey, R.G., Bermejo-Alvarez, P., Rizos, D., Gutierrez-Adan, A., Leese, H.J., et al. (2011): Elevated non-esterified fatty acid concentrations during bovine oocyte maturation compromise early embryo physiology. *PloS One*. 6 (8).
- [4] Pereira, M.H.C., Wiltbank, M.C., and Vasconcelos, J.L.M. (2016): Expression of estrus improves fertility and decreases pregnancy losses in lactating dairy cows that receive artificial insemination or embryo transfer. *Journal of Dairy Science*. 99 (3): 2237–2247.
- [5] Mann, G.E., Payne, J.H., and Lamming, G.E. (2001): Hormonal regulation of oxytocin-induced prostaglandin F<sub>2</sub> $\alpha$  secretion by the bovine and ovine uterus in vivo. *Domestic Animal Endocrinology*. 21 (2): 127–141.
- [6] Wiltbank, M., Lopez, H., Sartori, R., Sangsritavong, S., and Gümen, A. (2006): Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. *Theriogenology*. 65 (1): 17–29.
- [7] Spencer, T.E., Forde, N., and Lonergan, P. (2016): The role of progesterone and conceptus-derived factors in uterine biology during early pregnancy in ruminants<sup>1</sup>. *Journal of Dairy Science*. 99 (7).
- [8] Campanile, G., Di Palo, R., Neglia, G., Vecchio, D., Gasparrini, B., Prandi, A., et al. (2007): Corpus luteum function and embryonic mortality in buffaloes treated with a GnRH agonist, hCG and progesterone. *Theriogenology*. 67 (8): 1393–1398.
- [9] Tauck, S.A., Wilkinson, J.R.C., Olsen, J.R., Janitell, J.N., and Berardinelli, J.G. (2007): Comparison of controlled internal drug release device and melengesterol acetate as progestin sources in an estrous synchronization protocol for beef heifers. *Theriogenology*. 68 (2): 162–167.
- [10] Kuroiwak, T., Ishibashi, A.İ., Fukuda, M., Kim, S., Tanaka, T., and Kamomae, H. (2005): Estrus synchronization and conception rate after a progesterone releasing intravaginal

- device (PRID) treatment from the early luteal phase in heifers. *Journal of Reproduction and Development*. 507250023.
- [11] Howard, J.M., Manzo, R., Dalton, J.C., Frago, F., and Ahmadzadeh, A. (2006): Conception rates and serum progesterone concentration in dairy cattle administered gonadotropin releasing hormone 5 days after artificial insemination. *Animal Reproduction Science*. 95 (3–4): 224–233.
- [12] Santos, J.E.P., Thatcher, W.W., Pool, L., and Overton, M.W. (2001): Effect of human chorionic gonadotropin on luteal function and reproductive performance of high-producing lactating Holstein dairy cows. *Journal of Animal Science*. 79 (11): 2881–2894.
- [13] Rizos, D., Scully, S., Kelly, A.K., Ealy, A.D., Moros, R., Duffy, P., et al. (2012): Effects of human chorionic gonadotrophin administration on Day 5 after oestrus on corpus luteum characteristics, circulating progesterone and conceptus elongation in cattle. *Reproduction, Fertility and Development*. 24 (3): 472.
- [14] Stevenson, J.S., Portaluppi, M.A., Tenhouse, D.E., Lloyd, A., Eborn, D.R., Kacuba, S., et al. (2007): Interventions after artificial insemination: conception rates, pregnancy survival, and ovarian responses to gonadotropin-releasing hormone, human chorionic gonadotropin, and progesterone. *Journal of Dairy Science*. 90 (1): 331–340.
- [15] Besbaci, M., Abdelli, A., Belabdi, I., Benabdelaziz, A., Khelili, R., Mebarki, M., et al. (2018): Effects of GnRH or hCG on day 11 after artificial insemination in cows luteal activity. *Journal of the Hellenic Veterinary Medical Society*. 69 (4): 1227–1234.
- [16] Price, C.A. and Webb, R. (1989): Ovarian response to hCG treatment during the oestrous cycle in heifers. *Reproduction*. 86 (1): 303–308.
- [17] Schmitt, É.J.P., Barros, C.M., Fields, P.A., Fields, M.J., Diaz, T., Kluge, J.M., et al. (1996): A Cellular and Endocrine Characterization of the Original and Induced Corpus Luteum after Administration of a Gonadotropin-Releasing Hormone Agonist or Human Chorionic Gonadotropin on Day Five of the Estrous Cycle. *Journal of Animal Science*. 74 (8): 1915–1929.
- [18] De Rensis, F., López-Gatiús, F., García-Ispuerto, I., and Techakumpu, M. (2010): Clinical use of human chorionic gonadotropin in dairy cows: An update. *Theriogenology*. 73 (8): 1001–1008.
- [19] Chenault, J.R., Kratzer, D.D., Rzepkowski, R.A., and Goodwin, M.C. (1990): LH and FSH response of Holstein heifers to fertirelin acetate, gonadorelin and buserelin. *Theriogenology*. 34 (1): 81–98.
- [20] Lonergan, P. (2011): Influence of progesterone on oocyte quality and embryo development in cows. *Theriogenology*. 76 (9): 1594–1601.
- [21] Herzog, K., Brockhan-Lüdemann, M., Kaske, M., Beindorff, N., Paul, V., Niemann, H., et al. (2010): Luteal blood flow is a more appropriate indicator for luteal function during the bovine estrous cycle than luteal size. *Theriogenology*. 73 (5): 691–697.
- [22] Ferrouk, M., Boukenaoui-Ferrouk, N., Gharbi, I., Baaziz-Amami, D., Adel, D., Lafri, M., et al. (2019): Corpus luteum size and plasma progesterone concentration during the estrous cycle in cheurfa cow of Algeria. *Agricultura*. 111 (3–4).
- [23] Mann, G.E. (2009): Corpus luteum size and plasma progesterone concentration in cows. *Animal Reproduction Science*. 115 (1–4): 296–299.
- [24] Besbaci, M., Abdelli, A., Minviel, J.J., Belabdi, I., Kaidi, R., and Raboisson, D. (2020): Association of pregnancy per artificial insemination with gonadotropin-releasing hormone and human chorionic gonadotropin administered during the luteal phase after artificial insemination in dairy cows: A meta-analysis. *Journal of Dairy Science*. 103 (2): 2006–2018.