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Plasma progesterone concentration after first service is associated with individual genetic traits, postpartum phenotypes, and likelihood of conception in seasonal-calving pasture-based dairy cows

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ABSTRACT

The aims of this study were to (1) evaluate postpartum phenotypes, cow factors, and genetic traits associated with plasma progesterone (P4) concentrations after first artificial insemination (AI); (2) determine variation in daily plasma P4 concentrations between d 7 and 13 after first AI; and (3) evaluate associations between plasma P4 concentrations and pregnancy success after first AI. First and second parity (n = 2,797) spring-calving lactating dairy cows from 35 dairy herds were enrolled. Farm visits were performed every 2 wk during the postpartum period as follows: cows that were at wk 3 (range: 14–27 d in milk) and wk 7 (range: 42–55 d in milk) postpartum were examined. Farm visits were performed weekly during the breeding season, and cows that were between 7 and 13 d after the first AI were examined. Body condition score (BCS) was measured at each visit using a 1 to 5 scale [low ($\leq$2.75), target ($\geq$3.0)]. Transrectal ultrasound examinations were conducted at wk 3 and wk 7 postpartum visits to determine presence or absence of a corpus luteum (CL) and uterine tract score [scale of G_1 (best)–G_4 (worst)]. Blood samples were collected at each visit, and plasma concentrations of glucose, β-hydroxybutyrate, and fatty acids were analyzed. On the day of the weekly farm visit during the breeding season, blood samples for P4 determination were collected from all cows that were between 7 and 13 d after first AI during the breeding period. Cows that had a CL present and a G_1 uterine score at wk 7 postpartum had greater plasma P4 concentration after first AI (+0.67 ng/mL and +0.4 ng/mL, respectively) compared with cows with no CL present and with a uterine score $\geq G_3$. Cows with low BCS at wk 7 postpartum had lesser plasma P4 concentration after first AI than cows with target BCS. Each unit increase in plasma fatty acids and β-hydroxybutyrate concentration at AI was associated with 0.45 ± 0.33 ng/mL (estimate ± standard error) and 0.07 ± 0.04 ng/mL greater plasma P4 concentration after first AI, respectively. Regarding genetic merit traits, each unit increase in fertility subindex was associated with 0.005 ± 0.003 ng/mL greater P4 concentration. In addition, for every 1 ng/mL increase in plasma P4 concentration, the odds of estimated probability of pregnancy per AI increased by 3% (odds ratio = 1.03; 95% confidence interval = 1.00, 1.05). In conclusion, cows with superior genetic merit for fertility traits and milk production traits, favorable fertility phenotypes at wk 7 postpartum, (e.g., presence of a CL, a G_1 uterine score, and target BCS), and blood parameters indicative of better metabolic status at AI were all associated with greater plasma P4 concentration after AI. In turn, greater plasma P4 concentrations were associated with greater odds of successful pregnancy establishment. This study underlines the important associations between early postpartum fertility phenotypes (CL presence, uterine health status) and subsequent plasma P4 concentrations after first AI, and hence provides additional evidence of the mechanisms through which selection for fertility traits improves phenotypic fertility performance.

Key words: cattle, fertility, metabolic status, uterine infection, anestrous

INTRODUCTION

The ovarian cycle is characterized by repeated patterns of cellular proliferation, differentiation, and transformation that accompany follicular development and the formation and regression of the corpus luteum (**CL**). The CL is a temporary endocrine gland formed after ovulation, and using cholesterol as the precur-

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sor, it synthesizes and secretes the steroid hormone progesterone (**P4**) under the regulation of LH until luteolysis initiates the next follicular phase (Schams and Berisha, 2004). The main source of cholesterol for luteal cells is circulating lipoproteins, and in ruminants, it is primarily high-density lipoproteins (Wiltbank et al., 1990). Production of P4 from cholesterol utilizes only 2 steroidogenic enzymes (CYP11A1 and HSD3β), and is considered the simplest steroidogenic pathway to produce a biologically active steroid (Diaz et al., 2002; Atli et al., 2012; Wiltbank et al., 2014).

Progesterone plays a fundamental role in the establishment and maintenance of pregnancy (Lonergan, 2011). Endometrial function including conceptus-maternal interactions, pregnancy recognition, and uterine receptivity for implantation require the action of P4 (Lonergan et al., 2016). A significant proportion of embryo loss is attributable to inadequate circulating P4 concentrations, which has downstream consequences for endometrial gene expression and histotroph secretion into the uterine lumen (Morris and Diskin, 2008; Wiltbank et al., 2016). Elevated circulating concentrations of P4 in the immediate postconception period have been associated with accelerated conceptus elongation, greater embryonic interferon-tau production, and an increase in pregnancy per AI (**P/AI**) in cattle (Lonergan et al., 2007; Garcia-Ispierto and López-Gatius, 2017).

The concentration of P4 in circulation at any moment in time reflects the balance between luteal P4 production and metabolism of P4. Although P4 production by the CL is influenced by hormones that have both stimulatory effects (e.g., LH) and inhibitory effects (e.g., $PGF_{2\alpha}$; Niswender et al., 2000), luteal phase P4 production is affected by CL volume and the number of granulosa cells that luteinize into large luteal cells (Wiltbank et al., 2014). Metabolism of P4 depends on liver blood flow, where P4 catabolic enzymes are abundant (Wiltbank et al., 2006). Hence, circulating concentration of P4 is negatively associated with DMI (Sangsritavong et al., 2002; Vasconcelos et al., 2003).

Genetic merit for fertility traits affects several organs and tissues involved in fertility outcomes in dairy cows (Butler, 2014a). Cows with good genetic merit for fertility traits had greater DMI and BCS, superior metabolic status and uterine health (Cummins et al., 2012a; Moore et al., 2014a; Moran et al., 2017), earlier resumption of postpartum ovarian cyclicity, and greater luteal phase circulating concentrations of P4 (Cummins et al., 2012b; Moore et al., 2014a) compared with cows with poor genetic merit for fertility traits. Moreover, Rojas Canadas et al. (2020b) recently reported strong associations between individual genetic traits, postpartum phenotypes, and reproductive performance in seasonal-calving, pasture-based dairy cows. Intervention studies that increased circulating P4 concentrations (exogenous P4 injections, vaginal inserts containing P4, or injection of hCG to induce an accessory CL) have reported conflicting results and overall modest effects on likelihood of pregnancy establishment (Mann and Lamming, 1999; Parr et al., 2012; Nascimento et al., 2013; Sánchez et al., 2018). Therefore, the aims of this study were as follows: (1) to evaluate genetic traits, postpartum phenotypes, and cow factors associated with plasma P4 concentrations between d 7 and 13 after first AI; (2) to determine the variation in plasma P4 concentration on each day, and (3) to evaluate associations between plasma P4 concentration and pregnancy success after first AI. Specifically, the objectives were (1) to interrogate a large data set of cow records to evaluate the associations between plasma P4 concentration on d 7 to 13 after first service with individual genetic traits, parity, DIM at commencement of the breeding period and wk 3 and wk 7 postpartum, CL presence or absence, uterine health, BCS, and metabolic status; and (2) to evaluate associations between plasma P4 concentration and pregnancy success after first AI in seasonal-calving, pasture-based, lactating dairy cows.

MATERIALS AND METHODS

All experimental procedures involving cows were approved by the Teagasc Animal Ethics Committee and authorized by the Health Products Regulatory Authority, the competent authority in Ireland responsible for the implementation of European Union legislation (Directive 2010/63/EU) for the protection of animals used for scientific purposes.

Herds and Experimental Design

A prospective, observational, cross-sectional study was conducted on 35 pasture-based seasonal-calving dairy herds located in the province of Munster in Ireland. The study population included first (n = 1,671) and second (n = 1,126) lactation dairy cows (Holstein-Friesian and Holstein-Friesian × Jersey crossbreds), representing approximately 50% of the animals in each herd. Only cows that were ≥30 DIM on the planned farm mating start date (**MSD**) were enrolled. All cows enrolled calved during the spring season of 2 consecutive years (yr 1 = 24 herds; yr 2 = 11 herds). Cows were excluded or withdrawn from the study if the herdowners observed clinical diseases, which included mastitis, lameness, metabolic disorders, and displaced abomasum.

Individual cow examinations were conducted at specific times points of interest as follows: wk 3 postpartum (14–27 DIM), wk 7 postpartum (42–55 DIM), 7 to 13 d after first AI (breeding visit), and after the farm mating end date. All data recorded for the wk 3 and wk 7 measurements were captured before the breeding period began. To collect data at the desired time points, all herds enrolled were visited every 2 wk during the postpartum period, and every week during the first 4 to 6 wk of the breeding period. A single postbreeding visit to each herd was carried out between 34 and 50 d after the farm mating end date to determine pregnancy status and estimate fetal age. At the wk 3 and wk 7 visits, postpartum ovarian structures and uterine health were evaluated by transrectal ultrasound (8.5-MHz transrectal transducer; Ibex Pro, E.I. Medical Imaging). At each farm visit, cow BCS was evaluated by a single observer using a 1 to 5 scale (1 = emaciated; 5 = obese) with 0.25 increments (Edmonson et al., 1989). Cows were subsequently classified according to their BCS as low (BCS $\leq$ 2.75) or target (BCS $\geq$ 3.00).

Ultrasound Evaluation of Ovarian Status and Reproductive Tract Score

The presence or absence of a CL was recorded during the ultrasound evaluation. Ultrasound reproductive tract score (**URTS**) was determined by examining the uterine horns based on the following criteria outlined by Mee et al. (2009): G_1 = a typical spoke wheel–shaped lumen; G_2 = a spoke wheel–shaped lumen with an enlarged center filled with a small volume (>2 mm, ≤5 mm diameter) of fluid of mixed echogenicity; G_3 = a stellate-shaped lumen filled with a moderate volume (>5 mm, ≤10 mm diameter) of fluid of mixed echogenicity; G_4 = a circular-shaped lumen filled with a large volume (>10 mm diameter) of fluid of mixed echogenicity.

Blood Sampling and Metabolic Status Assays

At the wk 3, wk 7, and breeding visits, blood samples were collected via coccygeal venipuncture into evacuated 10-mL tubes (catalog #367820, BD Vacutainer, Becton Dickinson) and stored at 4°C during transportation. Samples were centrifuged for 15 min at 1,500 × *g* and 4°C, and plasma was harvested and stored at −20°C until assayed.

Concentrations of P4 in plasma were determined in samples collected during the breeding period from d 7 to 13 after first AI using a commercially available solid-phase RIA (PROG-RIA-CT kit; DIAsource ImmunoAssays S.A.). The inter- and intraassay coefficients of variation for low, medium, and high plasma P4 quality controls were 4.6% and 17.9%, 7.3% and 13.4%, and 4.7% and 9.2%, respectively. It was not logistically feasible to collect blood samples every day after AI from all cows during the breeding season. Hence, at each weekly breeding visit, any cows that were between d 7 and 13 after first service were selected for blood sample collection [d 7 (n = 404), d 8 (n = 431), d 9 (n = 409), d 10 (n = 428), d 11 (n = 399), d 12 (n = 353), and d 13 (n = 373)].

Plasma samples collected at wk 3 and wk 7 postpartum were assayed for concentrations of glucose, BHB, and fatty acids (**FA**) using enzymatic colorimetry (ABX Mira). The glucose kits were supplied by Horiba ABX; BHB kits were supplied by Randox Laboratories Ltd.; FA kits were supplied by Wako Chemicals GmBH.

Genetic Traits

The Economic Breeding Index is a single-figure profit index aimed at helping farmers identify the most profitable bulls and cows for breeding dairy herd replacements. It comprises the following 7 subindexes: production, fertility, calving, beef, maintenance, management, and health (O'Sullivan et al., 2020). Each subindex comprises individual genetic traits. For this study, the fertility subindex and specific individual genetic traits [PTA for calving interval, survival, milk (kg), and milk protein (%)] were selected for inclusion in the study. Calving interval and survival are defined as number of days between successive calving events and longevity in the herd, respectively.

Fertility Management and Reproductive Parameters

Detection of estrus during the breeding season was conducted using the standard reproductive management protocols within each farm, which typically involved 2 to 4 periods per day of visual observation of cows aided by assessment of tail paint removal. Artificial insemination procedures varied between farms, and AI was performed by either a commercial AI technician or by a trained and qualified member of the farm staff. The total duration of the breeding season was approximately 84 d. Reproductive records including farm MSD, service dates, and the mating end date were obtained from the Irish Cattle Breeding Federation profile for each participating herd. Pregnancy to first insemination (**P/AI1**) was coded as 1 if an animal received only 1 service and was diagnosed as pregnant at the end of the breeding season. Cows with more than 1 service, or where the cow was diagnosed as nonpregnant, were allocated a P/AI1 of 0.

Statistical Analysis

The initial data set of plasma progenesterone concentrations included data collected from 2,932 cows. To avoid use of nonphysiological plasma P4 concentrations that may have inadvertently arisen in the data set (e.g., incorrect date recorded for estrus, cow failed to ovulate after insemination), the cows in the bottom fifth percentile for plasma P4 concentration on each day were removed for both pregnant and nonpregnant cows, resulting in 2,797 cows included in the study. The cut-off of 5% was guided by examining the tails in the normal probability plot produced using the Univariate procedure of SAS and checking that P4 outlier values [defined as P4 $<Q_1 - 1.5$ (interquartile range)] were included in this range. The percentage of these animals that also had phenotype data recorded at wk 3 and wk 7 postpartum was 90.0% (n = 2,517) and 93.9% (n = 2,629), respectively. All statistical analyses were performed using SAS software v 9.4 (SAS Institute). To visualize the interquartile range for plasma P4 on each day (d 7–13), box plots for P4 concentration in pregnant and nonpregnant cows were created using PROC BLOXPOT. The sample unit used in this study was the individual cow. The assumption that errors were normally and independently distributed was evaluated in all models using PROC UNIVARIATE residual plots, and where transformation was necessary, the most appropriate Box-Cox transformation was implemented. Outliers were detected using PROC UNIVARIATE residual plots and were removed from the data set. For the first analysis, plasma P4 concentration (d 7–13) was the dependent variable, and the independent variables available included presence or absence of a CL (CL status) at wk 3 and wk 7 postpartum; URTS (uterine health) at wk 3 and wk 7 postpartum; plasma concentrations of glucose, BHB, and FA (metabolic status) at wk 3 and wk 7 postpartum and after AI; BCS at wk 3 and wk 7 postpartum and after AI; DIM at MSD; parity; the fertility subindex; and specific individual genetic traits [PTA for calving interval, survival, milk (kg), and milk protein (%)]. To evaluate possible correlations among predictor variables, the Variance Inflation Factor was calculated using the PROC REG procedure of SAS. Multicollinearity between fertility subindex, PTA for calving interval, and survival was detected. Independent variables associated with plasma P4 were assessed using mixed models (PROC MIXED) using the full list of independent variables outlined above as fixed effects, but the effects of fertility subindex, PTA for calving interval, and survival were assessed in separate models. Day after service (d 7–13) and year were included as covariates, and farm was included as a random effect. A manual backward elimination procedure was used to eliminate independent variables with $P > 0.15$ from the model. For the fertility subindex and specific individual genetic traits [PTA for calving interval, survival, milk (kg), and milk protein (%)], linear, quadratic, and cubic relationships with the dependent variable plasma P4 concentation were tested using PROC GLM in SAS separately for each day.

Marginal associations between the dependent variable P/AI1 and independent variables were evaluated using multiple logistic regression (PROC LOGISTIC) to calculate odds ratio and predicted probabilities. The model included plasma P4 and all independent variables indicated above. A manual backward elimination procedure was used to eliminate independent variables with $P > 0.15$ from the model. Day after service (d 7–13) and year were included as covariates. The receiver operating characteristics curve and the associated area under the curve and confidence interval were also calculated using the LOGISTIC procedure. A significant difference was considered as $P \leq 0.05$, whereas differences between $P > 0.05$ and ≤ 0.10 were considered a tendency.

RESULTS

Progesterone and Pregnancy at First Insemination

Pregnancy after first AI across all herds was 57.1% (1,598/2,797). There was a highly significant association ($P < 0.0001$) between plasma P4 concentration and day after AI when samples were collected (Table 1). Overall, mean plasma P4 concentration from d 7 to 13 after first AI was greater for pregnant compared with nonpregnant cows (10.1 ± 0.17 ng/mL vs. 9.4 ± 0.17 ng/mL; $P = 0.0004$). Figure 1 illustrates the interquartile range for plasma P4 concentration on d 7 to 13 post-AI in pregnant and nonpregnant cows. Across all days, for every 1 ng/mL increase in plasma P4 concentration, the odds of estimated probability of P/AI increased by 3% (odds ratio = 1.03; 95% CI = 1.00, 1.05; $P = 0.03$).

CL Status and Uterine Health

The associations between CL status and uterine health data collected at wk 3 and wk 7 postpartum with plasma P4 concentrations on d 7 to 13 after AI are summarized in Table 2. There were no associations between plasma P4 concentration between d 7 and 13 after first service and either presence of a CL or URTS at wk 3 postpartum ($P > 0.05$; Table 2). Conversely, cows that had a CL present at wk 7 postpartum tended to have greater plasma P4 concentration on d 7 to 13 after first service compared with cows that did not have

Table 1. Summary of plasma progesterone (P4) concentration (LSM ± SE; ng/mL) from d 7 to 13 after first service and pregnancy success after first service (P/AI1; %, number in parentheses)

Item	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13
P4 concentration	7.1 ± 0.10[a]	8.5 ± 0.13[b]	9.3 ± 0.13[c]	10.1 ± 0.17[d]	10.7 ± 0.19[e]	11.2 ± 0.20[f]	11.2 ± 0.21[f]
P/AI1	57.2 (231/404)	60.8 (262/431)	55.7 (228/409)	57.2 (245/428)	56.6 (226/399)	58.07 (205/353)	53.9 (201/373)

[a–f]Different superscripts within a row indicate significant differences ($P < 0.05$).

a CL present at wk 7. There was a tendency ($P = 0.10$) for an association between URTS at wk 7 and plasma P4 concentrations on d 7 to 13 after first AI, with greater plasma P4 concentrations in cows that had G_1 uterine score compared with cows that had G3 and G4 uterine scores (10.4 ± 0.1 ng/mL vs. 9.7 ± 0.2 and 9.4 ± 0.3 ng/mL, respectively; Table 2).

BCS and Metabolic Status

Table 3 summarizes the mean (± SD), minimum, and maximum values at wk 3 and wk 7 postpartum and at AI for plasma FA, BHB, and glucose concentrations. No associations were observed between plasma P4 concentrations on d 7 to 13 after AI with BCS at wk 3 postpartum or after first AI, or with indicators of metabolic status at wk 3 or wk 7 postpartum (plasma concentration of glucose, BHB, and FA, all $P > 0.05$; Table 2). Conversely, BCS at wk 7 postpartum was associated with plasma P4 concentrations, whereby cows with target BCS had greater plasma P4 concentrations on d 7 to 13 after AI compared with cows that had low BCS (10.2 ± 0.1 ng/mL vs 9.4 ± 0.09 ng/mL; $P = 0.03$, Table 2). Plasma FA and BHB concentrations after first AI were associated with plasma P4 concentration on d 7 to 13 after first AI (both $P < 0.05$; Table 2). Each unit increase in plasma FA and BHB concentrations was associated with 0.45 ± 0.33 ng/mL (estimate ± SE; $P < 0.0001$) and 0.07 ± 0.04 ng/mL (estimate ± SE; $P = 0.02$) greater plasma P4 concentration between d 7 to 13 after AI, respectively.

Genetic Traits

Each unit increase in fertility subindex was associated with greater ($P = 0.05$) plasma P4 concentrations on d 7 to 13 after first AI [0.005 ± 0.003 ng/mL (estimate ± SE)]. Conversely, each unit increase in PTA for calving interval (i.e., poorer genetic merit) was associated with lesser ($P = 0.03$) plasma P4 concentration after first service [−0.09 ± 0.04 ng/mL (estimate ± SE)]. A tendency ($P = 0.10$) for greater plasma P4 concentration on d 7 to 13 was observed for each unit increase in PTA for milk kilogram [0.0007 ± 0.0004 ng/mL (estimate ± SE)]. There were no associations between either PTA for survival ($P = 0.31$) or PTA for milk protein % ($P = 0.64$) and plasma P4 concentration on d 7 to 13 after first AI. Table 4 summarizes the mean (± SD), minimum and maximum values for fertility subindex, specific individual genetic traits [PTA for calving interval, survival, milk (kg), and milk protein (%)], and DIM at MSD. Moreover, fertility subindex had a linear relationship with P4 at d 7 ($P = 0.05$), d 8 ($P = 0.02$), and d 13 ($P = 0.01$), and a quadratic

Table 2. Phenotypic associations between plasma progesterone (P4) concentrations between d 7 and 13 after first AI with corpus luteum (CL) status, ultrasound reproductive tract score (URTS), BCS, and plasma metabolites at wk 3 (14–27 DIM) and wk 7 (42–55 DIM) postpartum, and after first service

	Wk 3		Wk 7		Post-AI	
Item	Estimate ± SE	*P*-value	Estimate ± SE	*P*-value	Estimate ± SE	*P*-value
CL presence[1]						
CL present	9.9 ± 0.11	0.75	9.9 ± 0.08	0.09	—	—
CL absent	9.7 ± 0.10		9.4 ± 0.16		—	
URTS[1,2]						
G_1	9.7 ± 0.5[a]	0.81	10.4 ± 0.1[a]	0.10	—	—
G_2	9.9 ± 0.1[a]		9.9 ± 0.1[ab]		—	
G_3	9.7 ± 0.09[a]		9.7 ± 0.2[b]		—	
G_4	9.4 ± 0.3[a]		9.4 ± 0.3[b]		—	
BCS[1,3]						
Low	9.5 ± 0.1	0.33	9.4 ± 0.09	0.03	9.6 ± 0.1	0.91
Target	10.0 ± 0.09		10.2 ± 0.1		9.9 ± 0.1	
Plasma FA[4]	0.009 ± 0.04	0.84	−0.01 ± 0.05	0.79	0.45 ± 0.33	<0.0001
Plasma BHB[4]	−0.002 ± 0.02	0.91	0.02 ± 0.04	0.23	0.07 ± 0.04	0.02
Plasma glucose[5]	−0.003 ± 0.005	0.50	−0.002 ± 0.005	0.64	−0.001 ± 0.005	0.80

[a,b]Different superscripts within a row indicate significant differences ($P < 0.05$).

[1]Plasma progesterone concentration (LSM ± SE) expressed in ng/mL.

[2]G_1 = a typical spoke wheel–shaped lumen; G_2 = a spoke wheel–shaped lumen with an enlarged center filled with a small volume (>2 and ≤5 mm diameter) of fluid of mixed echogenicity; G_3 = a stellate-shaped lumen filled with a moderate volume (>5 and ≤10 mm diameter) of fluid of mixed echogenicity; G_4 = a circular-shaped lumen filled with a large volume (>10 mm diameter) of fluid of mixed echogenicity.

[3]Evaluated by a single observer using a 1-to-5 scale (1 = emaciated; 5 = obese) with 0.25-point increments (Edmonson et al., 1989). Cows were classified according to their BCS as low (≤2.75) or target (≥3.0).

[4]Measurement units for FA and BHB were mmol/L.

[5]Measurement units for glucose was mg/dl.

relationship was observed on d 12 ($P = 0.04$). Similarly, PTA for calving interval had a linear relationship with P4 concentration on d 7, d 12, and d 13 (all $P < 0.02$). A linear relationship between PTA for milk kilogram and P4 concentration was detected on d 12 ($P = 0.05$) and d 13 ($P = 0.007$), and a quadratic relationship was detected on d 8 ($P = 0.03$) and d 11 ($P = 0.02$). Some of the relationships between genetic traits and plasma progesterone concentrations are depicted in Figure 2.

Parity and Calving Date

The mean DIM at MSD was 70.8 ± 15.1 d (range: 30–114 d). There were associations between both parity ($P = 0.03$) and DIM at MSD ($P = 0.003$) and plasma P4 concentration after first AI. Primiparous cows had a greater plasma P4 concentration on d 7 to 13 after first AI compared with second parity cows (10.00 ± 0.09 vs. 9.4 ± 0.11 ng/mL, respectively). Each unit increase in

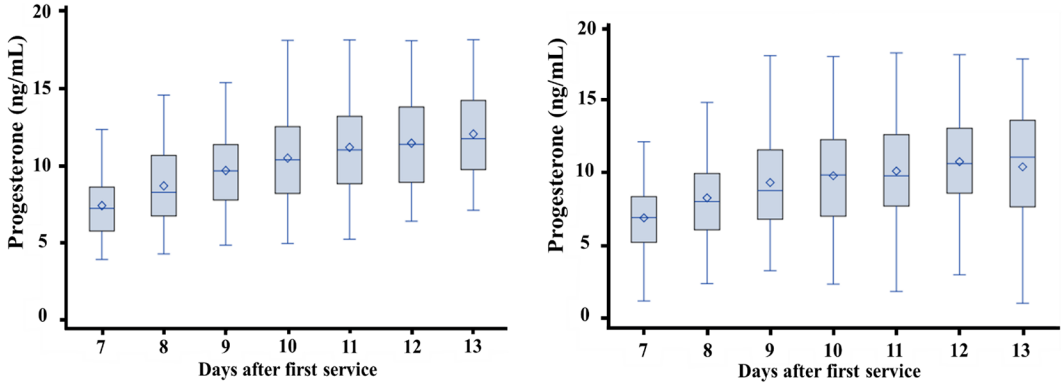

Figure 1. Box and whisker plots for plasma progesterone (P4) concentration (ng/mL) from d 7 to d 13 after first AI in cows that became pregnant (left) and failed to become pregnant (right). The ends of the whiskers represent the maximum and minimum plasma P4 concentration for an individual day. The upper and lower quartiles make up the boundaries of the box. The height of the box represents the interquartile range, and the median is indicated by the horizontal line within the box. The arithmetic mean plasma P4 concentration for each day is indicated by the diamond.

Table 3. Summary of the mean (± SD), minimum and maximum values at wk 3 and wk 7 postpartum and after first service (AI) for plasma fatty acids (FA), BHB, and glucose (Glu) concentrations

Item[1]	Mean ± SD	Minimum	Maximum
Wk 3			
FA	0.43 ± 0.2	0.02	1.8
BHB	0.88 ± 0.5	0.03	3.9
Glu	62.5 ± 15.1	25.9	101.4
Wk 7			
FA	0.41 ± 0.2	0.01	1.9
BHB	0.87 ± 0.4	0.01	4.2
Glu	63.1 ± 14.4	24.3	98.07
Post-AI			
FA	0.31 ± 0.21	0.02	2.07
BHB	0.76 ± 0.30	0.02	3.0
Glu	66.3 ± 12.9	32.1	96.01

[1]Measurement units for FA and BHB were mmol/L, and for glucose was mg/dL.

Table 4. Summary of the mean (± SD), minimum, and maximum values for fertility subindex, PTA values for specific individual genetic traits, and DIM at mating start date

Item	Mean ± SD	Minimum	Maximum
Fertility subindex (€)	49.1 ± 31.5	−78.9	180.7
PTA calving interval (d)	−2.4 ± 1.9	−11.8	4.6
PTA survival (%)	1.5 ± 0.9	−2.1	5.0
PTA milk (kg)	−2.8 ± 179.2	−926	657
PTA milk protein (%)	0.08 ± 0.06	−0.1	0.3
DIM at mating start date	72.4 ± 22.4	30.0	133.0

DIM at MSD was associated with greater plasma P4 concentrations on d 7 to 13 after first AI [0.08 ± 0.02 ng/mL (estimate ± SE)]. Moreover, a 2-way interaction was observed between parity and days post-AI ($P = 0.05$; Figure 3), whereby primiparous cows had greater plasma P4 concentrations on d 7 ($P = 0.08$) and d 8 ($P = 0.0003$) compared with second parity cows, but there were no differences between parities thereafter.

DISCUSSION

Associations between genetic traits, postpartum phenotypes, cow factors, and reproductive performance were previously described by Rojas Canadas et al. (2020a,b). The current manuscript further extends those findings and elucidates the biological mechanisms explaining the associations previously observed by describing the relationship between genetic traits, postpartum phenotypes, cow factors, and plasma P4 concentrations from d 7 to d 13 after first insemination in seasonal-calving, pasture-based, lactating dairy cows. In agreement with previous reports, a favorable association between plasma P4 concentrations after first AI and pregnancy success was also demonstrated [see review: Inskeep (2004); Stevenson and Lamb (2016)]. It is important to note that the present study used only a subpopulation of lactating dairy cows within each herd (first and second parity, no clinical disease, and calved at least 30 d before the farm MSD), which likely represented the most fertile cows within the herd. Moreover, blood samples for P4 concentration determination were not collected from the same set of cows on consecutive days of the estrous cycle (i.e., repeated measurements), but rather were collected on a single occasion from each cow between d 7 and 13 after first AI. Nonetheless, important associations were identified.

The preovulatory LH surge causes differentiation of follicular cells into luteal cells. The luteinization process is characterized by a switch from estradiol to P4 synthesis, and is mediated by a change in the enzymes responsible for synthesizing these steroid hormones (Dieleman et al., 1983; Wiltbank et al., 1990). After the preovulatory surge of LH and subsequent ovulation, 6 to 8 d are required for luteal tissue formation

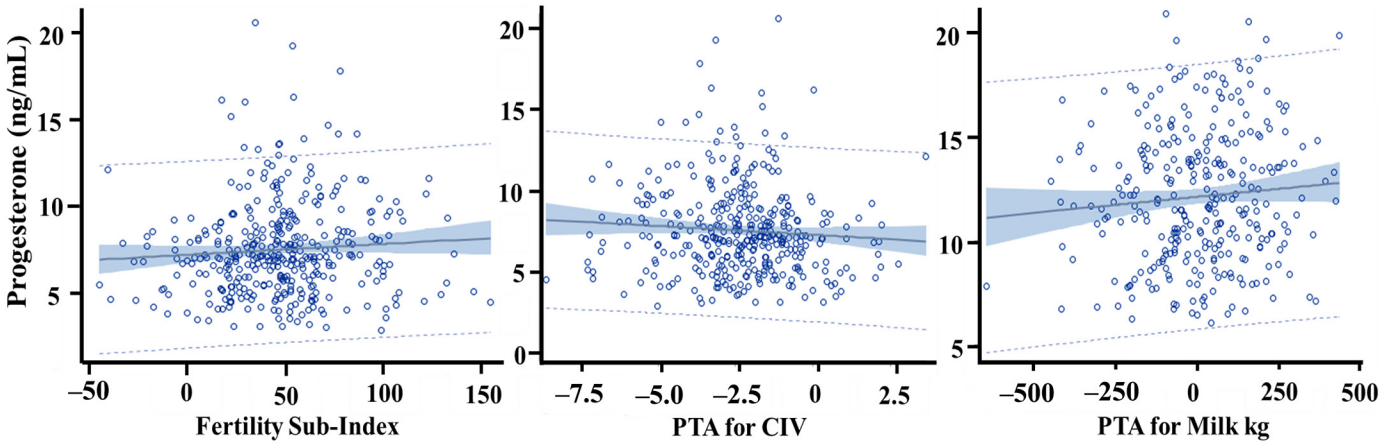

Figure 2. Linear relationships between fertility subindex (left; $P = 0.05$), PTA for calving interval (CIV; middle; $P = 0.02$), and PTA for milk (right; $P = 0.007$) with plasma progesterone on d 7, 7, and 13 after AI, respectively. The shaded area represents the 95% CI.

and angiogenesis, and for the CL to achieve mature physical size (Schams and Berisha, 2004). It is clear from the findings of the current study, however, that P4 concentration continues to increase daily from d 7 to 12 after AI, in agreement with previous reports comparing associations between P4 concentration, CL size, and day of the estrous cycle (Mann, 2009).

Although there is unequivocal evidence that there is an absolute requirement for P4 to support pregnancy establishment and maintenance [for review see: Lonergan et al. (2016); Spencer et al. (2016)], data on the relationship between plasma P4 concentrations after AI and pregnancy success in lactating dairy cows have been variable (Mann and Lamming, 1999; Nascimento et al., 2013; Sánchez et al., 2018). This study clearly demonstrated that mean plasma P4 concentration was greater in cows that became pregnant after first AI compared with cows that did not become pregnant, which is in agreement with previous studies that reported greater P/AI in heifers and cows that had increased circulating P4 concentrations during the early luteal phase after AI (Parr et al., 2012; Garcia-Ispierto and López-Gatius, 2017). Conversely, in some studies where cows were treated to induce greater plasma P4 concentrations (i.e., P4 supplementation or induced accessory CL), authors have reported no difference in P/AI (Monteiro et al., 2015) or even a deleterious effect of supplementary P4 on P/AI (Parr et al., 2014; Sánchez et al., 2018). Differences between studies may be explained by different sources of P4 (endogenous vs. exogenous), and a detrimental effect of both inadequate and excessive early luteal phase P4 concentrations on embryonic survival (Lonergan et al., 2016). Elevated plasma P4 concentrations exert an indirect effect on the developing embryo via changes induced in the endometrium (Clemente et al., 2009). Greater plasma P4 concentration during the early luteal phase stimulates earlier downregulation of P4 receptors in the endometrium (Okumu et al., 2010) and an advancement in the temporal changes that normally occur in the endometrium (Forde et al., 2009), resulting in chronologically appropriate conceptus elongation and secretion of interferon-tau. These physiological changes are a fundamental prerequisite for maternal recognition of pregnancy, which normally occurs around d 16 after ovulation in cattle [see review: Lonergan et al. (2016); Spencer et al. (2016)].

Cows with compromised postpartum uterine health have been previously reported to have a prolonged postpartum interval to resumption of ovarian cyclicity and longer luteal phases (Opsomer et al., 2000). Using data from the same animals as the current study, presence of a CL at wk 3 or wk 7 postpartum was associated with reduced likelihood of abnormal uterine health compared with cows without a CL at wk 3 or wk 7 (prevalence of these phenotypes is summarized in Supplemental Table S1, https://doi.org/10.6084/m9.figshare.15172701, Rojas Canadas, 2021; Rojas Canadas et al., 2020a). In the present study, we observed a tendency for greater plasma P4 concentrations after AI

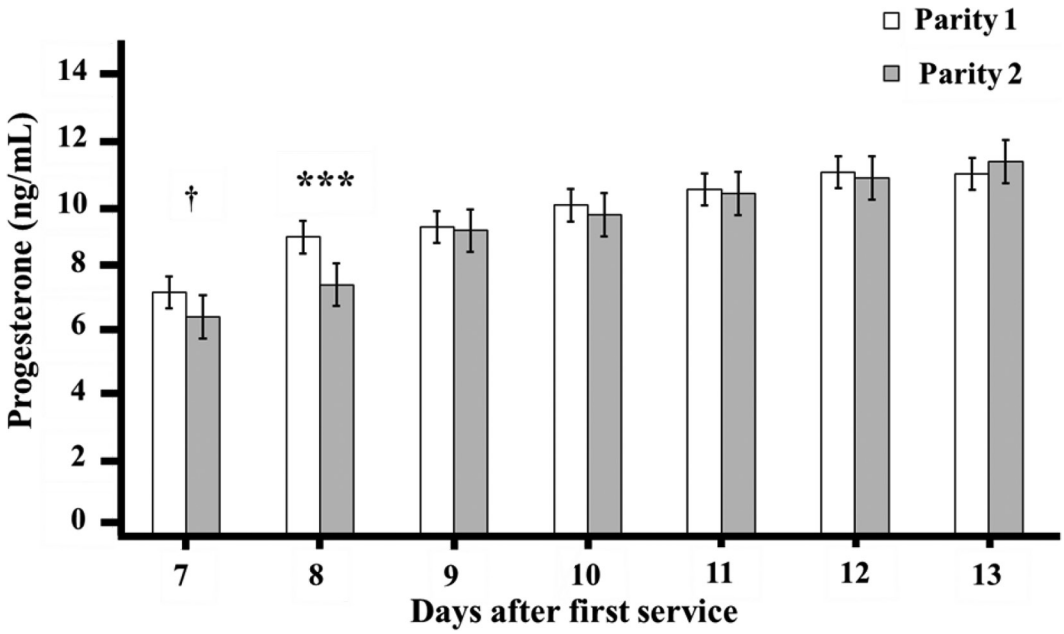

Figure 3. Two-way interaction between parity and days after first service on plasma progesterone concentration (ng/mL). Error bars indicate SEM. ***Indicates parity differed at $P < 0.001$ significance; † indicates a tendency ($0.05 < P \leq 0.10$).

in cows that had CL present at wk 7 compared with cows without CL, and also in cows that had G_1 URTS at wk 7 postpartum compared with cows that had G_3 and G_4 URTS. Although it was not logistically feasible to examine CL morphology or histology in the current study, it is likely that greater plasma P4 concentrations were associated with greater CL volume, a greater proportion of large luteal cells, or greater P4 synthetic capacity. Cows that have an earlier resumption of postpartum ovarian activity have more luteal phases before the start of the breeding period (Horan et al., 2005; Rojas Canadas et al., 2019). Conversely, uterine bacterial infections impair hypothalamus and pituitary function, and directly perturb ovarian granulosa cell steroidogenesis (Williams et al., 2007), mainly due to the presence of the *Escherichia coli*–derived lipopolysaccharide in follicular fluid (Sheldon et al., 2009a). Therefore, cows with uterine disease have reduced circulating estradiol and perturbed prostaglandin signaling, resulting in disruption of ovarian cyclicity and slower follicular growth (Bromfield et al., 2015). It is likely that an extended period of subclinical endometritis occurs in cows that were previously diagnosed as having clinical endometritis during the postpartum period, despite resolving clinical problems (Sheldon et al., 2009b). In the current study, cows with impaired uterine health at wk 7 postpartum were close to the start of the breeding period, and consequently were unlikely to have fully resolved clinical or subclinical endometritis at the time of the first AI. Therefore, cows that have more severe and prolonged postpartum uterine disease are likely to have lesser plasma P4 concentrations after first AI, and this may contribute to reduced likelihood of P/AI.

Metabolism of P4 is regulated primarily by the liver; hence, an increase in liver blood flow associated with elevated DMI results in a decrease in circulating P4 concentrations, without any change in luteal P4 production (Wiltbank et al., 2006). Therefore, milk yield and metabolic status have been previously reported to influence plasma P4 concentrations in dairy cattle (Kafi and Mirzaei, 2010; Lemley et al., 2010). In the current study, plasma P4 concentration was associated with metabolic status. We observed a positive association between BCS at wk 7 postpartum and plasma P4 concentrations on d 7 to 13 after first AI. These results may be reflective of a subtle long-term advantage in energy balance in cows with BCS $\geq$ 3.00 [see review: Roche et al. (2009)]. Moreover, there was also a positive association between both plasma FA and BHB concentrations after breeding and plasma P4 concentration on d 7 to 13 post-AI. Of note, in this study, overall mean plasma FA and BHB concentrations after first AI were basal (mean $\pm$ SD, 0.31 $\pm$ 0.004 mmol/L and 0.72 $\pm$ 0.004 mmol/L, respectively) and were not indicative of negative energy balance. Hence, the association between greater plasma concentrations of both FA and BHB with greater P4 concentrations after first AI may reflect greater absorption of butyrate and FA arising from dietary digestion. It is well known that ruminal butyrate is an important precursor of BHB in well-fed animals, which differs from the situation in early lactation negative energy balance when mobilized FA are converted to BHB by the liver (Van Soest, 1994). It is important to note that the plasma FA and BHB concentrations at the time of first AI were basal (i.e., not indicative of negative energy balance), but the subtle differences may indicate differences in DMI. If greater intake induces a better energy balance status, there may be a plausible link to greater luteal phase plasma P4 concentrations.

In the current study, the parity by day interaction revealed that multiparous cows had lesser plasma P4 concentrations on d 7 and 8 after first AI compared with primiparous cows. This may also be partially explained by greater milk yield and DMI in multiparous cows compared with primiparous cows (Wathes et al., 2007; Adrien et al., 2012), and hence greater hepatic blood flow and associated P4 metabolism. Days in milk at the start of the breeding season is an important factor affecting both production and reproduction in seasonal-calving, pasture-based systems (Dillon et al., 1995; see review: Butler, 2014b). Cows that calve at the start of the calving season are more likely to have resumed ovarian cyclicity, completed uterine involution, and passed peak milk production at the start of the breeding period. Collectively, this highlights the importance of DIM for improving P/AI, and based on our findings in the current study, the mechanism underpinning the improvement in P/AI may be explained, at least in part, by greater luteal phase P4 concentrations with increasing DIM.

A lactating dairy cow genetic model was recently developed by our group, where cows had similar genetic merit for milk production traits but either good or poor genetic merit for fertility traits (Cummins et al., 2012a). Cows with good genetic merit for fertility traits had greater luteal phase circulating P4 concentrations (Cummins et al., 2012b) and greater CL volume (Moore et al., 2014b) compared with cows with poor genetic merit for fertility traits. The results from the current study agree with these previous findings, and again highlight the strong association between both fertility subindex and PTA for calving interval and plasma P4 concentration from d 7 to 13 after first AI. We have previously reported that cows with good genetic merit for fertility traits had greater BCS, earlier resumption of ovarian cyclicity, superior metabolic status, and greater uterine health (Cummins et al., 2012a; Moore

et al., 2014a; Moran et al., 2017) compared with cows with poor genetic merit for fertility traits. In the current study, we detected associations between all those phenotypes with plasma P4 concentration after first AI. On the other hand, there are conflicting reports on the association between genetic merit for milk yield and fertility. Some studies reported a negative association [greater calving interval and interval to first service (Mackey et al., 2007), longer postpartum interval to commencement of luteal activity, and greater days open (Fulkerson et al., 2001)], whereas others have indicated superior fertility [greater submission rates and greater pregnancy success during first 21 and 84 d of breeding season (Rojas Canadas et al., 2020b)] in cows with greater genetic merit for milk production. In the current study, there was a positive association between genetic merit for milk (kg) and plasma P4 concentrations, which agrees with superior reproductive performance observed in cows with greater genetic merit for milk (kg) reported recently (Rojas Canadas et al., 2020b). Indeed, we previously observed greater phenotypic milk yield in cows with good genetic merit for fertility traits that also had greater luteal phase circulating P4 concentrations (Cummins et al., 2012a,b; Moore et al., 2014b). Conversely, other studies that used high-producing lactating dairy cows maintained in confinement systems reported reduced plasma P4 concentrations during the postpartum period in cows with greater genetic merit for milk production (Lucy and Crooker, 2001; Veerkamp et al., 2003). Differences between studies in the years when data were collected [throughout the 1990s for Lucy and Crooker (2001) and Veerkamp et al. (2003)], period of lactation (postpartum vs. breeding period), and dairy system (confinement vs. pasture-based) likely explain, at least in part, the contrasting findings. Of note, it is worth highlighting that the associations between postpartum phenotypes, cow factors, individual genetic traits, and plasma P4 concentrations observed in the current study are consistent with the associations between these variables and reproductive performance recently reported by our group (Rojas Canadas et al., 2020b).

CONCLUSIONS

The present study has clearly demonstrated that postpartum phenotypes and cow factors, bioenergetic status, fertility subindex, and PTA for calving interval and milk production (kg) were all associated with plasma P4 concentration on d 7 to 13 after first AI. Furthermore, plasma P4 concentration increased daily from d 7 to 12 after AI, with a strong positive association between plasma P4 concentration and pregnancy success after first service. Ensuring cows have an adequate intake at AI and target BCS at wk 7 postpartum, increasing the proportion of the herd with G_1 uterine tract score and CL presence by wk 7 postpartum, and selecting animals based on fertility subindex (and its component calving interval genetic trait) and PTA for milk production (kg) were all associated with greater plasma P4 concentration, and thus improve pregnancy success after first AI in seasonal-calving, pasture-based dairy cows.

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