Hormonal Response and Reproductive Outcome Following PGF2α Luteolysis and eCG Treatment in Diestrus Bitches

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Abstract

Abstract

Background: The prolonged diestrus phase in bitches poses a physiological barrier to reducing the inter-estrus interval, making hormonal induction a complex challenge lacking precise timing protocols. This study addresses this knowledge gap by evaluating the optimal stage for eCG/hCG intervention following PGF2α-induced luteolysis to determine how the diestrus phase influences estrus induction and conception rates and to evaluate the effect of the time elapsed from the day of nadir progesterone concentration to the initiation of the treatment on the efficiency of eCG-dependent protocol in the induction of estrus in diestrus bitches. Bitches (n=14) were allocated to receive 180 µg/kg of PGF2α twice daily for 4 days until luteolysis of corpus luteum (CL) occur, then equally divided two groups of bitches; group-1 early diestrus and group-2 late diestrus (n=7) receive 10 IU/kg of eCG daily for 9 days, followed by 500 IU hCG on day 10. Concentrations of LH and progesterone were evaluated every 48 hours from day -4 to day 14, with day 0 being the instigation of the treatment. Estrus induction rates were detailed at 71.4 %, 100 %, while conception rates were recorded at 14.2%. All treated bitches exhibited a progressive increase in LH and progesterone concentrations between days -4 and 14, Progesterone concentrations exceeded 3 ng/ml from day 12, while proestrus began on day 15. Using dosage of 10 IU/kg of eCG after luteolysis by PGF2alfa in late diestrus bitches was associated with a higher estrus induction, conception rate, and higher LH concentrations than early diestrus ones.

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Keywords: *Bitch; Estrus Induction; CL; PGF2alfa; eCG; Progesterone; LH.*

Introduction

Domestic female dogs are spontaneous ovulates, ovulate one or two times each year **(Kutzler, 2007)**. The interval between two successive estrus cycles normally ranges from 5-8 months (average 6 months) in fertile non-bred cycles **(Hoffmann et al., 2004; Concannon, 2011).** The inter-estrus interval consists of nearly two months of the diestrus stage followed by a persisted anestrus stage which extends from 4-6 months **(Volkmann et al., 2006).** The length of the luteal phase is almost identical in pregnant and non-pregnant bitches until shortly before parturition and prepartum luteolysis **(Concannon et al. 1989)**. During diestrus phase, the LH concentration is about 10 to 15 ng/ml and the FSH concentration is low ranging from 1 to 8 ng/ml**,** and ovaries contain corpus luteum that produces a high amount of progesterone **(Concannon, 2009)** The CL function is dependent on LH and prolactin from the pituitary **(Olson et al., 1989; Concannon, 1993; McBride et al., 2001).**

Prostaglandin F2 alfa therapy in diestrus also shortens the interval to the next estrus **(Oettle, 1982; Tsutsui et al., 1982; Oettle et al., 1985; Romagnoli et al. 1991)**, but it should be used with low multiple doses due to serious side effects such as vomiting, hypersalivation, frequent urination and diarrhea after administration **(Lein et al., 1989; Romagnoli et al., 1991; Kowalewski, 2016)**.

To the best of our knowledge, only two studies have endeavored to induce estrus in diestrus female dogs. In the first study, five diestrus female dogs were treated with Cabergoline, following treatment

initiation, 80% of the bitches entered pro-estrus. Despite this, none of the treated dogs conceived during the induced estrus cycle **(Jeukenne et al., 1997)**. The second study involved treating 15 diestrus female dogs initially with PGF2α to induce luteolysis. All treated dogs responded by entering clinical pro-estrus within 3 to 5 days post-treatment with deslorelin implant, but only one of them achieved pregnancy. **(Volkmann et al., 2006)**.

It is speculated that a significant challenge hindering conception in the induced estrus in diestrus female dogs is the unsuitability of the endometrium to support zygote implantation during this stage **(Diaz et al., 2002; Volkmann et al., 2006; Groppetti et al., 2010)**.

Studying the hormonal profile and clinical findings in diestrus bitches received 10 µg/kg dose of eCG after luteolysis by PGF2α may reduce the inter-estrous interval for the individual bitch.

Importance of the study:

The present study was designed to evaluate the effect of the time elapsed from the day of nadir progesterone concentration to the initiation of the treatment on the efficiency of eCG-dependent protocol in the induction of estrus in diestrus bitches.

Materials and Methods

Management of Bitches

Fourteen healthy mature mongrel female dogs weighing from 18 to 23 kg with an average age of 16 to 24 months, as determined by dentation, were randomly assigned to this experiment from August 2025 to June 2026. All bitches were housed in the experimental animal unit, Faculty of Veterinary Medicine at Zagazig University, beginning from six months before the induction of the treatment protocol up to 30 days after the end of the treatment regime or parturition. Prior to the initiation of the treatment, bitches were dewormed using Drontal tablets (Praziquantel 50 / Pyrantel embonate 144 / Febantel 150 mg tablet for dogs, Bayer, Germany) at a dosage of 1 tablet per 10 kg. To mitigate tick infestation, the bitches were administered a single dose of Bravecto chewable tablets (Fluralaner 500 mg, MSD, USA) at a dosage of 1 tablet for 10-20 kg. Throughout the entire duration of the study, female dogs were provided with a continuous supply of clean water, fed a diet of cooked meat supplemented with vegetable, and were subjected to natural light on regular basis.

Defining the stage of the estrous cycle

The mean pre-treatment estrus interval (days from onset of proestrus to onset of the next proestrus) was 180 ± 15 days (14 cycles of 14 bitches). To distinguish the phase of the estrous, female dogs were admitted to progesterone hormonal inspect and vaginal epithelium was microscopically examined two weeks before (day -14) and two days prior to initiation of the treatment (day -2). The diestrus stage is characterized by the existence of parabasal cells, Neutrophils, superficial cells, and squamous cells in vaginal cytology, and a progesterone concentration less than 20 - 80 ng/ml. **(Manda et al., 2004; Concannon, 2009; Concannon, 2011).**

Treatment Protocols

All Assigned bitches were treated with 180 µg/kg of PGF2α (Estrumate; cloprostenol 250 µg/ml, MSD, USA) administered subcutaneously twice daily for 4 days with a daily assessment of progesterone till luteolysis occurred as described by **(Romagnoli et al., 1996).**

The bitches were divided equally into two groups each of seven bitches, The first group (group 1) were received eCG-dependent estrus induction protocol beginning from 15 days after recording declined progesterone concentration on the fourth day of PGF2α administration (luteolysis), The estrus induction protocol included daily intramuscular injections of 10 IU/kg dose of eCG (Folligon; PMSG 5000 IU, MSD, USA) for 9 consecutive days, On the tenth day, all bitches will receive one intramuscular injection of 500 I.U. hCG. (Epifasi; hCG 5000 IU, EIPICO., Egypt) according to the method recommended by **(Bolghanabadi et al.,2023).** The second group (group 2) received same procedures applied on group 1 except that estrus induction protocol began 40 days after luteolysis.

The bitches were divided equally into two groups each of seven bitches, the first and the second groups were received eCG-dependent estrus induction protocol beginning from 15 (group 1) and 40 (group 2) days after recording progesterone concentration on the fourth day of PGF2α administration, The estrus

induction protocol included daily intramuscular injections of 10 IU/kg dose of eCG for 9 consecutive days, On the tenth day, all bitches will receive one intramuscular injection of 500 I.U. hCG. according to the method recommended by **(Bolghanabadi et al.,2023).**

Examination of the bitches:

Assessment of hormone concentrations:

Blood samples were collected from the cephalic vein of treated bitches beginning from day -2 till day 3 of PGF2α administration (with day zero being the first day of PGF2α treatment) and day -4 till day 14 (the day zero was the day of eCG treatment etiquette beginning). Blood was centrifuged at 3000 rpm in 10 minutes. The serum was withdrawn and kept at – 20 ºC until the estimation of hormone concentrations. The serum concentrations of LH and progesterone were measured using a chemiluminescence immunoassay (Elecsys®, Roche Diagnostics GmbH, Mannheim, Germany). The intra-assay coefficients of variation were below 4.5% and 1% for progesterone and LH, respectively. The inter-assay coefficients of variation were 3.66% and 1.98% for progesterone, and LH, respectively. The sensitivities were 0.3 ng/ml for progesterone, and 0.3 IU/L for LH.

Monitoring the behavioral and external signs of proestrus and estrus:

Female dogs were observed for the external symptoms of proestrus on daily basis. The onset of the proestrus was characterized by the appearance of obvious vulvar swelling and the existence of bloody-tinged discharge from the vulva. The onset of the estrus was defined when the vulvar discharge changed to a straw (pale yellow) color and acceptance to the male.

Mating and gestation detection

Female dogs that exposed the symptoms of proestrus were teased daily starting from the first day of proestrus to test their receptivity to the male. Female dogs that revealed male acceptance were mated on the first day of male acceptance and every 2 days until male rejection. The five males used in mating had a normal semen picture and approved fertility. Bitches were examined for pregnancy detection using ultrasound (Edan Dus 60 with micro-convex transducer 7 MHZ, CHINA) on day 25 of post-mating (figure 7). Pregnant female dogs were stayed up to the day of birth. The conception rate and the litter size were documented.

Statistical Analysis

Statistical analyses were performed using the R software version 4.3.3 (R Project for Statistical Computing, https://www.r-project.org/). Data were initially assessed for compliance with parametric assumptions. Normality was evaluated using the Shapiro–Wilk test, while homogeneity of variances was assessed using Levene's test. The most of variables did not met the assumptions for parametric analysis. Therefore, a consistent non-parametric approach was adopted, and hormone concentrations were expressed as median and interquartile range (IQR) and analyzed using the Friedman test for repeated measures, followed by Dunn's post hoc test with Bonferroni correction for multiple comparisons. A p-value < 0.001 was considered statistically significant. Graphs were generated using R software version 4.3.3.

Ethical Approval: The experimental research work was consented by the Institutional Animal Care and Use Committee, Zagazig University (ZU-IACUC, approval number ZU-IACUC/2/F/155/2024).

Results

Effect of specific dose of PGF2alfa on progesterone concentrations and luteolysis.

Bitches treated with 180 µg/kg of PGF2α exhibited a significant decline in progesterone serum levels starting from day 2 of treatment ($p < 0.001$). on day -2 the pretreatment serum median progesterone levels were 31.7 ng/ml which was substantially higher than progesterone median serum levels on day 2 of treatment 5.5 ng/ml. Blood collected from 14 bitches on the fourth day of treatment yielded a serum median progesterone concentration of 0.5 ng/ml **(Table 1)**, the drastic changes in progesterone serum levels across examination points in the individual bitches suggest successful luteolysis in twelve out of 14 bitches (85.7%) **(Figure 1)**. Only two out of 14 bitches (14.3%) showed a resurgence serum levels

of progesterone after two weeks of PGF2alfa treatment.

Variable side effects were observed on allocated bitches to PGF2alfa treatment like tremors, gastritis, diarrhea, nausea and vomiting.

Hormone concentrations throughout the study period in bitches treated with 10 IU/kg of eCG after 15 days of luteolysis (Group 1).

The leverage of eCG dosage in all seven bitches on overall serum levels of progesterone and LH was quite interesting, all (seven) bitches treated with 10 IU/kg dose of eCG after 15 days of luteolysis showed statistically significant changes in LH and progesterone serum levels across the different examination time points between day -4 and day 14. **(Table 2)**

The impact of eCG dosage on the overall concentration of LH was found to be statistically significant ($p < 0.05$) **(Figure 2)**. Bitches treated with 10 IU/kg of eCG exhibited a significant overall effect of time on LH concentrations in the seven bitches, Specifically, LH levels at D12 and D14 were significantly higher compared to D-2, D-4, and D0 (all adjusted $p < 0.05$).

Before the onset of eCG treatment protocol, two out of seven bitches showed a rebound in progesterone serum levels after 15 days of the last dose of PGF2alfa administration which indicates failure of luteolysis. The effect of eCG dosage on the overall concentration of progesterone was found to be statistically significant ($p = .032$). Bitches treated with 10 IU/kg of eCG after 15 days of luteolysis exhibited significantly higher concentrations of progestogen from day -2 to day 14. **(Figure 3)**

Progesterone (P4) levels were measured in group-1 of 7 bitches at 10 time points (D-4, D-2, D0, D2, D4, D6, D8, D10, D12, D14). The Friedman test revealed a significant effect of time on P4 concentrations ($\chi^2 = 58.359$, df = 9, $p < 0.0001$), indicating that P4 levels changed significantly over the study period **(Table 2)**. Post-hoc pairwise comparisons using Dunn's test with Bonferroni adjustment showed a significant increase in P4 from D-2 to D14 (adjusted $p = 0.032$). **(Figure 3)**

Effect of 10 IU/kg eCG dosage after 15 days of luteolysis on estrus induction rate, conception rates, days to proestrus, length of estrus, and litter size in group - 1 bitches.

Five out of seven bitches (71.4%) treated with 10 IU/kg of eCG after 15 days of luteolysis showed typical estrus signs (restlessness, swollen red vulva, bloody tinged vaginal discharge and licking of the rear end). However, none of the responded bitches accepted mating with fertile male. The length of estrus was abnormally short as it lasted for 3 to 4 days then it diminished abruptly. The other two bitches (28.5%) did not show any signs of estrus suggesting failure of luteolysis. Obviously, conception rate and litter size allover group 1 of seven bitches were both zero as no mating occurred.

Hormone concentrations throughout the study period in bitches treated with 10 IU/kg of eCG after 40 days of luteolysis (Group 2).

The impact of eCG dosage in all seven bitches on overall serum levels of progesterone and LH was thoroughly investigated, all seven bitches treated with 10 IU/kg dose of eCG after 40 days of luteolysis showed statistically significant changes in progesterone and LH serum levels across the different examination time points between day -4 and day 14. **(Table 3)**

The impact of eCG dosage on the overall concentration of LH was found to be statistically significant ($p < 0.0001$), specifically from day 8 to day 14 of treatment protocol. **(Figure 4)**

Progesterone (P4) concentrations were measured in group-2 bitches at 10 time points (D-4, D-2, D0, D2, D4, D6, D8, D10, D12, D14). The Friedman test indicated a significant overall effect of time on P4 levels ($\chi^2 = 62.86$, df = 9, $p < 0.0001$) **(Table 3)**.

The effect of eCG dosage on the overall concentration of progesterone was found to be statistically significant ($p < 0.0001$), treated bitches showed A significant increase in P4 levels was observed between early (D-2, D-4, D0) and late (D10, D12, D14) time points, as determined by Dunn's post-hoc test following a significant Friedman test (*p ≤ 0.05, Bonferroni-adjusted). **(Figure 5)**

Effect of 10 IU/kg eCG dosage after 40 days of luteolysis on estrus induction rate, conception rates, days to proestrus, length of estrus, and litter size in group - 2 bitches.

Seven out of seven bitches (100%) treated with 10 IU/kg of eCG after 40 days of luteolysis showed typical estrus signs staring from two to five days after the end of treatment protocol by eCG [Day 12 to

Day 15] (restlessness, swollen red vulva, bloody tinged vaginal discharge, accepting the male). Moreover, all the responded bitches accepted mating with fertile male. The average estrus phase in the 7 bitches continued for 10 days. Proestrus phase lasted for 9 days on average. Only two out of seven bitches were conceived (conception rate 28.5%), they successfully maintained full-term pregnancy until whelping. Average litter size was 2 puppies.

Effect of luteolysis by PGF2alfa on inter-estrus intervals in diestrus bitches.

A shortening in the inter-estrus intervals was recorded in group-2 bitches allocated to the current study of 60 ± 5 days post-treatment (luteolysis) by PGf2alfa and conducting the estrus induction protocol by eCG/hCG, which is significantly (p<0.0001) shorter than pre-treatment inter-estrus interval (180 ± 15 days).

Discussion

The current study was conducted to investigate the hormonal profile and clinical response in diestrus bitches that were treated with a specific dose of PGF2alfa then admitted to an eCG-based estrus induction regimen. The design of this study was motivated by the dearth of previous research work to evaluate the impact of the stage of diestrus on the efficiency of eCG-dependent protocol in the induction of estrus in diestrus bitches and reduction of the inter-estrus interval for individual bitch.

When comparing the luteolysis rate achieved in the current study in bitches treated with 180 µg/kg of PGF2α administered subcutaneously twice daily for 4 days to the results of the previous studies, we noticed that the luteolysis rate in the current study was quiet successful by 85.7%, closely approximated to the 90 % reported by some studies **(Romagnoli et al., 1991; Jeukenne et al., 1997; Volkmann et al., 2006; Ucar et al., 2018)**. Furthermore, the median serum progesterone levels rapidly declined on the second day of PGF2alfa administration (5.5 ng/ml) which is nearly like the 5.7 ± 4.3 ng/ml reported in other studies **(Romagnoli et al., 1991; Ucar et al., 2018)**. After application of the 4-day PGF2alfa based protocol in the current study, progesterone median serum levels decreased significantly to 0.5 ng/ml which were nearly like 0.3 ± 0.4 ng/ml recorded by **(Romagnoli et al., 1991)** and lower than 3.84 ± 0.06 ng/ml noted by **(Volkmann et al., 2006)**.

However, **(Jeukenne et al., 1997)** succeeded in termination of diestrus in non-pregnant bitches, but it took the researchers too long time (15-20 days) to decrease progesterone serum concentrations using prolactin antagonist; cabergoline at a daily dose of 5 µg/kg, which considered a very long time compared to usage of PGF2alfa in the current study and fail to achieve the ultimate goal of reducing inter-estrus interval in bitch.

Failure of luteolysis or the capability of the canine corpus luteum to salvage following the use of PGF2alfa has been reported in the current study in two out of 14 bitches (14%) and confirmed by histopathological findings on the ovaries, characterized by a temporary decrease of plasma progesterone concentrations followed by a rebound to normal values, this came along with cases observed by other previous studies **(Concannon et al., 1977; Van der Horst et al., 1977; Oettle 1982; Lein et al., 1989; Romagnoli et al., 1991)**.

Variable side effects to the use of PGF2alfa like nausea, vomiting, gastritis and tremors were observed in the current study which was like that recorded in previous studies **(Lein et al., 1989; Romagnoli et al., 1991; Kowalewski, 2016)**, these side effects took place due to full occupation of PGF2alfa receptors (PTGFR) in the CL resulting in serious side effects as clarified by **(Boiti et al., 2001; Ucar et al., 2018)**.

Induction of estrus in diestrus bitches facing many difficulties such as the ability to suppress the mechanism that stops the estrus cycle and the ability of the uterus to support embryonic development **(Davis et al, 2002; Linde-Forsberg et al., 2008; Marinelli et al., 2009)**. In the current study, treating diestrus bitches after luteolysis with mixture of 10 µg/kg dose of eCG for 9 successive days and single injection of 500 I.U. hCG. On the tenth day resulted in pronounced increase in progesterone and LH serum levels attaining induced estrus signs in 12 out of 14 bitches (85.7%) which was close to 100% recorded by **(Volkmann et al., 2006)** and higher than 80% noted by **(Jeukenne et al., 1997)**.

In the present study, Group-1 bitches which treated in early diestrus (20 days after end of estrus) showed

notable rise in serum median progesterone and LH levels (3.9 and 6 ng/ml respectively), these bitches did not exhibit follicular development exceeding 4 mm up to day 15 of treatment. Failure of ovulation led to vanished estrus signs and conception rate zero.

While group-2 bitches which treated in late diestrus (40 days after end of estrus) showed distinguished increase in serum progesterone and LH levels, successful ovulation and conception in two out of seven (28%) bitches was observed with maintaining pregnancy until full-term, which is significantly higher than 6% recorded by **(Volkmann et al., 2006)** and Zero stated by **(Jeukenne et al., 1997)**, referring to that **Volkmann et al., 2006** used deslorelin implant for nearly 2 months while **Jeukenne et al., 1997** used cabergoline for 15-20 days to achieve luteolysis.

Variability across different studies may be attributed to disparities in the biopotency of the hormonal preparations employed, the specific stage of diestrus at which the protocol was initiated, drug used for luteolysis or the breed of the bitches involved in the study.

The utilization of 10 IU/kg of eCG in the present study was found to be significantly associated with a higher estrus induction (100%) and conception rates (28%) in group-2 compared to the group-1 bitches (14% and zero respectively). the observation that conceived bitches across all treated cohorts, irrespective of the eCG dosage, exhibited elevated LH and progesterone concentrations staring from day 2 of the protocol.

Additionally, treated bitches showed a significant increase in progesterone concentrations starting from day 2, with average progesterone concentration on day 14 being 10.34 ng/ml in group-1 and 4.39 ng/ml in group-2.

The high rise in median progesterone and LH serum levels was pronounced on day 12 of treatment protocol in bitches that exhibited estrus and subsequently conceived (3 and 9.5 ng/ml respectively), which indicates that this increase does not interfere with normal ovulation and conception.

Shortening of the inter-estrus interval was reported for bitches treated with PGF2alfa in diestrus, A shortening of the inter-estrus interval of 43, 75 and 95 days was reported by **(Tsutsui et al., 1982; Oettle et al., 1985; Romagnoli et al. 1991)** respectively. which are nearly like the observations (60 ± 5 days) in our present study.

Conclusion

In conclusion, the time and stage of diestrus is a crucial factor in succession of eCG/hCG based estrus induction protocol, we recommend initiating the treatment protocol during late rather than early stage in diestrus bitches.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Data Availability

All data supporting the findings of this study are available within the manuscript.

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Table 1. Median (IQR) progesterone concentration at different days of examinations in bitches during and after PGF2alfa treatment.

Examination days	Progesterone concentration (ng/ml)
-2	31.7 (30.08-32.5)
0	15.55 (14.98-16.58)
1	15.7 (15.33-16.65)
2	5.5 (4.53-5.7)
3	0.5 (0.23-0.67)
Friedman test	
***p*-value**	< 0.0001
χ^2	51.886

Results are expressed as median (interquartile range, IQR).
*Day 0 is the treatment initiation day.

Table 2. Median (IQR) serum P4 and LH concentrations at different days of examinations in Group -1 bitches during and after eCG treatment.

Examination days	P4 concentration (ng/ml)	LH concentration (ng/ml)
-4	0.5 (0.45-6.5)	1.7 (1.45-3.5)
-2	0.3 (0.2-6.4)	1.7 (1.45-3.45)
0	0.5 (0.45-7.45)	2.6 (2.45-3.95)
2	0.7 (0.6-9.1)	3 (2.85-4.35)
4	0.9 (0.8-9.6)	3.6 (3.45-4.6)
6	1.3 (1.2-10.7)	4.4 (4.3-5.1)
8	1.6 (1.5-11.1)	4.8 (4.65-5.45)
10	2.6 (2.45-12.35)	5.3 (5.15-5.9)
12	3.5 (3.3-14.05)	5.8 (5.55-6.2)
14	3.9 (3.75-15.1)	6 (5.95-6.5)
Friedman test		
***p*-value**	< 0.0001	< 0.0001
χ^2	58.359	61.78

Results are expressed as median (interquartile range, IQR).
*Day 0 is the treatment initiation day.

Table 3. Median (IQR) serum P4 and LH concentrations at different days of examinations in Group-2 bitches during and after eCG treatment.

Examination days	P4 concentration (ng/ml)	LHconcentration (ng/ml)
-4	0.2 (0.1-0.25)	1.5 (1.45-1.65)
-2	0.2 (0.1-0.25)	1.5 (1.45-1.65)
0	0.4 (0.35-0.45)	1.8 (1.65-2.5)
2	0.7 (0.65-0.8)	2.1 (2-3.45)
4	1.2 (1.1-1.3)	2.4 (2.25-2.45)
6	1.6 (1.5-1.65)	2.7 (2.65-3.3)
8	2.3 (2.15-2.35)	3.3 (3.2-3.8)
10	2.8 (2.75-2.85)	3.7 (3.55-4.3)
12	3.2 (3.15-3.35)	4.3 (4.15-7)
14	4.4 (4.25-4.55)	4.7 (4.4-6.9)
Friedman test		
***p*-value**	< 0.0001	< 0.0001
χ^2	62.86	61.15

Results are expressed as median (interquartile range, IQR).
*Day 0 is the treatment initiation day.

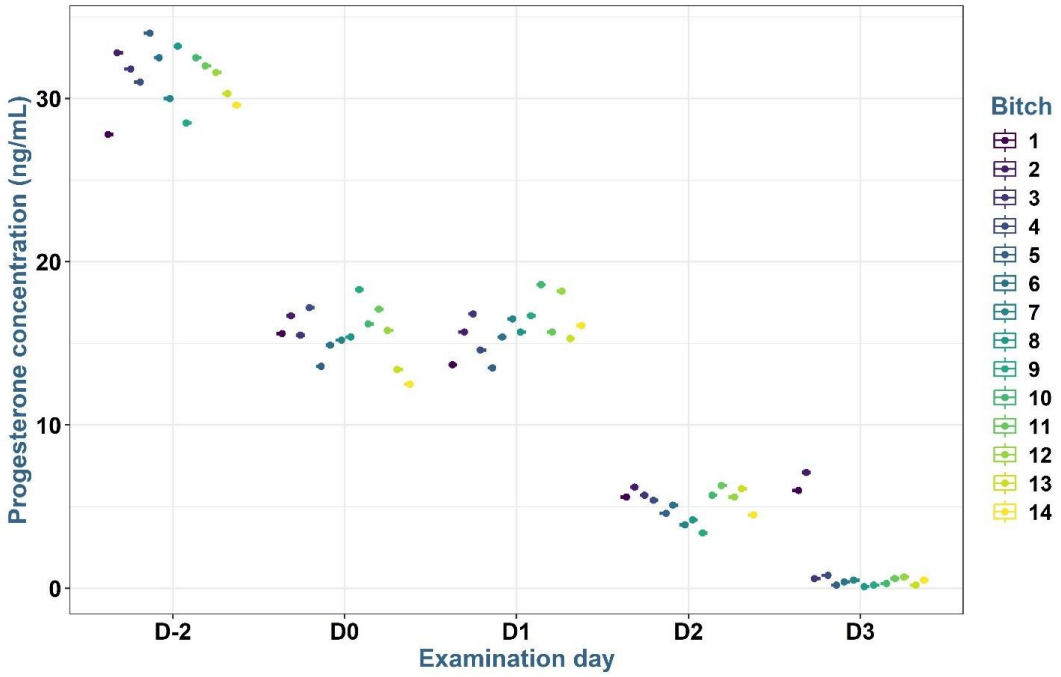

Figure 1. Changes in progesterone concentration across examination days in individual bitches during and after PGF2alfa treatment. Each point represents an individual measurement of a bitch. Data are presented as median ± interquartile range (IQR).
*Day 0 is the treatment initiation day.

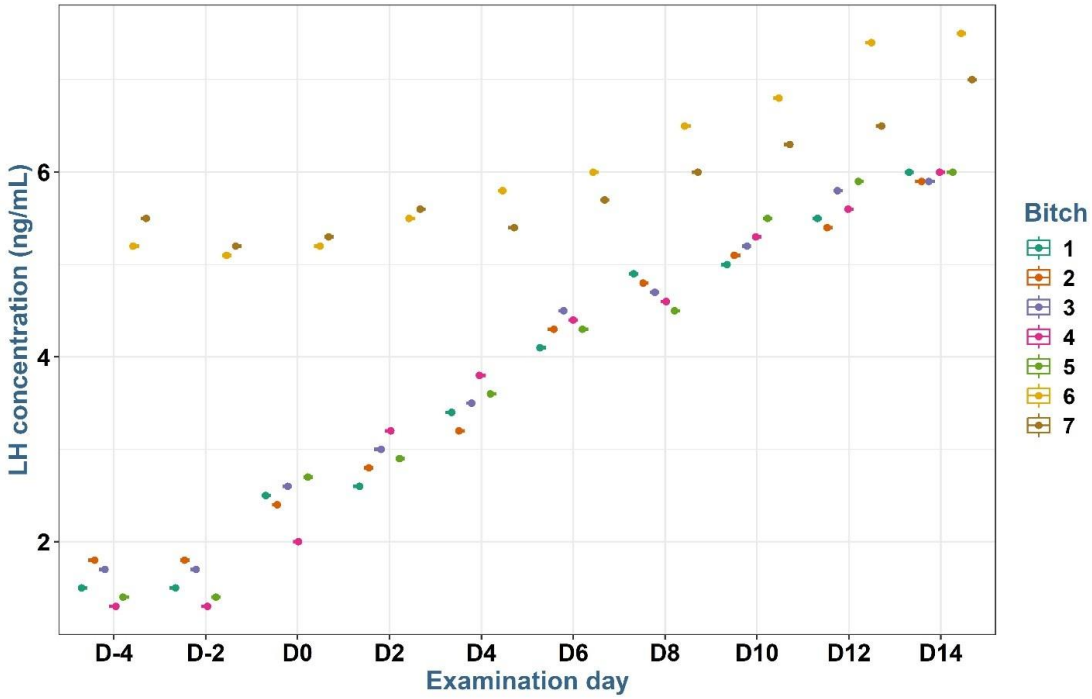

Figure 2. Temporal changes in LH concentrations in Group-1 (n=7) bitches across 10 time points during and after eCG treatment. Each point represents an individual measurement. Data are presented as median ± interquartile range (IQR).
*Day 0 is the treatment initiation day.

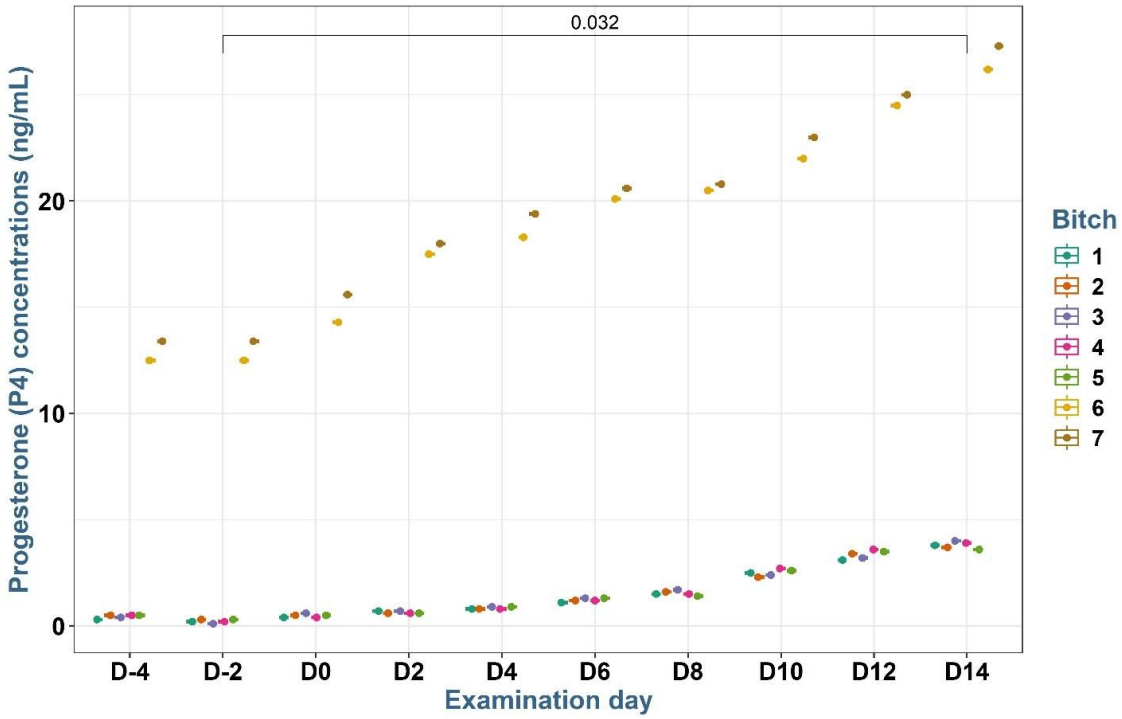

Figure 3. Progesterone (P4) concentrations in Group-1 bitches (n=7) over 10 time points after eCG treatment. Each point represents an individual measurement. Data are presented as median ± interquartile range (IQR). *Day 0 is the treatment initiation day. A significant increase in P4 was observed between D-2 and D14 (*p = 0.032, Dunn's test with Bonferroni correction).

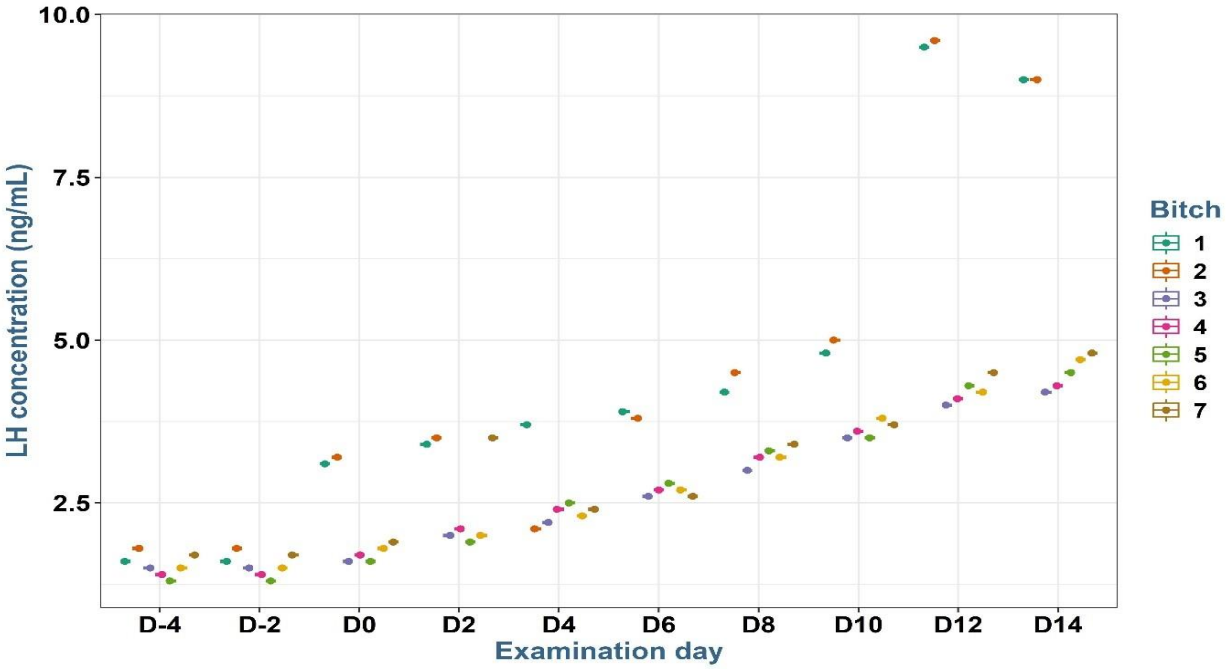

Figure 4. Temporal changes in LH concentrations in group-2 bitches (n=7) over 10 time points after eCG treatment. Data are presented as median ± interquartile range (IQR). *Day 0 is the treatment initiation day.

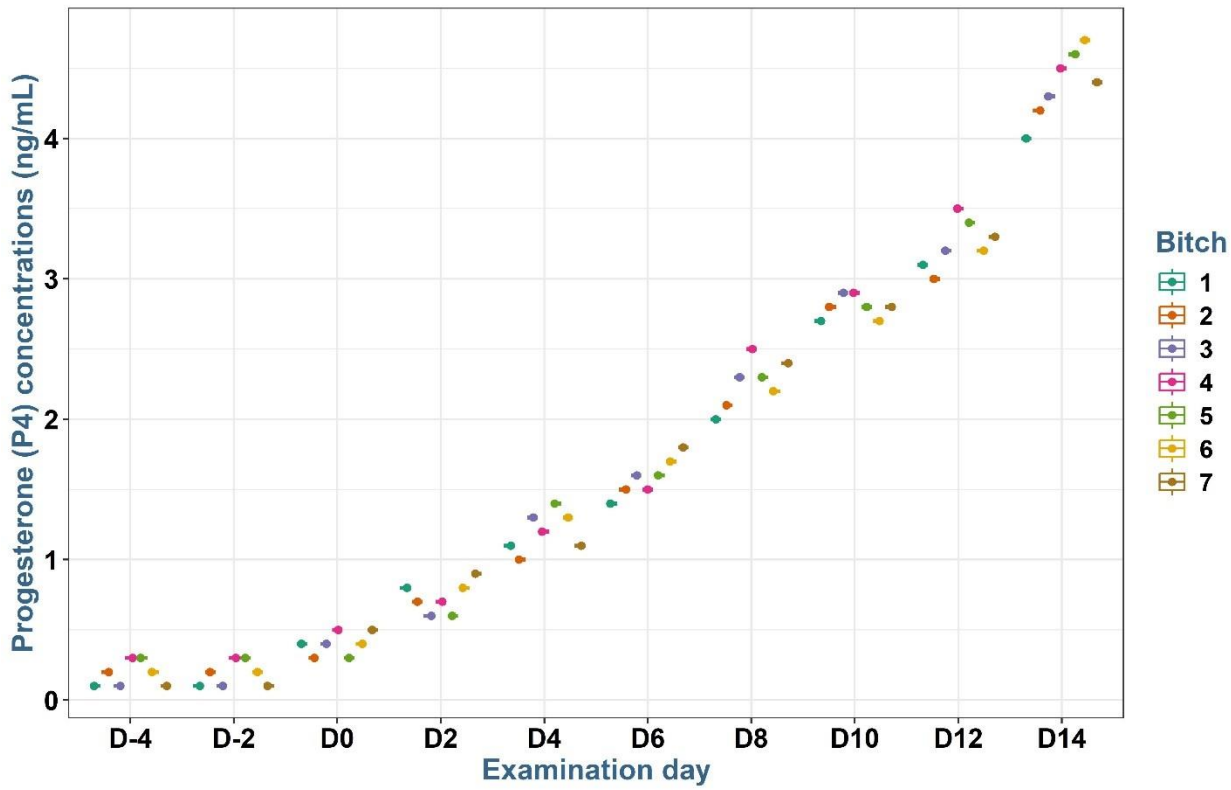

Figure 5. Temporal changes in progesterone (P4) concentrations in Group-2 bitches (n=7) over 10 time points during and after eCG treatment. Each point represents an individual measurement for a bitch. Data are presented as median ± interquartile range (IQR).

*Day 0 is the treatment initiation day.