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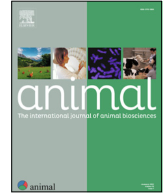

The effect of natural and induced calving of beef heifers on stress-related gene expression and maternal health and immunity

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ABSTRACT

The peri-partum processes can exert stress on a cow on many levels. There is little evidence about acute stress around the calving event and subsequent potential effects for the cows' immunological status or subsequent reproductive health. To investigate this, 55 crossbred recipient beef heifers carrying purebred Simmental embryos were assigned to one of three groups on day 285 of gestation: (i) control (no parturition induction treatment; n = 19); (ii) induction of parturition with corticosteroid (n = 20) and (iii) induction of parturition with corticosteroid plus prostaglandin (n = 16). Interval from induction of parturition to calving and calving ease was recorded. Reproductive tract examinations were conducted on Day 21 (**D21**) and Day 42, and a sample was obtained for the determination of uterine cytology on D21. Blood samples were taken from the dams two weeks before parturition, one day after parturition (**D1**) and two weeks after parturition (**D14**) for gene expression and cortisol and calcium concentration determination. Calves were weighed at birth and subsequently every week until they were 10 weeks of age. A colostrum sample was taken immediately after calving and stored for subsequent Immunoglobin G (**IgG**) concentration analysis. Data were analysed using ANOVA with posthoc Tukey, Spearman correlation and stepwise backwards linear regression using SAS. Quantitative reverse transcription PCR was performed on the following immune genes: *Interleukins IL1a* and *b*, *IL2*, *IL4*, *IL8*, *Tumour Necrosis Factor Alpha*, *Interferon-gamma*, *Lymphotoxin*, *Toll-Like Receptor*, *Nuclear factor of kappa light* polypeptide gene enhancer in B cells 1 and 2, *glucocorticoid receptor alpha*, as well as the neutrophil genes that regulate inflammation: *Fas*, *L-selectin*, *MMP-9* and *BPI*. The results show that compared with non-induced contemporaries, induction has no negative effect on dystocia or subsequent calf weight gain but can have a positive effect on colostral IgG concentration. Blood calcium concentrations on both D1 and D14 postcalving are associated with subsequent uterine health. Parturition events were reflected in temporal changes in the expression of the cytokines *IFNγ*, *TNFα*, *IL1b*, *IL4*, *IL8* and *Haptoglobin* in the dams' blood, all of which are associated with the immune competence of the cow during this period. The conclusion is that induction of calving can have a positive effect on colostral IgG concentration. Calcium concentrations postcalving are associated with subsequent reproductive tract health. Events associated with the peri- and postpartum period are all reflected in temporal changes in immune function-related cytokines.

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Implications

Calving and the processes associated can be regarded as stress-inducing events. Research presented here shows that induction of calving has no negative effects on dystocia or subsequent calf weight gain but can have a positive effect on colostral IgG concentration. Calcium concentrations postcalving are associated with subsequent reproductive health. Events associated with calving are reflected in temporal changes in expression of the cytokines *IFNγ, TNFα, IL1b IL4, IL8* and *Haptoglobin*, leading to the conclusion that calving does lead to a stress response in the animal. This should be considered when managing a cow around calving time.

Introduction

Cows can experience multiple types of stress: these can be categorised as acute or short term stress and chronic or long term stress (Dobson and Smith, 2000). Stress can have a negative effect on reproduction (Crowe and Williams, 2012), specifically on the resumption of cyclicity in beef cows (Crowe, 2008; Crowe et al., 2014). Stress can also have a subsequent effect on calf health

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(O'Loughlin et al., 2011), but there is scant information about the effect of acute stress around the actual calving event and the effects for cow health.

There is little published data on the effect of acute stress around the actual calving event or dystocia on subsequent possible knock-on effects for the cows' immunological status and or subsequent reproductive health. Chronic stress and pain have been associated with disturbances of the hypothalamic–pituitaryadrenal axis. This can lead to disrupted patterns of pulsatile GnRH release, potentially depriving developing ovarian follicles of sufficient gonadotrophin support (Dobson and Smith, 2000; Walker et al., 2008). Stress in all its forms is a major suppressor of the immune system with stressed animals being more susceptible to disease (Carroll and Forsberg, 2007). The immune function of a cow during the calving period is negatively affected, which can lead to uterine disease postcalving (LeBlanc et al., 2011). There is also an increased risk of the development of mastitis and suppression of appetite, all with subsequent effects for the health and reproductive potential of the animal (Dobson et al., 2008). The incidence and effects of dystocia as a stressor can be minimised by monitoring and controlling the parturition (Adams, 1969; Wagner et al., 1974). This can be achieved with the pharmacological induction of parturition, thereby reducing the range of parturition timing and thus ensuring maximisation of supervision of parturition and a decrease in dystocia and the associated stress (Mee, 2004; Villarroel and Lane, 2010).

Induction of parturition, however, can have the potential negative effects as most methods include the use of a corticosteroid which can have immunosuppression as an unwanted side effect (Mansell et al., 2006). The immune status of an animal in relation to stress can be determined by measuring systemic parameters such as cortisol and calcium. Cortisol is the primary hormone that is released by the adrenal glands in response to stress and as such plasma cortisol concentrations rise abruptly when an animal is experiencing stress (Sapolsky et al., 2000). The rise of cortisol leads to a modulation in response of the normal immune system with the main effect being the inhibition of pro-inflammatory cytokines (Hughes et al., 2014). The negative effect of stress on subsequent health and growth in cattle has been well reported with an increased susceptibility to disease as the effect of the exposure to stressors (Ting et al., 2004; Buckham Sporer et al., 2007; O'Loughlin et al., 2011). While there are some studies reporting differences in gene expression profiles between animals that have experienced stress and those that have not (Ting et al., 2004, Buckham Sporer et al., 2007), there is limited knowledge available about the components of the immune system that are affected during the peri-partum period. Calcium concentrations around parturition can affect the immune system via a reduced immune cell activation due to reduced intracellular calcium signalling as well as a reduction of phagocytic capacity of neutrophils (Ducusin et al., 2003; Kimura et al., 2006; Goff, 2008).

The immune response is affected around parturition, determining the relative functionality of the immunological cells (i.e., leucocytes) and their potential to mount an immunological defence during the peri-partum period (Crowe and Williams, 2012). The effects of calcium and cortisol concentrations on the immune response would help to avoid or manipulate the potential negative effects of parturition and a stressor on future productive and reproductive performance of the cow (Crowe and Williams, 2012).

Colostrum is the first secretions from the mammary gland after calving and contains the vital immune components for the naive immune system of the calf. The calf relies on the passive immunity derived from colostrum until its own immune system is functional at 1–2 months of age. Colostrum contains antibodies or immunoglobulins necessary to provide the calf with protection from disease (Lorenz et al., 2011). There are many factors that affect the quality of the colostrum in cattle, such as age of the cow and interval from cessation of the previous lactation (Conneely et al., 2013). Disease or other stressors can also affect colostrum quality in a negative way, thus potentially depriving the calf of adequate priming of the immune system (Gulliksen et al., 2008). The aim of this study was to determine the effect of induced calving of full-term beef heifers' immune status via a targeted gene expression study of genes previously associated with stressful events such as weaning and transport and to investigate the effect of the parturition as stressor on subsequent reproductive health as well as on colostrum IgG concentration in order to elucidate what events take place within the cows' system during this time. Our hypothesis was that calving would have a negative effect on (i) the general health of the cow and the associated gene expression as well (ii) on colostrum quality. Preliminary data of this study have been published both in abstract and poster form (Beltman et al., 2016a; 2016b).

Material and methods

Experimental design

The experimental design used for this study has been previously described in detail (Šavc et al., 2016) and is briefly outlined here. Crossbred pregnant Bos Taurus beef heifers (n = 55) with a mean BW of 555 kg were used and these heifers received embryos from purebred Simmental cows through non-surgical transfer, seven days after a synchronised oestrus. The research herd from which the embryos were sourced has been described previously (Lawrence et al., 2011). Pregnancy was confirmed at 35 days and again at 90 days of pregnancy using transrectal ultrasonography using a 7.5-MHz linear-array transducer, and foetal sex was determined at this latter scan. All heifers were managed as a single group throughout pregnancy and were fed a grass silage on an *ad libitum* basis prior to calving, fortified with 100 g of a commercial mineral supplement/day. All animals were body condition scored using a five-point system (Edmonson et al., 1989) two weeks before calving, at calving and two weeks after calving. Based on the average gestation length of the herd, the predicted transmitting ability (**PTA**) of the sires used for gestation length, and the proportion of male and female foetuses an average gestation length of 287 days were estimated for the group of 55 heifers. At day 285 of gestation, heifers were blocked by live-weight, body condition score, foetal gender and foetal sire PTA for gestation length and assigned to one of three groups: (i) Control (no induction treatment; **CON**; n = 19); (ii) induction with corticosteroids (**CORT**; n = 20) or (iii) induction with corticosteroids plus prostaglandin (**CORT + PG**; n = 16). Both CORT and CORT + PG received 0.08 mg dexamethasone per kg BW, subcutaneously (Colvasone®, Norbrook Laboratories, Newry, Co. Down, Ireland) while CORT + PG also received 500 μg of cloprostenol (Estrumate®, MSD Animal Health, Dublin, Ireland), intramuscularly. Following administration of treatment drugs, all heifers received 24 h supervision and calvings were assisted, where necessary. Interval to calving from induction, calving ease and level of assistance were recorded on a scale of 1–5: 1, no assistance; 2, slight assistance; 3, severe assistance; 4, veterinary assistance and 5, caesarean section (Lombard et al., 2007) and the incidence of retained foetal membranes (retention > 24 h) was also recorded. Blood samples were collected at 2 weeks before parturition (**D-14**; day 272 of gestation), 1 day after parturition (**D1**) and 2 weeks after parturition (**D14**) for subsequent analysis of cortisol and calcium concentrations. Calves were weighed at birth and a colostrum sample was taken from the dam within 3 hours after calving and stored at −20 °C for subsequent analysis. All calves were fed a minimum of 1 litre colostrum from their

own dam within 3 hours of birth. As the calves were destined for another study, the objective of which was to examine the biological control of feed efficiency, all calves were weaned from their dams between 4 and 7 days of age. They were subsequently weighed weekly until they were 10 weeks of age. Cows were fed grass silage at a restricted rate in the first week after weaning to ensure prompt drying off and mammary gland involution. Subsequently, the cows were kept on a diet of grass silage offered *ad libitum* and supplemented with three kilograms of concentrates daily for the remainder of the experimental period. All animals received a reproductive tract examination using an ultrasound scanner (BCF Technology, Easi-scan®, 7.5-MHz linear-array transducer) on day 21 (**D21**) and again on day 42 (**D42**) postcalving to assess uterine involution and cyclicity using a scale as described by Šavc et al. (2016). Briefly, the amount of fluid, the echogenicity of the fluid and the visibility of the endometrial folds on cross-sectional view of the uterine horns were scored on a scale of 1–5. At each scan, cows were considered to have resumed ovarian cyclicity if the presence of the corpus luteum was confirmed (Forde et al., 2011).

A uterine cytology sample was obtained on D21 to assess uterine infection (LeBlanc, 2008) as described by Šavc et al. (2016). This involved the insertion of a cytobrush through the cervix to collect a cytology sample, which were subsequently transferred to slides, dried and stained followed by evaluation.

Description of critical methods

A vaginal mucus sample was taken on both D21 and D42 by means of a Metricheck® and scored on a scale of 1–3 as described by (Sheldon et al., 2006). Using this system, a score 0 was given if clear mucus was collected, a score 1 if the mucus collected was clear with small flecks of pus, a score 2 if the mucus collected was ≤50 ml with ≤50% purulent material and score 3 if it was ≥50 ml with ≥50% purulent material and/or had a bloody appearance.

A cytological sample of the uterine lumen was also taken on D21 using a previously published technique (Barlund et al., 2008). After collection, intrauterine smears were air dried, and slides were marked with date and animal identification number prior to analysis. Smears were fixed using anhydrous methanol and were stained using an Aerospray® Haematology Stat automatic slide stainer. Slides were stained using eosin (SS-035C), thiazin azure B/methylene blue (SS-035/049B) and rinsed using light eosin rinse (SS-035A), all in an aqueous solution (approx. 98%). A positive cytological sample was defined as the presence of >18% neutrophils in the sample (Sheldon et al., 2006).

All colostrum samples were analysed using a Biopanda Bovine IgG1 ELISA kit at 1/1000 K dilution as per the manufacturer's protocol. The inter- and intra-assay coefficients of variation (CV) for colostrum were 9 and 3%, respectively.

Blood samples were collected into TEMPUS® tubes D-14, D1 as well as D14 after parturition. RNA was isolated from blood samples using the Tempus Spin RNA isolation reagent kit, according to the manufacturer's guidelines (Applied Biosystems, USA). qPCR was performed on a selection of genes (Interleukins *IL1a* and *b*, *IL2*, *IL4*, *IL8*, *Haptoglobin*, Tumour Necrosis Factor Alpha (*TNFα*), Interferon-gamma (*IFNv*), *Lymphotoxin*, Toll-Like Receptor 4 (*TLR4*), Nuclear factor of kappa light polypeptide gene enhancer in B cells 1 and 2 (*NFkB1*, *NFkB2*), glucocorticoid receptor alpha (*GRa*), Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Zet (*YWHAZ*), *L-selectin* (*CD62L*) and Cyclin-dependent kinase inhibitor 1 (*p21*)as well as the neutrophil genes that regulate inflammation such as *Fas*, *L-selectin*, *MMP-9* and *BPI*) based on recently published research (Buckham Sporer et al., 2007; Eckersall and Bell, 2010; O'Loughlin et al., 2011). Primer sequences of all genes used are displayed in Table 1. PCR efficiencies were determined for all primers used through serial dilutions of pooled cDNA samples, ensuring that only primers with RNA efficiencies of between 90 and 110% were used in this study. Real-time quantitative PCR was performed on all genes using an Applied Biosystems 7500 Fast Real-Time PCR system. Reactions were carried out in triplicate in a total volume of 20 μl with all melting curves assessed for all genes to verify amplification of only the target gene, as previously described by O'Loughlin et al. (2011).

Validation and quality assurance

Plasma cortisol concentrations were determined by Radioimmunoassay (**RIA**) using a previously validated method (Fisher et al., 1996). The intra-assay CV for samples containing 2.95, 9.78 and 34.94 pg/ml were 11.9, 3.9 and 4.6%, respectively, and the inter-assay CV (n = 2 total assays) for the same samples were 7.9, 4.2 and 3.1%, respectively. The concentration of calcium was measured using a Randox RX Imola multichannel autoanalyzer (Randox Laboratories Ltd., Crumlin, Co. Antrim, Northern Ireland) by a colorimetric method as per the manufacturer's instructions. The minimum detectable concentration for Ca was 0.01 mmol/L, and the intra-assay CV were 4.35, 2.99, and 2.16% for high-, medium-, and low-quality control sera, respectively. Total area under the curve (AUC) was calculated for cortisol concentrations of each individual heifer in each treatment group.

The yield and quality of the resultant RNA were assessed through evaluation of the absorbance at 260 nm on a Nanodrop spectrophotometer (Nanodrop Technologies, DE, USA) and RNA integrity number (**RIN**), established through the Agilent Bioanalyser (Agilent Technologies Ireland Ltd.), respectively. All RNA samples were of sufficient high-quality RIN > 7. Complimentary DNA (**cDNA**) was synthesised from 1 μg of total RNA per sample using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Dublin, Ireland). GeNorm software (GenEx, MultiD analyses, Sweden) was employed to determine the most stable expressed reference genes based on reference gene Ct values. In the present study, the genes Actin beta (*ACTB*), Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and Succinate Dehydrogenase Complex Flavoprotein Subunit A (*SDHA*) were selected as the most stable for subsequent analyses. Raw Ct values were then imported into GenEx software (MultiD analyses, Sweden) for the following procedures: inter-plate calibration, correction of primer PCR efficiency, and Ct values of all genes of interest were normalised to reference gene Ct values. A log2 transformation of normalised Ct values was also undertaken in GenEx to provide relative abundance of the mRNA for each gene.

Statistical analysis of results

A Spearman's rank-order correlation was run using SPSS 20 for Windows to determine the association between variables. Correlated variables were subsequently excluded from the multiple linear backwards regression analysis used to develop a model for predicting IgG concentrations. Body condition score, cow weight, calf weight, calf gender, induction treatment group together with serum concentrations of calcium on D-14, D1 and D14 and serum concentrations of cortisol on D1 and D14 were included as predictor variables. ANOVA and Tukey's pairwise comparisons were made to show significant differences ($P < 0.05$) between treatment groups for blood parameters and gene expression data. Gene expression data were first checked for normality within SAS software (PROC UNIVARIATE, SAS v9.4). Data that were not normally distributed were subsequently transformed. The appropriate lambda transformation value was determined through a Box-Cox transformation analysis using the TRANSREG procedure of SAS. All gene expression data were analysed using the MIXED procedure

Table 1
Quantitative real-time PCR primer pairs used for the quantification of expression of genes associated with the immune system in calved beef cows.

Gene name	Forward sequence 3′–5′	Reverse sequence 5′–3′
IL1a	GCCGCCCTCGATCACTGACTTT	TCAGCACATGCTCAGCGAGTGA
IL1β	CAGTGCCTACGCACATGTCT	CCAGGGATTTTTGCTCTCTG
IL2	GTGAAGTCATTGCTGCTGGA	GGCGCGTAAAAGTCAAATGT
IL4	CTGCCCCAAAGAACACAACT	TCGTCTTGGCTTCATTCACA
IL8	TGGGCCACACTGTGAAAATTC	CCTTCTGCACCCACTTTTCC
IFNγ	TTCAGAGCCAAATTGTCTCC	AGTTCATTTATGGCTTTGCGC
TNFα	TGGAGGGAGAAGGGATTCTT	CCAGGAACTCGCTGAAACTC
Lymphotoxin	GCTGCATCCCTAAGAACAGC	CATCCGGCTCAAAAAATCAGT
TLR4	TGGTAAACCCCAGAGTCCAG	GCACAATGCTTGGTACATGG
NFkB1	GCACCACTTATGACGGGACT	TCCTCATCCCAGGAGTCATC
NFkB2	ATCTGAGCATTGTGCGACTG	CTTCAGGTTTGAGGCTCCAG
GRα	CCATTTCTGTTCACGGTGTG	CTGAACCGACAGGAATTGGT
Fas	AGTTGGGGAGATGAATGCTG	CCTGTGGATAGGCATGTGTG
Haptoglobin	TGGTCTCCCAGCATAACCTC	AGGGTGGAGAACCACCTTCT
BPI	TTCAGAAATGATCCAAACATGAAAC	GCCCTTGGAAGAAACAATTCC
MMP-9	TGTACGGCCCGAAGCT	CCATCGAAGGGATACCC ATCT
L-selectin	ACGGGAAAAAAGGATTACT ATGGA	GCCTATAGTTGCATATGT ATCAAATTTTCA
ACTB	ACTTGCGCAGAAAACGAGAT	CACCTTCACCGTTCCAGTTT
SDHA	AACTGCGACTCAACATGCAG	TGTCGAACGTCTTCAGATGC
GAPDH	GGGTCATCATCTCTGCACCT	GGTCATAAGTCCCTCCACGA
YWHAZ	*GCATCCCACAGACTATTTCC*	*GCAAAGACAATGACAGACCA*
CD62L	*CCGATTGCTGGACTTACCAT*	*CCAAGTCCACACCCCTTCTA*
p21	*TCCAAGGACTTTTTCCATTTGC*	*TCTGACTCCTTCAGCTGTTATTCAA*

in SAS, including terms for treatment and time-point as well as their interaction within the statistical model. Time-point was used as the repeated measure, with treatment and time-point used as fixed effects within the statistical model. The covariance structure of the statistical model was determined based on the magnitude of the Akaike information criterion (**AIC**) for models run under compound symmetry, unstructured, autoregressive, heterogeneous first-order autoregressive covariance structures. The model with the lowest AIC coefficient was selected, which for all gene expression variables was compound symmetry. Differences between treatment groups were determined by F-tests using type III sums of squares, with the PDIFF command incorporating the Tukey test applied to evaluate pairwise comparisons between treatment means. Associations between relative gene expression values and also between gene expression values and blood analytes were generated through the CORR procedure in SAS.

Results

Gestation length, calving difficulty score, retained foetal membranes, mucus score, cyclicity, uterine involution and cytology are reported in Table 2. Calving difficulty score did not differ between the two groups of animals, with 12 animals in the CON group needing veterinary assistance, 7 in the CORT group and 8 in the CORT + PG group. There was no difference in the incidence of retained foetal membranes between the three groups but there were significantly more ($P < 0.006$) animals with a mucus score of 2 or higher on D21 postpartum in both the CORT and CORT + PG groups when compared with CON group. The cytology results on D21 showed the same trend; however, this was not significant ($P < 0.08$). There was no difference between the three groups with regard to return to cyclicity by D21, but by D42, there were significantly more animals cycling in the CON group when compared with the CORT and CORT + PG groups ($P < 0.04$).

Cortisol concentrations at D-14, D1 and D14 of all groups are displayed in Fig. 1. Cortisol concentrations were not different between groups on D-14 but on D1, the CON group had greater cortisol concentrations ($P < 0.04$) compared with the two other treatment groups. On D14, the CORT + PG group had higher concentrations of cortisol when compared with the CON group, but these concentrations were not different than those of the CORT group. The AUC for the CORT concentration was also not different between groups.

Ca concentrations were not different ($P > 0.05$) between groups but there was a trend towards significance on D1 where CON had lower Ca concentrations than CORT ($P = 0.08$). The area under the curve (**AUC**) for the Ca concentration was also not different between groups. There was a positive correlation ($R = 0.863$,

Table 2
Characteristics of the beef cows in the three different treatment groups: Control (CON, n = 19), corticosteroids (CORT, n = 20) and corticosteroids combined with prostaglandin F2alpha (CORT + PG, n = 16).

Item	Sampling day	CON	CORT	CORT + PG
Average gestation length (days)		291	287	286
Calving assistance (1–5)		3.0 ± 0.32	3.1 ± 0.30	3.2 ± 0.26
Retained foetal membranes (n)		1	1	0
Mucus positive (%)	Day 21	52	70	52
	Day 42	17	26	16
Resumed cyclicity (%)	Day 21	52[a]	59[a]	29[b]
	Day 42	93[a]	89[a]	67[b]
Uterine involution (%)	Day 21	69[a]	48[b]	32[b]
	Day 42	86	85	80
Cytology positive (%)	Day 21	24[a]	48[b]	48[b]

[a,b] Indicates significant differences ($P < 0.05$).

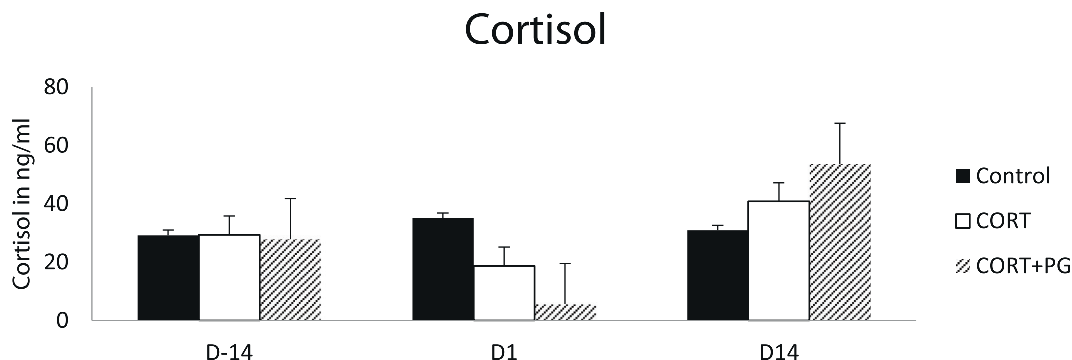

Fig. 1. Cortisol concentrations at D-14, D1 and D14 postcalving in the three different beef cow treatment groups. Induction of parturition took place on day 285 of gestation with corticosteroids (0.08 mg/kg dexamethasone subcutaneously, CORT; n = 20) corticosteroids plus prostaglandin (0.08 mg/kg dexamethasone subcutaneously combined with 500 µg of cloprostenol intramuscularly, CORT + PG; n = 16). The control group (CON; n = 19) did not receive any treatment. Concentrations were not different between groups on D-14 but on D1, the CON group had higher cortisol concentrations ($P < 0.04$) compared with either treatment groups.

$P < 0.04$) between Ca concentrations on D1 and D14 as well as a negative correlation between Ca concentrations on D14 and mucus score and cytology score on D21.

IgG concentrations for all three treatment groups are displayed in Fig. 2. IgG concentrations at calving were all above 50 g/L (McGuirk and Collins, 2004). IgG concentrations at calving did not differ between the two induced groups but were lower ($P < 0.01$) for CON compared with for either of the treatment groups. Treatment group and Ca concentrations on D-14 were strong predictors of IgG concentration.

Calf weight at birth was not different between groups and neither was the total weight gain over 10 weeks. Calf weight at birth was positively correlated with the mucus score ($R = 0.685$, $P < 0.05$), negatively correlated with uterine involution ($R = -0.426$, $P < 0.03$) and positively correlated to cytology on D21 of the dams ($R = 0.581$, $P < 0.04$).

Gene expression

Relative gene expression across treatment groups and across the three sampling time points are displayed in Fig. 3. At D1, there was increased expression of *L1b, Haptoglobin* and *IL8* ($P < 0.01$).

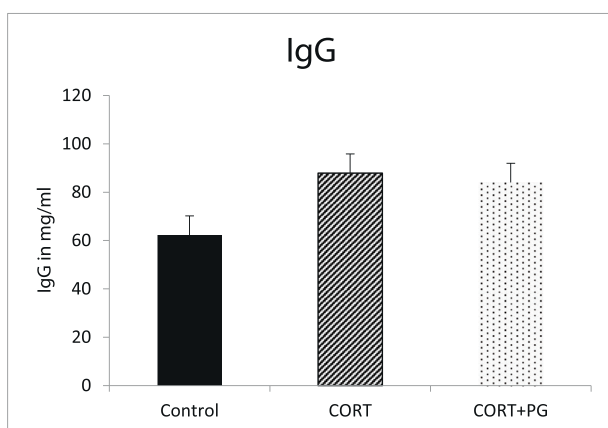

Fig. 2. Colostrum Immunoglobin G (IgG) concentrations in the three different beef cow treatment groups. Induction of parturition took place on day 285 of gestation with corticosteroids (40 mg dexamethasone subcutaneously, CORT; n = 20) and corticosteroids plus prostaglandin (40 mg dexamethasone subcutaneously combined with 500 µg of cloprostenol intramuscularly, CORT + PG; n = 16). The control group (CON; n = 19) did not receive any treatment. IgG concentrations at calving did not differ between the two induced groups but were significantly lower in the CON group ($P < 0.05$) when compared with the induced.

Immunosuppression occurred with *IFNγ* between D-14 and D1 ($P < 0.01$) and D1 and D14 ($P < 0.001$). *Lymphotoxin* and *IL4* showed a decreased expression at D1 and an increased expression at D14 ($P < 0.001$). At D1, both assisted and non-assisted induced animals had increased gene expression of *Haptoglobin*. There was an increase in gene expression of *lymphotoxin* between D1 and D14 for assisted animals induced with CORT + PG ($P < 0.01$). Calf weight ($R = -0.216$) and gender ($R = -0.271$) were negatively associated ($P < 0.05$) with the expression of *IL1b* on D1, with dams who produced male calves with a larger birth weight having an increased expression of *IL1b. IL8* was negatively associated ($R = -0.228$; $P < 0.05$) with cortisol concentrations on D1. Weight gain of the calf in week 1 was positively associated ($R = 0.223$; $P < 0.05$) with the expression of *Haptoglobin*. There was a positive association ($R = 0.242$; $P < 0.05$) between *TNα* and cyclicity on D42 but a negative association ($R = -0.278$; $P < 0.05$) between *TNFα* and mucus score at D21.There were also time-point effects ($P < 0.01$) for *BP1, TLR4, YWHAZ, MMP-9, NFKB1, NFKB2, GRa, IL1a, Fas, L-selectin, CD62L, IL2, p21*, whereby expression reduced as time progressed.

Discussion

Induction of parturition in cattle is a relatively common practice but does not necessarily have a good reputation when it comes to outcomes and animal welfare. As such, New Zealand, where induction was and is used mainly in the seasonal dairy industry, introduced strict regulations to ensure calf and cow welfare in their Code of Recommendations and Minimum Standards for the Welfare of Dairy Cattle (NAWAC 1992). In beef cattle, induction tends to not be used to fit seasonality, but to combat foetal oversize. While a number of authors report on the effect of induction on postparturient diseases such as retained foetal membranes (Villarroel and Lane, 2010; Hartmann et al., 2013) and others report on the effect of uterine disease on subsequent reproductive performance (Dubuc et al., 2011; LeBlanc, 2012; Šavc et al., 2016), very little is reported about stress around calving and subsequent knock-on effect of this stress for both cow and calf health.

In our study, we did not see an effect of induction on the incidence of retained foetal membranes as reported by others such as Nasser et al (1994) and Benedictus et al (2011). Uterine involution was more complete in the CON group when compared with the other two treatment groups on D21. While there was no residual effect of treatment on uterine involution score by D42, we found that there was an increased incidence of resumption of ovarian cyclicity by D42 in the CON and CORT groups coincidental to heifers that undergone uterine involution at D21. *TNFα* expression was decreased in animals with an increased cytology score on D21.

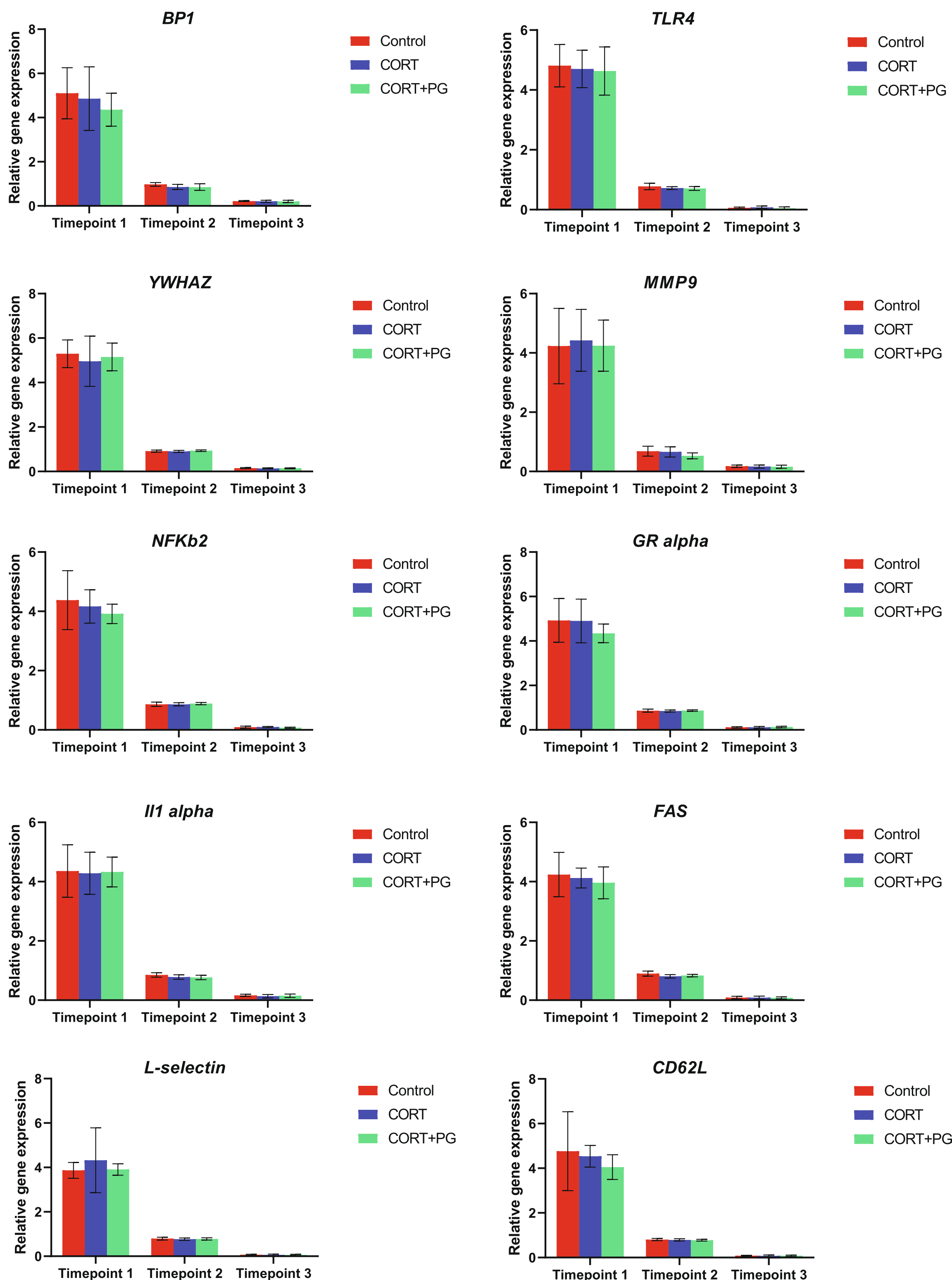

Fig. 3. Relative gene expression of all genes of interest across the three beef cow treatment groups: Control (CON, n = 19), corticosteroids (CORT, n = 20) and corticosteroids combined with Prostaglandin F2α (CORT + PG, n = 16). Blood samples were collected from the dams two weeks before (D-14; time point 1), one day after (D1; time point 2) as well as two weeks after parturition (D14; time point 3). *Haptoglobin* on time point 1 (before parturition) and *IL4* on time point 1 (before parturition) both displayed significance for treatment × day interaction ($P < 0.01$).

Increased cytology numbers indicate increased inflammation in the uterus, and therefore, the expectation would be that *TNFα* expression would be increased instead of decreased. However, *TNFα* is a cytokine that is involved in the early phases of healing so tends to be quickly released, initiates healing and then decreases again to basal expression levels. Therefore, our finding of increased cytology numbers with lower TNF expression is likely caused by the fact that *TNFα* initiated the immune response early on and then

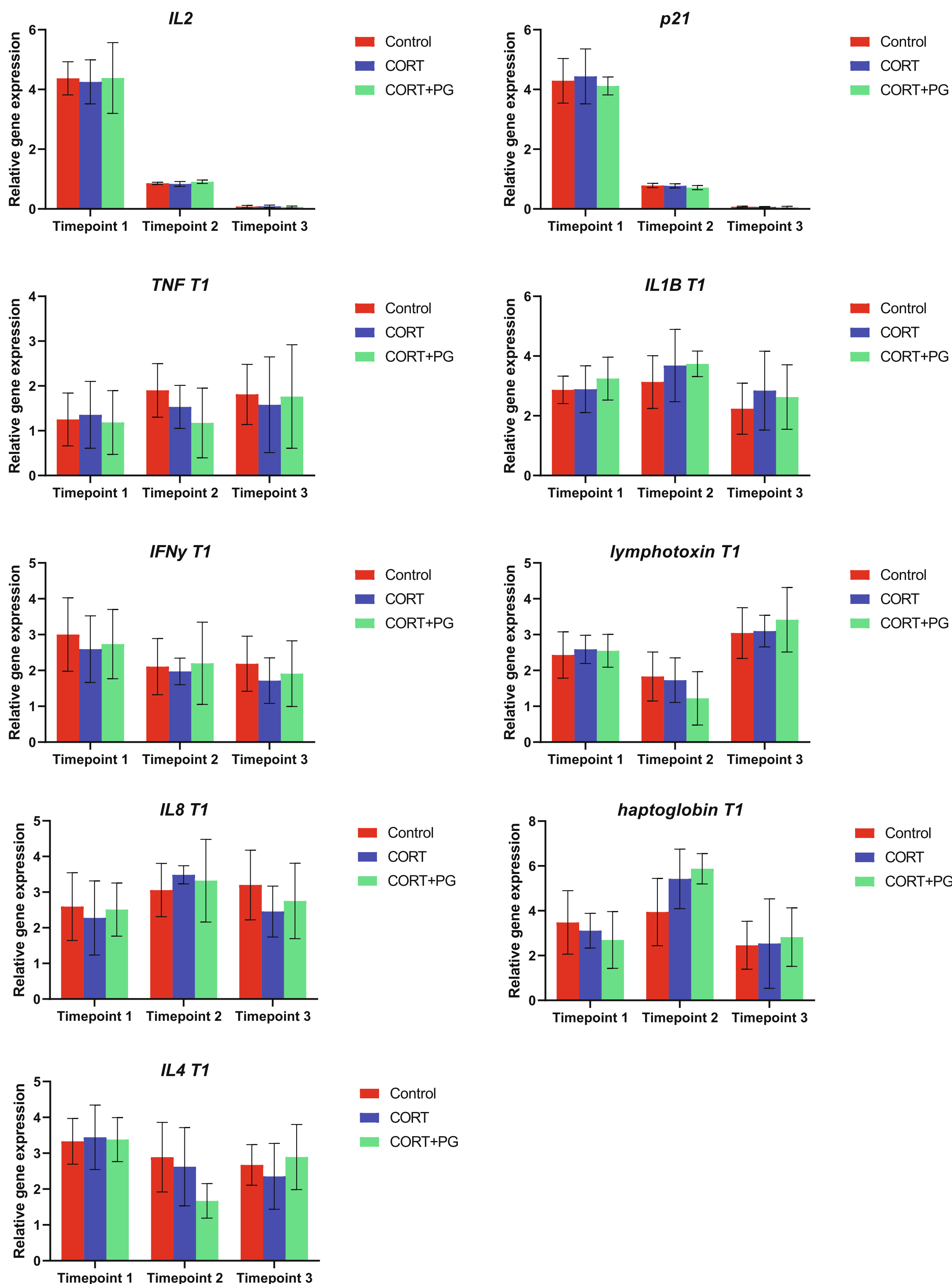

Fig. 3 (*continued*)

the expression returned back to basal level (Ritsu et al., 2017). *TNFα*expression was positively associated with cyclicity on D42, supporting the suggestion that early expression of this cytokine may stimulate the immune function of the uterus postpartum and therefore lead to better recovery of the uterus and subsequently improved resumption of cyclicity (Šavc et al., 2016, Velázquez et al., 2019). This finding is similar to those of Mee et al. (2009) who reported that in dairy cows, non-completion of uterine involution and therefore postpartum recovery of the uterus at the onset of the breeding season led to lower pregnancy rates in those animals. This will in turn affect the length of the postpartum interval to first ovulation which is one of the key reproductive targets for beef cows (Diskin and Kenny, 2014).

Though CON received no induction treatment, IgG concentrations in the colostrum were decreased compared with both CORT groups. It is possible that the induction with steroids led to a decrease in milk production likely leading to increased concentrations of IgG in the milk collected. This hypothesis would be supported (Conneely et al., 2013) that colostrum quality and concentration are related to the amount of milk produced. Unfortunately, we were not able to measure total milk production on the day of calving as the calves stayed with their dams as sucklers. The calves were monitored for weight gain but as the weigh-in of the calves took place 7 days postcalving, calf weight gain cannot be used here as a measure of milk quality and quantity. All colostrum samples had more than 50 g/L IgG in them and as such can be classified as good quality (McGuirk and Collins, 2004). Weight gain of the calf in week one went together with increased expression of *Haptoglobin* in their dams. *Haptoglobin* is a major acute phase protein that is produced in response to infection, inflammation, tissue damage or stress (Petersen et al., 2004; Marcato et al., 2021). Studies have shown that increased *Haptoglobin* expression are associated with either the calving process or colostrum quality (Orro et al., 2008) but we were unable to find this association in our study. We were also unable to find that the calves from dams with the higher *Haptoglobin* expression did receive lower quality colostrum or experienced disease in this first week, as shown by (Godson et al., 1996; Murray et al., 2014). Our only explanation for the increased *Haptoglobin* expression in the cows with larger calves is that the increased expression is a carry-over effect of the stress on the dam from calving due to the calf already being large in utero and subsequently continuing to have a high growth rate in its first week of life.

We found that there was no significant difference in Ca concentrations on either D-14, D1 or D14 suggesting that the cows did not suffer from subclinical hypocalcaemia which could have impaired their immune response (Kimura et al., 2006). However, we did find a strong correlation between Ca concentrations on D1 and D14 and subsequent mucus and cytology scores on D21, both indicators of uterine infection. This hypothesis is supported by some of the literature comparing Ca concentrations between beef and dairy cows with muscle weakness showing a clear difference between the two animal groups (Hanif et al., 1990). Our finding that the calcium concentrations on D1 appeared to be a good predictor for both mucus score and cytology score is supportive of our suggestion as it is well known that Ca concentrations around parturition will have a direct effect on uterine disease as the Ca concentrations directly affect the general but also local immune system (Goff, 2008).

With regard to cortisol, one of the main hormones associated with stress response (Carroll and Forsberg, 2007), we found no differences between groups on D-14 but on D1, both treatment groups had significantly lower circulating cortisol than the CON group. Considering calving would be regarded as a stressful event, it is more likely that the cortisol concentrations in the CON group were normal and that the cortisol concentrations in the treatment groups were low due to suppression of the endogenous steroid production or increased clearance rate after the induction with corticosteroids approximately 72 hours before the sampling took place. This effect has previously been shown in sheep that received corticosteroid infusions to mimic stressful situations (Rhind et al., 1999). This suppression or clearance is temporary however as the cortisol concentrations on D14 postcalving were not different between the groups. Higher cortisol concentrations led to a decrease in *IL8* expression, meaning that the production of this chemokine, which stimulates chemotaxis and phagocytosis, was reduced. This would fit in with the general immunosuppressive effect of corticosteroids which was also found by others who investigated the uterine immune response in relation to steroid treatment (Lander Chacin et al., 1990; Velázquez et al., 2019). While not affected by the cortisol injections, the fact that we also saw a temporary time-point effect ($P < 0.01$) for the immune response-related genes *BP1, TLR4, YWHAZ, MMP-9, NFKB1, NFKB2, GRa, IL1a, Fas, L-selectin, CD62L, IL2, p21*, whereby expression reduced as time progressed, fits into a similar picture. The immune system of the animals was highly active immediately postpartum with recovery of the reproductive tract and this response reduced as time postpartum progressed.

Our finding that calf weight at birth was correlated to mucus score, uterine involution and cytology on D21 is not surprising as larger calves could lead to higher mucus and cytology score and less involuted uteri due to the fact that the calving process is slower as there is more stretching as well as potential damage to the tract (Potter et al., 2010; LeBlanc et al., 2011). The finding that the expression of *IL1b* was increased in male calves with a higher birth weight would fit into this hypothesis as this cytokine is an important mediator of the inflammatory response and therefore the dams which produced larger calves had a higher mucus and cytology score would need this increased inflammatory response.

In conclusion: Induction of calving, using corticosteroid in the absence of, or in addition to, prostaglandin F2α has no negative effects on dystocia or subsequent calf weight gain but can have a positive effect on colostral IgG concentration, compared with that of non-induced contemporaries. Calcium concentrations on both D1 and D14 postcalving are associated with subsequent reproductive health. Events associated with the peri- and postpartum period are all reflected in temporal changes in expression of the cytokines *IFNγ*, *TNFα*, *IL1b, IL4*, *IL8* and *Haptoglobin*, which are all associated with the immune function of the cow around this time.

Ethics approval

All experimental procedures involving animals were approved by the Animal Research Ethics Committee of University College Dublin (AREC-13-31) and were licensed by the Health Products Regulation Authority in accordance with the Cruelty to Animals Act and European Community Directive 2010/63/EU (AE19132/P005). The study was conducted at the Teagasc Animal and Grassland Research and Innovation Centre, Grange Dunsany, Co. Meath, Ireland.

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available from the authors upon request.

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Declaration of interest

None.

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