The Effect of A PRID®Delta-Based Estrus Synchronization Protocol On Pregnancy Rates In Cows With No Estrus Symptoms

Murat Can Demir (murat_can_demir@hotmail.com)
Faculty of Veterinary, University of Kafkas https://orcid.org/0000-0002-3803-069X

Cihan Kaçar
Faculty of Veterinary, University of Kafkas

Umut Çağın Arı
Faculty of University of Kafkas

Semra Kaya
Faculty of Veterinary, University of Kafkas

Oğuz Merhan
Faculty of Veterinary, University of Kafkas

Mushap Kuru
Faculty of Veterinary, University of Kafkas

Savaş Yıldız
Faculty of University of Kafkas

Research Article

Keywords: cow, estrus, anestrus, progesterone, eCG

Posted Date: January 14th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1199382/v1

Abstract

The present study aimed to determine the effects of progesterone-based gonadotropin-releasing hormone (GnRH), prostaglandin F2α (PGF2α), and equine chorionic gonadotropin (eCG) injections on progesterone profiles and pregnancy rates in cows with no estrus symptoms within 60 days after parturition. A total of 80 cows were included in the study. All animals had the progesterone-releasing device PRID®Delta placed intravaginally for nine days with an injection of GnRH. On the eighth day, PGF2α was injected, and PRID®Delta was removed from the vagina on day nine. Artificial insemination was carried out 60 hours after PRID®Delta removal. In half of the animals (n = 40), 600 IU of eCG was injected when PRID®Delta was removed on the ninth day before artificial insemination 60 hours later. Blood samples were taken from the tail vein on days 0 and 8 to determine progesterone levels. The pregnancy rate in the group that received eCG was 37.5%, while it was 27.5% in those that did not (P = 0.4). While the dominant follicle diameter was 15.5 mm in cows injected with eCG during timed artificial insemination, the follicle diameter was 12.4 mm in cows with no eCG injection (P <0.001). There were no differences in serum progesterone values in blood samples taken until the time of artificial insemination. However, progesterone values in the blood taken during artificial insemination were 0.94 ng/ml in the eCG- group and 0.72 ng/ml in the eCG+ group (P <0.05). As a result, it was determined that eCG injections, in addition to progesterone-based GnRH and PGF2α applications, increased the pregnancy rates in cows without symptoms of estrus. The dominant follicle diameter was larger in cows treated with eCG during artificial insemination; however, follicle size did not increase the pregnancy rate.

Introduction

Due to the increase in milk yield in cows in recent years, observing estrus symptoms has been challenging. Accordingly, pregnancy rates at the first insemination have decreased to 35%. Observing the symptoms of estrus and applying artificial insemination on time are essential to increasing fertility. Due to the difficulties in determining estrus symptoms, timed artificial insemination protocols have been developed.

Research has shown that many factors affect ovarian functions in cows in the postpartum period. These factors include inadequate food intake in the pre and postpartum periods (Baruselli et al., 2004, Opsomer et al., 2000), a negative energy balance, and low body reserve in connection with high milk yield and feed intake in the early postpartum period (Leroy et al., 2008; Beam and Butler, 1999; Opsomer et al., 2000). The high metabolic clearance rate of steroid hormones (Wiltbank et al., 2006), the lower circulating concentrations of progesterone and estradiol in lactating cows compared to non-lactating cows (Sartori et al., 2004; Sangsritavong et al., 2002), and low luteinizing hormone (LH) secretion due to stress and malnutrition (Butler and Smith, 1989) are also influential. Ovulation is delayed due to the decrease in estrogen production of the dominant follicle due to the inhibition of LH release frequency and suppression of blood glucose, insulin, and insulin-like growth factor-1 (IGF-1) concentrations in cows (Butler, 2000).

It is ideal to have a minimum negative energy balance in the postpartum period. The duration of postpartum anestrus is determined not by the emergence of follicular waves but by follicular deviation and the dominant follicle. Under optimum conditions, follicular growth is a cycle formed by the selection of a dominant follicle, maturation of the follicle, luteolysis, and resumption of ovarian activity, shaping pregnancy. Failure of any of these processes prolongs postpartum anestrus. Cows that do not exhibit any estrus symptoms or have mild estrus symptoms can have an increased incidence of anestrus (Peter et al., 2009). Different hormone combinations have been used in cows with postpartum anestrus, including gonadotropin-releasing hormone (GnRH) with the Ovsynch protocol (Kacar et al., 2006; Bartolome et al., 2005), and progesterone-estradiol-prostaglandin F2α (PGF2α)-equine chorionic gonadotropin (eCG) (Núñez-Olivera et al., 2014; Barreiros et al., 2014; Sales et al., 2011). In timed artificial insemination in cows, follicle development (Prata et al., 2017; Dorneles Tortorella et al., 2013), ovulation (Prata et al., 2017; Núñez-Olivera et al., 2014), and pregnancy rate have been reported to increase (Dorneles Tortorella et al., 2013; Sá Filho et al., 2010).

The present study aimed to determine the effects of progesterone (PRID®Delta)-based GnRH, PGF2α, and eCG injections on progesterone profiles and pregnancy rates in cows with no estrus symptoms within 60 days after birth.

Materials And Methods

Animals

The present study was conducted at the Kafkas University Veterinary Faculty Ali Rıza AKSOY Research and Application Farm in Kars, Turkey, with 80 cows that did not show any estrus symptoms within two months after giving birth. Brown Swiss cows, housed in a closed barn system, fed a mixed ration (meadow grass, sainfoin, cattle milk feed, molasses) and with an average daily milk yield of 13-17 liters during lactation, were included in the study. Body condition scores (BCS) were evaluated using a 5-point grading system according to the definition given by Edmonson et al., (1989).

Protocol

Cows that did not show any estrus symptoms within two months after parturition were included in the study after their ovaries were examined by ultrasound (Draminski Iscan, Poland 7.5 MHz). The selected cows were randomly divided into two groups. The first group (n = 40) received 2 ml GnRH injected intramuscularly (Ovarelin®, Ceva, Istanbul, Turkey) on day 0, and the intravaginal progesterone-releasing device, PRID®Delta (1.55 g progesterone, Ceva, Istanbul, Turkey) was placed using an applicator after the lips of the vulva were cleaned with a cotton ball soaked in Baticonol antiseptic solution. The applicator was also cleaned with Baticonol and water before each application. Lubricating gel was applied, and PRID®Delta was placed into the vagina. On day 8, one day before the removal of PRID®Delta, 5 ml of PGF2α (Estrumate® RTM, Ceva, Istanbul, Turkey) was injected intramuscularly. PRID®Delta was removed from the vagina on the ninth day, and the cows were inseminated rectovaginally at the following 60th

hour. The second group of cows (n = 40) received 600 IU of eCG, intramuscularly (Oviser®, Hipra, Istanbul Turkey) upon removal of PRID®Delta, and artificial insemination was performed rectovaginally 60 hours later (Figure 1).

To determine the progesterone levels created by PRID®Delta, 5-ml blood samples were taken from the tail vein into EDTA-free tubes using vacuum injectors on day 0 (the day PRID®Delta was applied) and day 8 (just before the PGF2α injection). After disinfection, the procedures were carried out using disposable needles on appropriately restrained animals, which is the most practical and risk-free method for both the animal and practitioner.

Pregnancy diagnosis was made by ultrasound (Draminski Iscan, Poland 7.5 MHz) 30 ± 2 days after artificial insemination.

Obtaining the Blood Serum

The blood samples were centrifuged at 3000 rpm for 10 minutes, and the obtained sera were stored at -20^{0}C until analysis. Serum progesterone levels were measured at Kafkas University Faculty of Veterinary Medicine, Department of Biochemistry using commercial ELISA kits.

Statistical Analysis

Statistical analysis of the data was carried out using SPSS® software (SPSS 20, IL, USA). Normal distribution was assessed with the Kolmogorov–Smirnov test. Follicle diameters between the groups were compared using the Mann–Whitney U test. Pregnancy rates were compared using a chi-square analysis program. Changes in progesterone levels within the group were compared with the repeated measures ANOVA test. Comparison of progesterone levels between groups was made with the Student's t-test if parametric and with the Mann–Whitney U test if non-parametric. Results were analyzed as X±SE. Values with P <0.05 were considered statistically significant.

Results

In cows with no estrus symptoms, the pregnancy rate was 37.5% in the eCG+ group, while it was 27.5% in the eCG- group. Although there was a 10% difference between the two groups, it was not statistically significant (P = 0.4, Table 1). The dominant follicle diameter was 15.5 mm in eCG+ cows injected during timed artificial insemination and 12.4 mm in eCG- cows. Thus, the dominant follicle diameter in cows treated with eCG was much larger, and a statistical difference was found between the groups in terms of follicle size (P <0.001, Table 1).

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

Parameters	eCG -	eCG +	P
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

With the application of the progesterone-releasing intravaginal device (PRID®Delta), serum progesterone concentrations below 1 ng/ml increased above 1 ng/ml on days 8 and 9. No differences were determined in serum progesterone values in blood samples taken until the time of artificial insemination. However, the progesterone values in the serum taken during the artificial insemination application were 0.94 ng/ml in the eCG- group and 0.72 ng/ml in the eCG+ group. It was observed that the progesterone concentration during artificial insemination was much lower in the group in which the eCG was applied with a statistically significant difference (P <0.05) (Figure 2).

No differences were found in pregnancy rates in cows with BCS ≥ 2.75 or < 2.75 in either group. Similar results were found when eCG+ and eCG- groups were compared (Table 2).

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

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

Discussion

In P4/E2-based protocols, it was observed that eCG applications had positive effects on fertility in cows undergoing timed artificial insemination (Sá Filho, Ayres, et al., 2010; Dorneles Tortorella et al., 2013; Núñez-Olivera et al., 2014; Bilbao et al., 2015). It was thought that treatment with eCG would increase gonadotropin support in follicle development and thus increase the size of the dominant follicle and the possibility of ovulation, thereby increasing fertility in grazing purebred or crossbred beef and dairy cattle (Prata et al., 2017). It has been reported that eCG application had no significant effect on fertility in cyclic or anestrus Nelore cows (Barreiros et al., 2014). It has also been reported that Nelore heifers treated with

eCG had more ovarian response and pregnancy than heifers not treated with eCG (Sá Filho, Torres-Júnior, et al., 2010) Similarly, in Nelore cows in the early postpartum period undergoing progesterone-based timed artificial insemination with appropriate body condition, it was observed that eCG application and removal of the calf from the mother did not affect the pregnancy rate (Pinheiro et al., 2009). In addition, eCG treatment in the first synchronized estrus improved reproductive performance in the short term; however, it did not affect it after subsequent inseminations (Rowe et al., 2019). In another study, no significant differences were found between the follicular dynamics of cyclic and anestrus groups treated with TCR (56 hours breastfeeding) or eCG, despite the differences in the cyclicity of the animals. Follicular diameter, follicular growth rate, corpus luteum size, and P4 concentrations of anestrous cows treated with eCG or TCR were similar (Barreiros et al., 2014).

In the present study, the effect of eCG injection on pregnancy rates after intravaginal application with progesterone, GnRH, and PGF2α-based protocols and timed artificial insemination was evaluated in Brown Swiss cows. With the removal of the progesterone-releasing intravaginal device and application of eCG, development of the dominant follicle was accelerated, and ovulation was stimulated with the LH peak. According to the present study results, pregnancy rates were 10% higher in cows treated with eCG; however, no statistical differences were found. The higher pregnancy rate was associated with possible corpus luteum lysis with PGF2α injection one day before PRID®Delta removal and accelerated follicle development with eCG application. The research was conducted on cows that did not show any symptoms of estrus within two months postpartum. Cows exited the early postpartum period when the postpartum negative energy balance was established. In the early postpartum period, cows may have insufficient gonadotropin support, and therefore fertility may be positively affected in response to eCG treatment. After cows are out of negative energy balance in the early postpartum period, additional gonadotropin supplementation may not be required. Therefore positive effects of eCG may not be observed in cows after 70 days postpartum (Prata et al., 2017).

It has been reported that eCG application during the removal of the vaginal device in cows with anestrus increased the ovulation rate and improved luteal functions (Núñez-Olivera et al., 2014) and eGG administration can reduce differences in ovulation timing (Cavalieri et al., 1997). However, another study reported that ovulation rates did not increase after eCG (Pulley et al., 2013). The eCG treatment yields a larger corpus luteum and higher progesterone concentrations in cows that ovulate after synchronization (Núñez-Olivera et al., 2014). In the present study, it was seen that intravaginal devices containing progesterone could increase serum progesterone concentration in cows without estrus symptoms. For the cyclic activity to develop healthily and the follicular activity in the ovary to continue, the genital organs must be under the influence of progesterone for a certain period. The progesterone value was lower in cows treated with eCG during artificial insemination. The low level of progesterone leads to the peak of LH release and increases the likelihood of ovulation. This low concentration of progesterone may have caused the difference in pregnancy rates.

Long-term progesterone treatment did not increase the pregnancy rate in cows with high BCS; however, the combination of long-term progesterone and eCG treatment increased the pregnancy rate per

preovulatory follicle and artificial insemination in cows with low BCS (Bilbao et al., 2015). The effect of eCG applications 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 VKS, and it has been reported that this may lead to increased fertility in cows. It has been observed that cows with a BCS <2.75 have increased follicle growth and fertility with eCG treatment in those with insufficient LH support (Souza et al., 2009). In the present study, no differences were found in pregnancy rates of anestrus cows with or without eCG injection, with a VKS ≥ 2.75 or a VKS < 2.75. Different factors affect the anestrus mechanism in cows other than VKS. In lactating dairy cows under 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 pregnancy rate 48 hours after application. It was thought that the dose and timing of eCG treatment could be delayed to affect the maturation period of the preovulatory follicle (Pulley et al., 2013). One study has reported that eCG application did not increase the ovulation rate. However, in another study, it was found that follicle size, corpus luteum volume, serum P4 concentrations, and pregnancy rates were higher when eCG was applied two days before the removal of the intravaginal progesterone device rather than when eCG was applied during the removal of the device in cows undergoing timed artificial insemination. (Dorneles Tortorella et al., 2013). During the application of timed artificial insemination, the diameter of the largest follicle found in the ovaries was larger in heifers treated with eCG than in untreated heifers (Sá Filho, Ayres, et al., 2010). Follicle diameters of anestrous cows and cyclic animals treated with eCG were similar. It has been shown that cows with eCG or anestrus treated by removing calves from suckling for 56 hours showed follicular diameter and a follicular growth rate similar to cyclic cows (Barreiros et al., 2014). The addition of eCG to progesterone and estradiol-based treatments increased the ovulation rate of the dominant follicle in anestrous cows due to the stimulating effect of eCG on growth rate (Núñez-Olivera et al., 2014). The most important result obtained in the present study was that the follicle diameter at the time of artificial insemination was larger in cows that did not show signs of estrus with eCG injection compared to cows that were not injected with eCG. It was observed that this difference in follicle diameter did not affect pregnancy rates, however, it is thought that eCG application 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 the treated animals (Núñez-Olivera et al., 2014).

In conclusion, it was determined that progesterone (PRID®Delta)-based GnRH, PGF2α application in addition to eCG injections increased the pregnancy rate numerically in cows without symptoms of estrus. It was also determined that the largest follicle diameter was greater in cows treated with eCG during artificial insemination; however, follicle size did not increase the pregnancy rate.

Declarations

Author contribution The experiments were designed by Murat can DEMIR and Cihan KACAR. The experiments were performed by Murat Can DEMIR, Cihan KACAR, Umut Cağın ARI, Semra KAYA, Oğuz MERHAN, Mushap KURU and Savaş YILDIZ. The data were analyzed by Cihan KACAR, Oguz MERHAN and Mushap KURU. The paper was written by Murat Can DEMIR, Cihan KACAR, Semra KAYA and Savaş YILDIZ. All authors read and approved the manuscript.

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

Data availability Not applicable.

Ethics approval The ethics committee document for this research was provided from KAÜ-HADYEK with the number 2017/32.

Conflict of interest The authors declare no competing interests.

References

1. Barreiros, T.R.R., Blaschi, W., Santos, G.M.G., Morotti, F., Andrade, E.R., Baruselli, P.S. and Seneda, M.M., 2014. 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, 81, 651–656 (Elsevier)
2. Bartolome, J.A., Sozzi, A., McHale, J., Melendez, P., Arteche, A.C.M., Silvestre, F.T., Kelbert, D., Swift, K., Archbald, L.F. and Thatcher, W.W., 2005. 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, 63, 1628–1642 (Elsevier)
3. Baruselli, P.S., Reis, E.L., Marques, M.O., Nasser, L.F. and Bó, G.A., 2004. The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates In:, Animal Reproduction Science, (Elsevier)
4. Beam, S.W. and Butler, W.R., 1999. Effects of energy balance on follicular development and first ovulation in postpartum dairy cows.
5. Bilbao, M.G., Massara, N., Ramos, S., Zapata, L.O., Farcey, M.F., Pesoa, J., Turic, E., Vázquez, M.I. and Bartolome, J.A., 2015. 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, 86, 536–544 (Elsevier)
6. Butler, W.R., 2000. Nutritional interactions with reproductive performance in dairy cattle In:, Animal Reproduction Science, (Elsevier)
7. Butler, W.R. and Smith, R.D., 1989. Interrelationships Between Energy Balance and Postpartum Reproductive Function in Dairy Cattle Journal of Dairy Science, 72, 767–783 (Elsevier)

8. Cavalieri, J., Rubio, I., Kinder, J.E., Entwistle, K.W. and Fitzpatrick, L.A., 1997. Synchronization of estrus and ovulation and associated endocrine changes in Bos indicus cows Theriogenology, 47, 801–814 (Elsevier)
9. Dorneles Tortorella, R., Ferreira, R., Tonellotto dos Santos, J., Silveira de Andrade Neto, O., Barreta, M.H., Oliveira, J.F., Gonçalves, P.B. and Pereira Neves, J., 2013. 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, 79, 1204–1209 (Elsevier)
10. Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T. and Webster, G., 1989. A Body Condition Scoring Chart for Holstein Dairy Cows Journal of Dairy Science, 72, 68–78 (Elsevier)
11. Kacar, C., Yildiz, S., Pancarci, S.M., Kaya, M., Oral, H., Gurbulak, K. and Gungor, O., 2006. Administration of GnRH treatment prior to ovsynch protocol to stimulate ovarian cycle in cows with functional anoestrus Bulletin of the Veterinary Institute in Pulawy, 50, 497–501
12. Leroy, J.L.M.R., Vanholder, T., Van Knegsel, A.T.M., Garcia-Ispierto, I. and Bols, P.E.J., 2008. Nutrient Prioritization in Dairy Cows Early Postpartum: Mismatch Between Metabolism and Fertility? Reproduction in Domestic Animals, 43, 96–103 (John Wiley & Sons, Ltd)
13. Núñez-Olivera, R., De Castro, T., García-Pintos, C., Bó, G., Piaggio, J. and Menchaca, A., 2014. Ovulatory response and luteal function after eCG administration at the end of a progesterone and estradiol' based treatment in postpartum anestrous beef cattle Animal Reproduction Science, 146, 111–116 (Elsevier)
14. Opsomer, G., Gröhn, Y.T., Hertl, J., Coryn, M., Deluyker, H. and De Kruif, A., 2000. Risk factors for post partum ovarian dysfunction in high producing dairy cows in Belgium: A field study Theriogenology, 53, 841–857 (Elsevier)
15. Peter, A.T., Vos, P.L.A.M. and Ambrose, D.J., 2009. Postpartum anestrus in dairy cattle (Elsevier)
16. Pinheiro, V.G., Souza, A.F., Pegorer, M.F., Satrapa, R.A., Ereno, R.L., Trinca, L.A. and Barros, C.M., 2009. Effects of temporary calf removal and eCG on pregnancy rates to timed-insemination in progesterone-treated postpartum Nellore cows Theriogenology, 71, 519–524 (Elsevier)
17. Prata, A.B., Pontes, G.C.S., Monteiro, P.L.J., Drum, J.N., Wiltbank, M.C. and Sartori, R., 2017. Equine chorionic gonadotropin increases fertility of grazing dairy cows that receive fixed-time artificial insemination in the early but not later postpartum period Theriogenology, 98, 36–40 (Elsevier)
18. Pulley, S.L., Wallace, L.D., Mellieon, H.I. and Stevenson, J.S., 2013. Ovarian characteristics, serum concentrations of progesterone and estradiol, and fertility in lactating dairy cows in response to equine chorionic gonadotropin Theriogenology, 79, 127–134 (Elsevier)
19. Rowe, S.M., Pryor, L., Tranter, W.P., Hosie, J. and Cavalieri, J., 2019. Effect of equine chorionic gonadotropin on reproductive performance in a dairy herd in Northern Queensland, Australia Theriogenology, 125, 30–36 (Elsevier)
20. 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. and Baruselli, P.S., 2010. Equine chorionic gonadotropin and gonadotropin-

releasing hormone enhance fertility in a norgestomet-based, timed artificial insemination protocol in suckled Nelore (Bos indicus) cows Theriogenology, 73, 651–658 (Elsevier)

21. Sá Filho, M.F., Torres-Júnior, J.R.S., Penteado, L., Gimenes, L.U., Ferreira, R.M., Ayres, H., Castro e Paula, L.A., Sales, J.N.S. and Baruselli, P.S., 2010. Equine chorionic gonadotropin improves the efficacy of a progestin-based fixed-time artificial insemination protocol in Nelore (Bos indicus) heifers Animal Reproduction Science, 118, 182–187 (Elsevier)
22. Sales, J.N.S., Crepaldi, G.A., Girotto, R.W., Souza, A.H. and Baruselli, P.S., 2011. 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 Animal Reproduction Science, 124, 12–18 (Elsevier)
23. Sangsritavong, S., Combs, D.K., Sartori, R., Armentano, L.E. and Wiltbank, M.C., 2002. High feed intake increases liver blood flow and metabolism of progesterone and estradiol-17β in dairy cattle Journal of Dairy Science, 85, 2831–2842 (Elsevier)
24. Sartori, R., Haughian, J.M., Shaver, R.D., Rosa, G.J.M. and Wiltbank, M.C., 2004. Comparison of ovarian function and circulating steroids in estrous cycles of Holstein heifers and lactating cows Journal of Dairy Science, 87, 905–920 (Elsevier)
25. Souza, A.H., Viechnieski, S., Lima, F.A., Silva, F.F., Araújo, R., Bó, G.A., Wiltbank, M.C. and Baruselli, P.S., 2009. Effects of equine chorionic gonadotropin and type of ovulatory stimulus in a timed-AI protocol on reproductive responses in dairy cows Theriogenology, 72, 10–21 (Elsevier)
26. 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, 17–29 (Elsevier)

Figures

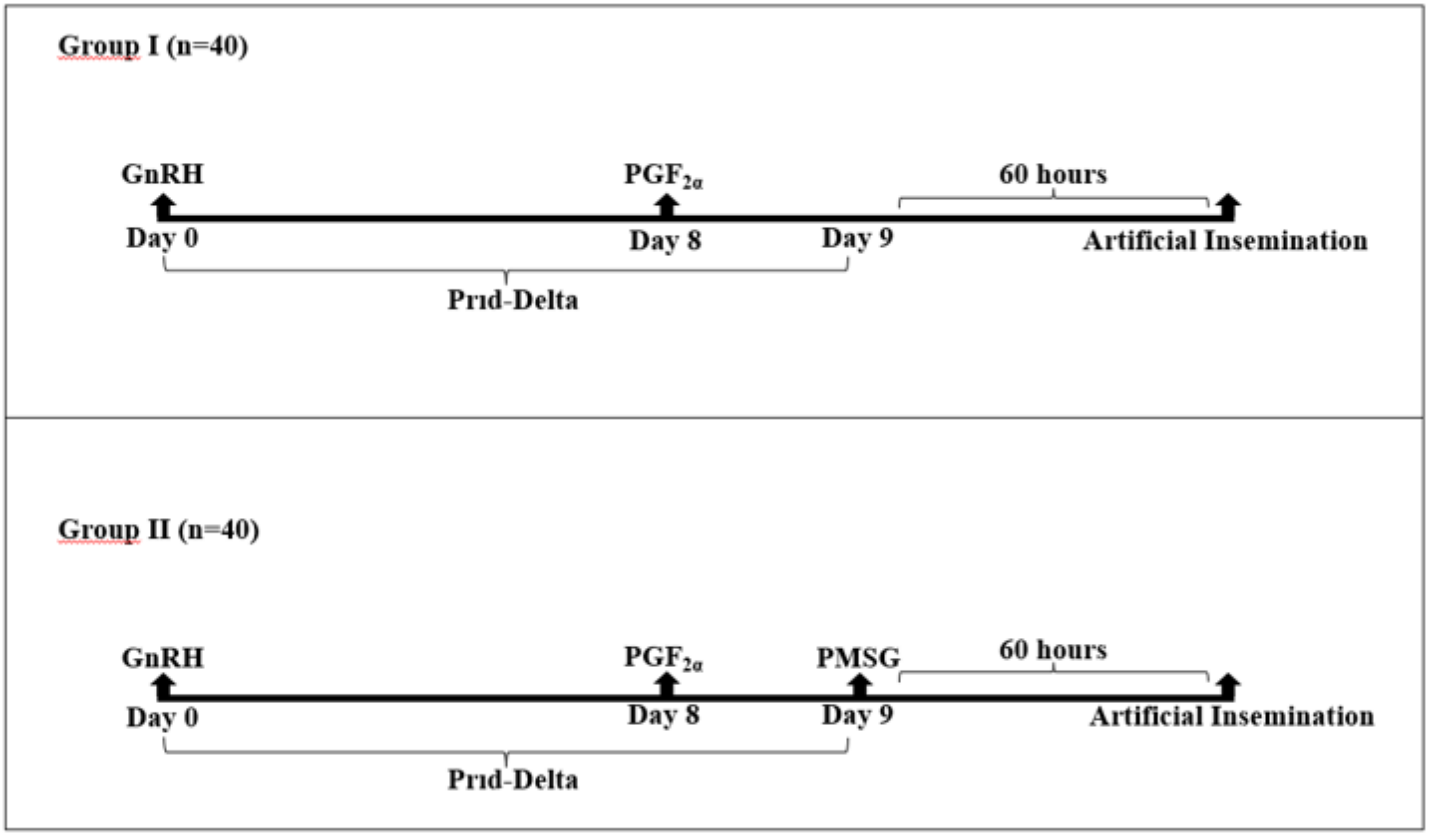

Figure 1

Hormone application protocol prior to artificial insemination

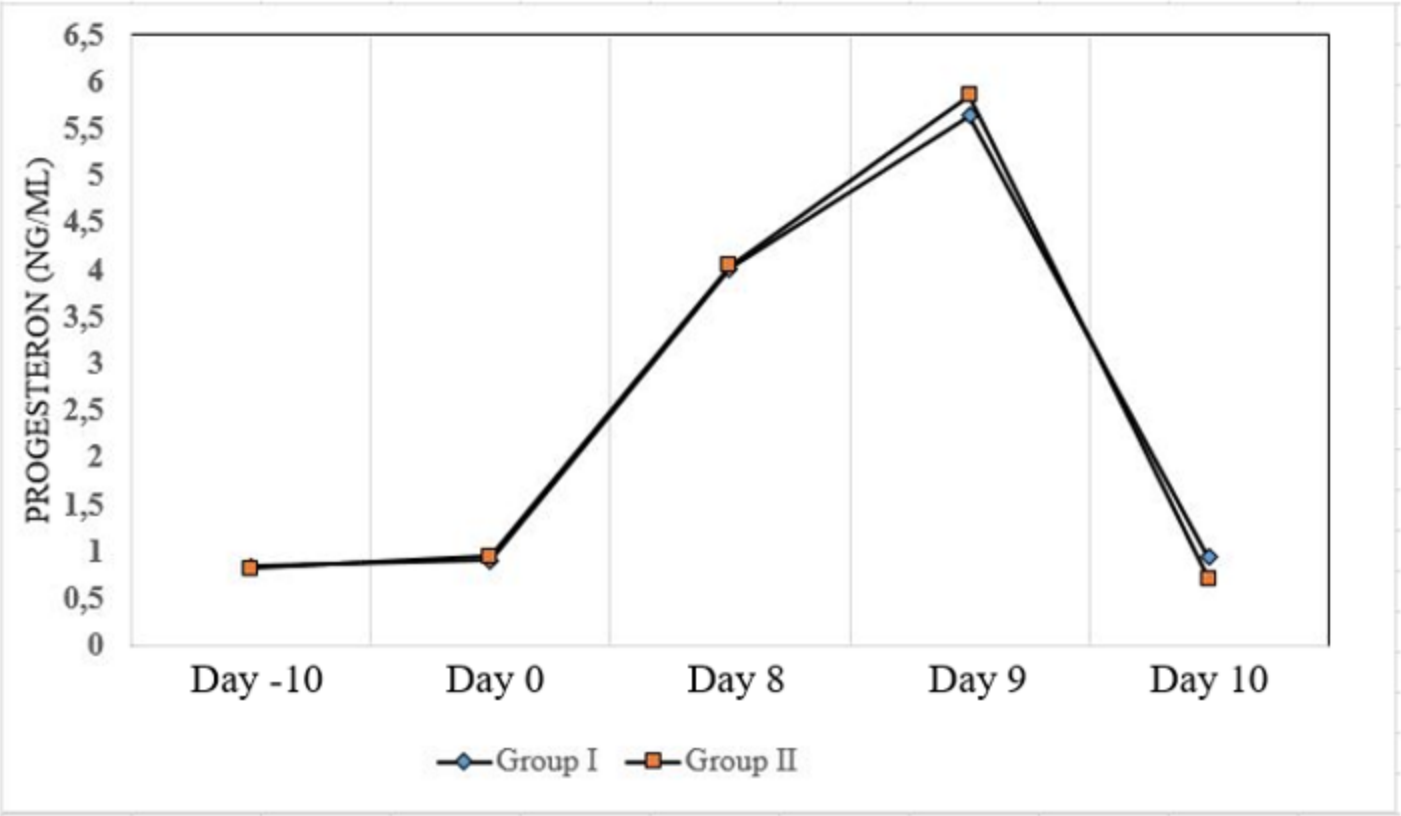

Figure 2

Progesterone values with and without eCG (Group I and Group II) treatment in cows without symptoms of estrus