Relationships between prostaglandin concentrations, a single nucleotide polymorphism in *HSD17B12*, and reproductive performance in dairy cows

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ABSTRACT

Prostaglandins are involved in multiple processes important for fertility, with previous work in mice highlighting a potential role for the *HSD17B12* gene in prostaglandin biosynthesis. This study aimed to determine the associations among circulating prostaglandin concentrations, a missense SNP in the *HSD17B12* gene predicted to disrupt protein function, and fertility traits in first-lactation Holstein-Friesian dairy cows. We used a study population of approximately 500 animals specifically bred to have either a positive (POS, +5%) or negative (NEG, −5%) genetic merit for fertility (FertBV). Genotypes of a previously identified SNP (rs109711583) in *HSD17B12* were determined, with 116 animals genotyped as AA, 215 genotyped as AG, and 153 genotyped as GG. Plasma concentrations of prostaglandin E₂ and the PGF_{2α} metabolite PGFM were determined at 3 time points (12 mo of age, 4 d postpartum, and 5 wk postpartum during first lactation) in a selection of animals with AA and GG genotypes from both the POS and NEG FertBV groups (n = 33–40 in each genotype for each FertBV group). Binary reproductive traits (yes or no) examined included submission for artificial breeding in the first 3 or 6 wk of the seasonal breeding period; conception to first service; conception during the first 6 wk of the breeding period; and pregnant at the end of the breeding period. Uterine health at 6 wk after calving was examined by evaluating the percentage of polymorphonuclear leukocytes following uterine cytology and by scoring vaginal discharge based on the presence of purulent material. The 3-wk submission rate was increased in animals that carried the G allele of the missense SNP in *HSD17B12*, but no differences were present among genotypes for 6-wk submission rate. The trait was additive, with each increase

of the G allele increasing the 3-wk submission rate by 6 to 7%. We did not observe any consistent associations between SNP alleles and circulating PGE₂ concentrations; however, a complex 3-way interaction among time, fertility group, and SNP allele was present for PGFM concentrations. Plasma concentrations of PGE₂ were increased approximately 40% at 5 wk postpartum in animals that were submitted for breeding within 3 or 6 wk of the start of the breeding season, and in those that conceived during the first 6 wk of breeding, compared with those that did not. Plasma concentrations of PGFM were decreased approximately 20% in those animals that conceived to their first service and tended to be decreased in animals that were pregnant at the end of the breeding period, compared with those that were not. In summary, associations were observed between the SNP in *HSD17B12* and submission rate by d 21 of the breeding season, as well as between circulating prostaglandin concentrations and fertility traits, but the SNP was not consistently linked to changes in prostaglandin concentrations. Thus, the association between submission rate by d 21 of the breeding season and the SNP in *HSD17B12* were unlikely driven by changes in prostaglandins.

Key words: *HSD17B12*, prostaglandin E₂, prostaglandin F_{2α}, fertility

INTRODUCTION

In dairy cattle, fertility is important to maintain regular calving cycles and efficient milk production (Burke and Verkerk, 2010; Cabrera, 2014). Cow fertility is especially important in seasonal systems that are constrained to a 365-d calving interval, where failure to become pregnant within a defined breeding period may result in the animal being culled. Following calving, resumption of regular reproductive cycles and expression of estrus, as well as the ability to conceive, are key factors driving pregnancy rates. Hence, understanding the genetic and environmental factors that support a

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positive pregnancy outcome is important to develop strategies to improve pregnancy rates in dairy cattle worldwide (Burke and Verkerk, 2010; Cabrera, 2014).

Prostaglandins are important regulators of fertility that are involved in multiple processes, including uterine involution and uterine disease progression, luteal function, ovulation, early embryo development, and parturition (Weems et al., 2006). The uterus is a major source for prostaglandin production during the early postpartum period (Guilbault et al., 1984a,b). Unsurprisingly, differential expression of prostaglandins or prostaglandin-related genes has previously been linked to differences in fertility. In endometrium or uterine luminal flushes of subfertile cattle, abundance of mRNA encoding PTGS2 or prostaglandins were decreased (Jaureguiberry et al., 2020; Mazzoni et al., 2020; Moraes et al., 2020). These relationships indicate that animal genetics or management interventions that alter aspects of prostaglandin biosynthesis could be used to improve cow fertility.

In cattle, a missense SNP in *HSD17B12* (rs109711583) has been associated with differences in fertility (Cochran et al., 2013; Ortega et al., 2016). This SNP results in a change of the amino acid at position 243, toward the C-terminus of the protein, from arginine to glycine, that is predicted to alter the function of the protein (SIFT score 0.01; Ng and Henikoff, 2003). Hydroxysteroid (17 β) dehydrogenases are multifunctional proteins, involved in roles such as conversion of steroids between low- and high-activity forms, peroxisomal β -oxidation of fatty acids, elongation of long-chain fatty acids, and mitochondrial oxidation of 3R-hydroxyacyl-groups (Saloniemi et al., 2012; Hiltunen et al., 2019). It is possible that this SNP affects fertility in cattle through altering prostaglandin biosynthesis, as deficiency of *Hsd17b12* in mouse embryonic stem cells has been associated with a reduced production of arachidonic acid (Rantakari et al., 2010), which plays a key role in prostaglandin biosynthesis. Furthermore, mice with haploinsufficiency of the *Hsd17b12* gene have reduced fertility, associated with an extended diestrus phase, fewer litters, and smaller-sized litters (Kemiläinen et al., 2016). Although complete knockout of the *Hsd17b12* gene is lethal during the embryonic period (Rantakari et al., 2010), *Hsd17b12*^{+/-} mice were shown to have reduced ovarian concentrations of arachidonic acid and downstream prostaglandins, including PGE₂ and PGF_{2 α} , but not 17 β -estradiol, androstenedione, testosterone, or progesterone (Kemiläinen et al., 2016).

We hypothesized that the missense SNP in cattle *HSD17B12* would be associated with alterations in circulating prostaglandin concentrations and that prostaglandin concentrations would differ between animals of varying reproductive performance. To test this hy-

pothesis, we examined the associations of the SNP in *HSD17B12* with plasma prostaglandin concentrations as well as prostaglandin concentrations in animals with differing fertility outcomes.

MATERIALS AND METHODS

Experimental Design and Sample Collection

All studies involving animals were approved by the Ruakura Animal Ethics Committee (Hamilton, New Zealand; AE application nos. 13574 and 14200) and in compliance with New Zealand animal welfare regulations.

The animal model used for this study consisted of Holstein-Friesian cows ($n = 523$) with either an estimated negative (**NEG**, -5%) or positive (**POS**, +5%) genetic merit for fertility (**FertBV**), and no or marginal differences in genetic merit for other traits such as milk production or body weight. The establishment and management of this animal model is described elsewhere, along with data collection methodologies and differences in fertility and reproduction traits as both growing nulliparous heifers and lactating cows during first and second lactations (Meier et al., 2021a,b). Fertility traits used in the current study were collected during the first lactation (2-yr-old) and included the following binary (yes or no) measures: submission for artificial breeding during the first 3 or 6 wk of the seasonal breeding period (**SR21**; **SR42**); conception to first AI (**CR1stAI**), pregnancy occurring during the first 6 wk of the breeding period (**PR42**), and pregnancy at the end of a seasonal breeding period lasting 98 d (**PRFin**). Uterine health at 6 wk after calving was also examined by evaluating the PMN percentage following uterine cytology (**PMN42**) or the degree of purulent material within the vagina (**PVD42**). The PVD42 score was classified as not affected (score of 1) or affected (score >1), with animals where a sample was not collected excluded from analysis. The PMN42 score was classified as affected (PMN $\geq 2\%$) or not affected (PMN <2%; McDougall et al., 2020).

Determination of Genotype of the Missense SNP in *HSD17B12*

DNA was isolated from blood samples taken from each animal when they were approximately 13 to 15 mo of age, and the genotype of the SNP in *HSD17B12* was determined as previously described (Campbell et al., 2015). The primers used for the assay were GCCCTTCTTTGTTGCAACCA and CGCCAATG-GTTTAAATGGCAC. Analysis was successful in 484 of 523 samples assayed, with 116 animals genotyped

as *AA*, 215 genotyped as *AG*, and 153 genotyped as *GG*. Insufficient sequence reads were obtained from the remaining 39 animals so accurate genotyping of those animals was not possible.

Measurement of Prostaglandin E_2 and 13,14-Dihydro-15-Keto Prostaglandin $F_{2\alpha}$

As the majority of $PGF_{2\alpha}$ is metabolized in a single pass through the lungs to 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ metabolite (**PGFM**), measurement of PGFM was used as a proxy for $PGF_{2\alpha}$. Although most PGE_2 is also metabolized in a single pass through the lungs to 13,14-dihydro-15-keto prostaglandin E_2 (Ferreira and Vane, 1967), sufficient quantities are found in plasma for direct measurement. Concentrations of PGE_2 and PGFM (pg/mL) were measured in duplicate directly from bovine plasma samples collected at approximately 12 mo of age and at 4 d and 5 wk postpartum using ELISA kits (PGE_2 , 500141; PGFM, 516671; Cayman Chemical) according to the manufacturer's instructions and as described previously (Almughlliq et al., 2017, 2018). These 3 time points were chosen to represent the median age of puberty (12 mo; Meier et al., 2021b), a time of elevated prostaglandin synthesis (4 d postpartum; Guilbault et al., 1984a,b), and following resolution of uterine inflammation after calving in healthy cows (Dadarwal et al., 2017). Animals with either the *AA* or *GG* SNP genotype were chosen for analysis, given these 2 genotypes would contain only one form of the *HSD17B12* protein (wild-type or mutated). Samples were chosen from animals that had samples from all 3 collection time points available, with representations from both the POS and NEG fertility groups. The PGE_2 and PGFM assay sensitivities were 36 and 15 pg/mL, and intra-assay variations were 6.4% and 10.8%, respectively. Approximately 10% of samples were above the top standard of the PGFM assay, and these extrapolated assay values were included in final analysis with the data for both PGFM and PGE_2 $\log_{10}$ transformed before statistical analysis to normalize distribution; median values are reported.

Statistical Analysis

Statistical analysis was undertaken in GenStat version 20. Of the original 523 animals assayed for genotypes, 39 were removed from the data set because genotype was unable to be determined, and an additional 41 animals were removed due to having no information recorded as 2 yr olds because they either failed to become pregnant as heifers and thus were culled (22) or they died or were culled early in their first lactation. Instances where data were not available due to missing

samples or assay failure were treated as missing data. A total of 3 animals, 2 from the POS fertility group and 1 from the NEG fertility group, all of the *AG* genotype, were bred by a Herford bull while segregated into the "sick group" (Meier et al., 2021a). These animals were retained in the data set. Thus, the final data set contained 443 animals, with the POS fertility group consisting of 53 *AA*, 109 *AG*, and 77 *GG* animals. The NEG fertility group contained 53 *AA*, 90 *AG*, and 61 *GG* animals. To determine the associations between the identified SNP and the binomial traits, data were fitted with logit link via hierarchical generalized linear models. Baseline models included SNP genotype (*AA*, *AG*, or *GG*) and fertility group (NEG or POS) for all models and Julian calendar calving day (SR21, SR42, PVD42, PMN42, and PR42) or calving day and synchronization treatment (CR1stAI and PRFin). The interaction between SNP and fertility group was also included. Sire was fitted as a random effect, but only SR21 and SR42 converged, so sire was removed from the remaining models. The SNP was originally fitted as a category, and in cases where a tendency ($0.05 < P < 0.10$) for differences between the SNP categories was present, a linear model (i.e., *AA* = 0, *AG* = 1, and *GG* = 2) was fitted.

Plasma concentrations of PGFM and PGE_2 were measured on 3 separate occasions for each animal, and so were analyzed as repeated measures data. The most parsimonious variance-covariance structure was selected by comparing the reduction in model deviances relative to the degrees of freedom (df) via chi-squared change in deviance tests. Data were modeled on the $\log_{10}$ scale. For PGFM, the model selected had uniform correlation structure with heterogeneous variances ($P < 0.001$). The most appropriate variance-covariance structure for $\log_{10}(PGE_2)$ was an order-1 autoregressive model with heterogeneous variances ($P = 0.007$). The model to determine the effects of different SNP genotypes on PGFM or PGE_2 concentrations included time of sample collection (12 mo of age, 4 d postpartum, or 5 wk postpartum), fertility group, and SNP genotype, as well as all interactions. A significant 3-way interaction existed for PGFM; thus, each time point was analyzed separately for both hormones. For the sample collected at 4 d postpartum, a fertility group by SNP genotype interaction was observed for PGFM; thus all 3 time points were examined with fixed effects of fertility and SNP genotype as well as the interaction for both PGFM and PGE_2 .

The relationships between PGFM and PGE_2 concentrations and the reproductive traits were also examined. Fixed effects for both models included time, fertility group, and the reproductive trait, as well as all interactions. Interactions between time and SR21,

Table 1. Association of different genotypes of SNP in *HSD17B12* with the adjusted mean (95% CI) of various reproductive traits in 2-yr-old Holstein-Friesian cows

Trait ¹	Genotype			P-value
	AA	AG	GG	
Number of observations	87–106	166–199	116–138	
SR21 (%)	64.7 (55.1, 73.3)	72.0 (64.2, 78.6)	78.3 (69.8, 84.9)	0.021
SR42 (%)	79.9 (71.2, 86.5)	82.0 (74.8, 87.5)	86.1 (78.6, 91.3)	0.379
CR1stAI (%)	46.9 (38.6, 55.4)	47.8 (40.7, 55.0)	50.8 (42.2, 59.3)	0.804
PR42 (%)	48.3 (39.7, 57.1)	50.8 (42.4, 58.2)	55.8 (46.9, 64.4)	0.487
PRFin (%)	76.1 (67.9, 82.8)	71.9 (64.6, 78.2)	75.1 (66.4, 82.1)	0.679
PVD42 (% score >1)	17.8 (11.9, 25.8)	16.8 (11.9, 23.3)	25.7 (18.5, 34.4)	0.156
PMN42 classification (% >2% PMN)	42.2 (34.0, 50.9)	36.0 (29.2, 43.4)	39.6 (31.4, 48.5)	0.539

¹SR21, SR42 = submitted for breeding in the first 21 or 42 d of the breeding period, respectively; CR1stAI = conceived to first artificial breeding; PR42 = became pregnant within the first 42 d of the breeding period; PRFin = pregnant at the end of the 98-d breeding period; PVD42 = purulent vaginal discharge score at approximately 42 d postpartum; PMN42 = PMN in vaginal discharge at approximately 42 d postpartum.

SR42, and PR42 were observed for PGE₂ so analysis was undertaken for each time separately for these traits. An interaction was also observed between time and PVD42 score classification for PGFM, so each time was also analyzed separately for this trait for PGFM. For PGE₂, to determine whether PGE₂ concentrations were associated with resumption of reproductive cycles at 5 wk postpartum, cows were classified into 2 groups (yes, resumed reproductive cycles; no, had not resumed reproductive cycles) based on concentrations of progesterone in milk samples as described (Meier et al., 2021a). Analysis was limited to PGE₂ concentrations 5 wk postpartum, and the model included resumption of reproductive cycles at 5 wk classification, fertility group, and the interaction between classification and fertility group. Concentrations of PGE₂ on the log₁₀ scale were assessed using ANOVA. Estimated means were back-transformed and presented with 95% confidence intervals.

Data are presented as means (reproductive traits) or median (PGE₂ and PGFM) values with 95% confidence intervals that were back-transformed from the model-adjusted predictions.

RESULTS

Associations Between *HSD17B12* SNP and Reproductive Traits

The SR21 tended ($P = 0.07$) to differ among the different genotypes of the *HSD17B12* SNP. Examining the differences between genotypes with a linear model provided evidence that SR21 increased from AA through AG to GG animals ($P = 0.021$), with a 14-percentage-point difference between the AA and GG animals (Table 1). By 6 wk of breeding, differences between the genotypes no longer reached significance ($P = 0.379$; Table 1). No associations were observed between the other reproductive traits and genotype of

HSD17B12 SNP (Table 1). We found no interactions ($P \geq 0.10$) between fertility group and SNP for any of the traits examined.

Changes of PGE₂ and PGFM Over Time

Both PGE₂ and PGFM concentrations in plasma changed over time ($P < 0.001$; Table 2), but concentrations were similar between fertility groups ($P > 0.43$; data not presented). No interaction was observed between fertility group and time for either prostaglandin ($P > 0.56$). Plasma concentrations of both PGE₂ and PGFM were similar at 12 mo of age and 5 wk postpartum ($P > 0.16$; Table 2). Plasma concentrations of PGE₂ were approximately 15% lower ($P < 0.001$) at 4 d postpartum than those observed at 12 mo of age or 5 wk postpartum (Table 2). Concentrations of PGFM in plasma at 4 d postpartum were increased ($P < 0.001$) approximately 15-fold compared with values obtained at 12 mo of age or 5 wk postpartum (Table 2).

Associations Between *HSD17B12* SNP and PGE₂ and PGFM Concentrations

For PGFM, a 3-way interaction among time, fertility group, and SNP genotype was observed ($P < 0.001$). This interaction related to differences at 4 d postpartum (Table 3). Concentrations of PGFM in plasma were similar ($P > 0.05$) between SNP genotypes and fertility groups at 12 mo of age and 5 wk postpartum. At 4 d postpartum, concentrations were approximately 2- to 3-fold different ($P < 0.05$) between the AA and GG genotypes; however, the direction of this difference was opposite in the NEG fertility group compared with the POS fertility group (Table 3). For PGE₂, no 2- or 3-way interactions among time, fertility group, or SNP were observed ($P < 0.16$), nor was there a significant main effect of *HSD17B12* SNP genotype ($P = 0.422$).

Table 2. Changes in median (95% CI) concentrations of prostaglandin E₂ (PGE₂) and 13,14-dihydro-15-keto prostaglandin F_{2α} (PGFM) in plasma over time

Hormone	n	Time			P-value
		12 mo of age	4 d postpartum	5 wk postpartum	
PGE ₂ (pg/mL)	146	233 (209, 260)	207 (189, 227)	250 (224, 277)	<0.001
PGFM (pg/mL)	146	173 (145, 206)	2,729 (2,102, 3842)	197 (170, 228)	<0.001

Differences in PGFM and PGE₂ Concentrations Between Animals with Different Classifications for the Reproductive Traits

For PGE₂, no 2- or 3-way interactions (with fertility group or time or both) were observed ($P > 0.20$) for PVD42 score, PMN42 classification, CR1stAI, and PRFin. Furthermore, no overall differences in PGE₂ concentrations were observed between reproductive trait classifications ($P \geq 0.44$; Table 4). For SR42 and PR42, 2-way interactions were observed ($P < 0.05$) between reproductive trait classification and time. For SR21, a 3-way interaction between time, reproductive trait classification, and fertility group was observed ($P < 0.002$). Therefore, data were assessed for each time point separately for these traits. For SR21 and PR42, at 12 mo of age, a 2-way interaction between trait and fertility group was observed ($P < 0.05$). Nevertheless, further analysis of each fertility group within each time point failed to detect significant ($P > 0.07$) differences within the individual fertility groups; thus, data presented are the average of the fertility groups. For all 3 traits, animals that were submitted for breeding or that had conceived in the first 6 wk of the breeding period had approximately 1.4-fold greater ($P < 0.05$) concentrations of PGE₂ at 5 wk postpartum compared with those who were not (Table 5). This difference was not observed in samples collected at 4 d postpartum or when these

animals were approximately 12 mo of age, although a trend ($P = 0.088$) was observed for animals that were submitted for breeding within the first 3 wk to have greater PGE₂ concentrations at 4 d postpartum (Table 5). Plasma concentrations of PGE₂ [geometric mean (95% CI)] at 5 wk postpartum were not different ($P = 0.586$) between those animals that had resumed reproductive cycles at 5 wk postpartum [230.14 (172.27, 307.47); $n = 26$] and those that had not [251.19 (217.17, 290.54); $n = 103$], with no interaction ($P = 0.631$) with fertility group observed.

For PGFM, no 3-way interactions were observed between reproductive trait classification, fertility group, and time ($P > 0.22$). Examining 2-way interactions, after dropping the 3-way interaction term, highlighted an interaction between PVD42 score classification and time ($P = 0.037$). Therefore, each time point was analyzed separately. However, significant differences between PVD42 classifications were not observed at any time point ($P > 0.15$) or overall ($P = 0.844$); thus, the data are presented in Table 6 for all time points combined. Plasma concentrations of PGFM were increased approximately 1.2-fold ($P = 0.023$), on average, in cows that failed to conceive on their first service compared with those that did conceive (Table 6). A similar trend ($P = 0.056$) was observed, whereby cows that were not pregnant at the end of the breeding period had greater PGFM concentrations, on average, compared with those that were pregnant (Table 6).

Table 3. Adjusted median concentrations (95% CI) of prostaglandin E₂ (PGE₂) and 13,14-dihydro-15-keto prostaglandin F_{2α} (PGFM) in plasma at each time point for each *HSD17B12* SNP genotype (*AA* or *GG*) within each fertility breeding value group

Parameter ¹	Positive fertility group		Negative fertility group	
	<i>AA</i> (n = 32–33)	<i>GG</i> (n = 39–40)	<i>AA</i> (n = 33)	<i>GG</i> (n = 38–40)
PGE ₂ (pg/mL)				
12 mo old	224 (177, 281)	246 (200, 303)	226 (180, 284)	234 (189, 289)
4 d PP	194 (160, 235)	243 (204, 290)	199 (164, 241)	193 (162, 230)
5 wk PP	229 (183, 286)	280 (229, 343)	264 (211, 329)	228 (186, 279)
PGFM (pg/mL)				
12 mo old	164 (114, 238)	192 (137, 268)	176 (121, 254)	160 (115, 224)
4 d PP ²	1,726 (1,017, 2,928)	4,732 (2,927, 7,648)	3,828 (2,242, 6,537)	1,750 (1,083, 2,828)
5 wk PP	214 (156, 293)	185 (139, 245)	206 (151, 281)	190 (142, 253)

¹PP = postpartum.

²Values differ between genotypes within each fertility group, $P < 0.05$.

Table 4. Adjusted median (95% CI) concentrations of prostaglandin E₂ (pg/mL) in plasma in 2-yr-old Holstein-Friesian cows classified for various reproductive traits

Trait ¹	n	No	n	Yes	P-value
PVD42	99	227 (205, 250)	26	256 (209, 312)	0.440
PMN42	83	228 (204, 254)	59	236 (206, 268)	0.967
CR1stAI	72	230 (204, 258)	73	232 (206, 260)	0.791
PRFin	41	233 (195, 277)	105	230 (208, 253)	0.997

¹PVD42 = purulent vaginal discharge classified as affected (yes; score >1) at approximately 42 d postpartum; PMN42 = PMN in vaginal discharge classified as affected (yes; PMN ≥2%) at approximately 42 d postpartum; CR1stAI = conceived to first artificial breeding; PRFin = pregnant at the end of the 98-d breeding period.

DISCUSSION

In this study, we examined the relationships between prostaglandin concentrations, SNP genotypes in *HSD17B12*, and reproductive traits in seasonally managed Holstein-Friesian dairy cows with divergent genetic merit for fertility. We determined associations between the SNP in *HSD17B12* and fertility traits, as well as between circulating prostaglandin concentrations and fertility traits.

Our results are consistent with a potential role for *HSD17B12* in reproductive performance in dairy cattle. The SR21 was increased in animals that carried the G allele of the missense SNP in *HSD17B12*. Furthermore, the effect was additive, with each increase of the G allele increasing SR21 by 6 to 7%. Failure to express estrus during the first 3 wk of the breeding season delays the opportunity to become pregnant for the cow. In seasonal systems, where cessation of lactation is at a set date for all animals in the herd, a delay in conception decreases milk production through reduced number of days in milk (Lindley et al. 2021). By 6 wk into the breeding season, no significant associations between the SNP and percentage of animals submitted for breeding was observed, indicating that the relationship declined over time. Given the seasonal nature of breeding in the

New Zealand dairy system, this is likely due to the increased time from calving, as all animals would have an additional 21 d to resume their reproductive cycles. It is, however, important to note that the animals assessed in this trial were at the extremes for FertBV. Potentially, observations from these animals may not be upheld in animals not selected on FertBV. However, no interaction was observed between FertBV group and this SNP, and distribution of genotypes were similar between the FertBV groups, supporting that the relationship with the SNP and fertility would be observed in animals with varying FertBV. In non-seasonally managed dairy cattle, this SNP has been associated with daughter pregnancy rate (Cochran et al., 2013; Ortega et al., 2016), with the G allele also being associated with more positive outcomes. Although no significant association was detected with PR42 nor PRFin in the current study, we observed a linear numerical trend for a 7.5-percentage-point increase in PR42 rate from AA to GG alleles, consistent with the relationship observed for SR21 and previously published reports of an association with daughter pregnancy rates (Cochran et al., 2013; Ortega et al., 2016). It remains to be determined whether this relationship will reach statistical significance if tested across a larger study population. It is also important to consider the differences in manage-

Table 5. Adjusted median (95% CI) concentrations of prostaglandin E₂ (pg/mL) in plasma of 2-yr-old Holstein-Friesian cows classified for various reproductive traits at 12 mo of age, 4 d postpartum, and 5 wk postpartum

Parameter ¹	n	No	n	Yes	P-value
SR21					
12 mo old	50	236 (193, 288)	95	244 (214, 279)	0.394 ²
4 d PP	50	180 (151, 215)	95	219 (195, 246)	0.088
5 wk PP	50	201 (164, 247)	96	290 (254, 332)	<0.001
SR42					
12 mo old	39	257 (185, 358)	106	244 (215, 277)	0.262
4 d PP	39	174 (130, 233)	106	215 (192, 241)	0.186
5 wk PP	39	180 (129, 251)	107	288 (254, 328)	<0.001
PR42					
12 mo old	70	235 (201, 275)	75	244 (210, 285)	0.703 ²
4 d PP	71	204 (178, 235)	74	211 (185, 242)	0.736
5 wk PP	71	219 (187, 258)	75	293 (251, 343)	0.011

¹SR21, SR42 = submitted for breeding in the first 21 or 42 d of the breeding period, respectively; PR42 = became pregnant within the first 42 d of the breeding period; PP = postpartum.

²Interaction ($P < 0.05$) between fertility group and reproductive trait.

Table 6. Adjusted median plasma concentrations (95% CI) of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (pg/mL) from all time points in plasma of 2-yr-old Holstein-Friesian cows classified for various reproductive traits

Trait ¹	n	No	n	Yes	P-value
PVD42	99	454 (378, 545)	26	513 (356, 740)	0.844
PMN42	83	409 (335, 500)	59	546 (430, 693)	0.148
SR21	50	461 (343, 620)	96	441 (363, 534)	0.709
SR42	39	290 (178, 471)	107	461 (384, 554)	0.475
CR1stAI	72	500 (403, 620)	73	419 (339, 518)	0.023
PR42	71	469 (373, 590)	75	437 (350, 545)	0.432
PRFin	41	532 (387, 733)	105	415 (347, 497)	0.056

¹PVD42 = purulent vaginal discharge classified as affected (yes; score >1) at approximately 42 d postpartum; PMN42 = PMN in vaginal discharge classified as affected (yes; PMN $\geq 2\%$) at approximately 42 d postpartum; SR21, SR42 = submitted for breeding in the first 21 or 42 d of the breeding period, respectively; CR1stAI = conceived to first artificial breeding; PR42 = became pregnant within the first 42 d of the breeding period; PRFin = pregnant at the end of the 98-d breeding period.

ment systems between the studies. The seasonal nature of pasture-based dairying places different constraints on the reproductive process. Importantly, however, all 3 studies have observed positive associations between the G allele of this SNP and fertility outcomes. Thus, 3 separate studies have consistently linked the G allele of this SNP to improved fertility in cattle, which, when considered together, provide considerable evidence for a potential role for *HSD17B12* in regulating fertility and indicate that further investigations into this genotype are warranted.

The substitution of A for G in amino acid 243 of the protein results in arginine residue, which contains a positively charged R group, being replaced with glycine, with a polar but uncharged R group. Based on SIFT scoring, this is predicted to alter protein function. Comparison of the sequence among species confirms that arginine is commonly, but not exclusively, conserved in this position across a wide range of species, with glycine very uncommon. Although previous studies have also observed associations between this SNP and reproductive traits (Cochran et al., 2013; Ortega et al., 2016), to our knowledge, the effect of this amino acid substitution on enzyme function has not been determined. Given that complete knockout of the *Hsd17b12* gene is lethal during gestation in mice (Rantakari et al., 2010), and many animals homozygous for the G at this SNP were present in the population, it is highly unlikely that this variation results in a nonfunctional protein. Various approaches have also associated variation in the *HSD17B12* gene with obesity and type 2 diabetes (Hachim et al., 2020), longissimus muscle area (Medeiros de Oliveira Silva et al., 2017), and cancer outcome (Sun et al., 2011; Dai et al., 2019; Lin et al., 2020). One study identified transcriptional regulation as a potential functional effect of a SNP found in the region of the *HSD17B12* gene in humans that was associated with obesity and type 2 diabetes (Hachim et al.,

2020), but the functional significance of other identified SNP has not been delineated.

In mice, animals with haploinsufficiency of *Hsd17b12* were shown to have reduced ovarian concentrations of arachidonic acid as well as PGD₂, PGE₂, and PGF_{2 α} (Kemiläinen et al., 2016). We observed a complex relationship between the SNP in *HSD17B12* and prostaglandins in cattle. Although we found no evidence of a difference in circulating PGE₂ concentrations, differences were observed in PGFM between animals with either the AA or GG genotypes at 4 d postpartum when concentrations of PGFM were markedly elevated immediately following parturition. Given that *HSD17B12* is involved in arachidonic acid availability (Rantakari et al., 2010; Kemiläinen et al., 2016), it could be that the effect of the SNP may only be observed at times when supply of arachidonic acid is limiting, as might be expected to occur with the peak PGFM concentrations observed shortly after calving. It is unclear, however, why differential effects of the SNP genotype would be observed between fertility groups. In the POS fertility group, cows with the GG genotype had greater PGFM concentrations than those with the AA genotype, but the opposite relationship occurred in the NEG fertility group. Arachidonic acid is increased in pregnant animals on d 17 of gestation compared with animals on d 17 of the estrous cycle, and increased concentrations of eicosatrienoic acid were observed in animals with increased fertility based on genetic strain (Meier et al., 2009), demonstrating some potential for interactions with genetics. Alternatively, given the large variability in PGFM concentrations at d 4, the observed interaction could be an anomaly of the current data set, potentially related to the selection of animals at the extremities for fertility breeding values, unrelated to biological function. Examining all time points, with interactions removed, no main effect of the SNP on either PGFM or PGE₂ was observed; thus, it seems

unlikely that the change of the amino acid caused by the SNP resulted in a direct effect on prostaglandin synthesis. Thus, our original hypothesis, that the SNP would alter circulating prostaglandin concentrations, was not consistently supported. Additional studies to better understand the potential effects of the variation in the *HSD17B12* sequence on protein function are warranted. This includes potential effects on arachidonic acid availability and prostaglandin synthesis as well as steroid synthesis, as *Hsd17b12* has also been linked to changes in estrogens and androgens (Luu-The et al., 2006; Bellemare et al., 2010), although this was not observed in all studies (Kemiläinen et al., 2016).

Concentrations of PGE₂ and PGFM differed over time, with altered circulating concentrations at 4 d postpartum compared with those observed at either 12 mo of age or 5 wk postpartum. Plasma concentrations of PGE₂ were slightly suppressed, whereas PGFM concentrations were elevated. Increased concentrations of PGFM during the early postpartum period have been observed previously, and the uterus has been identified as the major source of PGF_{2α} and capable of metabolizing it to PGFM (Guilbault et al., 1984a,b). The uterus has also been shown to produce PGE₂ and its metabolite PGEM during the early postpartum period (Guilbault et al., 1984a). These prostaglandins are thought to be involved in uterine involution (Madej et al., 1984), and, given the dramatic rise in PGFM associated with a small decrease in PGE₂ at d 4 postpartum, synthesis of PGF_{2α} would seem to dominate, potentially at a slight cost to PGE₂ synthesis, compared with other time points. Several studies have examined the potential for exogenous PG administration during the early postpartum period to improve fertility—and have observed limited success (Hirsbrunner et al., 2006; Haimel et al., 2012, 2018; Stephen et al., 2019).

Our results, however, provide some evidence that greater PGE₂ concentrations during the postpartum period may be linked to earlier submission for breeding and, therefore, earlier conception. We determined that cows that were submitted for breeding during the first 3 or 6 wk of the breeding period had greater PGE₂ at 5 wk postpartum, with those that were submitted within 3 wk also tending to have greater PGE₂ at 4 d postpartum. Given that plasma concentrations of PGE₂ did not differ between animals that had reinitiated reproductive cycles by 5 wk postpartum and those that had not, this did not appear to be linked to an association between cyclicity at 5 wk postpartum, which would be expected to increase the chance of expression of estrus during the first 3 and 6 wk of the breeding season, and PGE₂ concentrations. Although further work is required to understand the causal relationship between greater PGE₂ postpartum and greater submission

rates during breeding in seasonally managed dairy cows, altered PGE₂ has previously been associated with changes in reproduction-associated parameters. Highly fertile animals, based on serial embryo transfer, have increased PGE₂ concentrations (Moraes et al., 2020). Additionally, mRNA encoding enzymes important for prostaglandin synthesis, as well as receptors for PGE₂, are upregulated in animals with higher fertility based on genetic strain (Walker et al., 2012). Increases in prostaglandin concentrations are also associated with ovulation in animals with induced uterine infection, although PGFM concentrations were more associated with ovulation than PGEM (Kaneko and Kawakami, 2009). Furthermore, treatment of bovine endometrial epithelial cells with PGE₂ reduced the inflammatory response induced by LPS treatment (Shen et al., 2018). Increased understanding of the role of PGE₂ in uterine health and involution, inflammation, and resumption of reproductive cycles during the postpartum period may provide new insights for improving fertility in cattle.

In contrast, PGFM concentrations were inversely linked to conception irrespective of when they were measured, as animals that conceived to their first service had lower PGFM concentrations, on average, with the same trend observed for those that were pregnant at the end of breeding. These relationships appear to contrast with prior work whereby high-fertility heifers had greater PGF_{2α} compared with those of low fertility, based on classification of serial embryo transfer (Moraes et al., 2020). However, PGF_{2α} has been shown to have a negative effect on the early embryo even with exogenous progesterone support, potentially through interactions with the corpus luteum and stimulation of oxytocin release (Buford et al., 1996; Seals et al., 1998).

We did not observe any significant associations with the *HSD17B12* SNP and measurements of uterine infection, nor differences in either PGFM or PGE₂ concentrations when we classified the animals as affected or non-affected according to PVD score or PMN percentage. The associations between prostaglandins and uterine infection are unclear. Some previous studies have indicated that concentrations of PGFM and PGEM are increased early postpartum in animals that develop puerperal metritis or clinical endometritis (Baithalu et al., 2019). Others have reported that endometrial epithelial cells exhibited reduced PGF_{2α} production when treated with exosomes isolated from plasma of cows with a uterine infection compared with those treated with exosomes isolated from healthy cows (Almughliq et al., 2017). Additionally, concentrations of PGFM tended to be lower in cows with endometritis (based on Metricheck reading) than healthy cows (Kawashima et al., 2018). Differences between these studies could be due to differences in cow breeds, methods of classifica-

tion of endometritis or uterine infection, and in vitro versus in vivo analysis.

CONCLUSIONS

We determined that the missense SNP in *HSD17B12* predicted to affect protein function is associated with earlier submission for seasonal breeding, perhaps due to earlier resumption of cyclicity postpartum. Whether the *HSD17B12* SNP might be linked to differences in prostaglandin concentrations, as was previously observed in mice with haploinsufficiency, was uncertain given the complex interactions observed between fertility group and time of sample collection; however, no consistent effect of genotype over time on plasma concentrations of either PGE₂ or PGFM were observed. Plasma concentrations of PGE₂ and PGFM differed over time, with much larger quantities of PGFM produced shortly after calving but, conversely, slightly suppressed PGE₂ at this time. These changes in PGFM and PGE₂ are likely associated with involution of the uterus. We observed positive associations between PGE₂ concentrations during the postpartum period and submission for breeding. In contrast, PGFM concentrations were negatively associated with conception traits. Further work to understand both the effect of the missense SNP in *HSD17B12* and the role of prostaglandins during the postpartum period on fertility seems warranted.

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