

## **Chapter 4 Management Study**

Negative energy balance (NEB) was defined by Wathes *et al.*, (2007b), as a “metabolic disorder affecting high yielding cows that can impair health and have carry over effects on fertility some months later”. The aim of this trial was to use a model of bovine NEB to investigate the metabolic and endocrine disruptions (Figure 1.8, page 45) that lead to ovarian cyst development. Specifically, this was a retrospective study of the endocrine and metabolic changes occurring during the transition period. Furthermore, from a managerial perspective, the implications of early cyst detection on reproductive management were examined. This included an investigation on the incidence of cysts in animals before the routine pre-breeding examination (week +8 post-calving). The effects of NEB have severe consequences for dairy cows including udder problems, mastitis, locomotive problems, laminitis, digestive problems and sub-fertility (Collard *et al.*, 2000). Determining the energy status of an individual cow on the farm can be complex, while accurate rations are calculated by nutritionists on an individual cow basis, cows are often fed *ad libitum* thus DMI is likely to be different from that assumed in ration calculations (Huxley, 2004). Furthermore, energy content of the feed can vary depending on processing, climate and storage (Reist *et al.*, 2002). Additionally, there is great variability between cows in how they adapt to the metabolic stress experienced during the transition period (Kessel *et al.*, 2008). To establish the energy status of the animal it may, therefore, be more appropriate to measure metabolite concentrations in serum, (i.e. NEFA, BHB and urea).

#### 4.1 Serum parameters used in energy profiling

Non-esterified fatty acids (NEFAs) are released during lipolysis, and are taken up either by the mammary gland (to be used as milk fat) or the liver (Bell *et al.*, 1995). Under normal conditions in the liver, NEFAs are oxidised to form ketone bodies which are used as an energy source, however when liver glycogen stores have been depleted, NEFAs are esterified to triglycerides which accumulate in hepatocytes. Elevated NEFAs (>0.4mmol/l) seven days prepartum are positively associated with a greater incidence of postpartum ketosis, displaced abomasum, and retained fetal membranes (Dyk *et al.*, 1995).

There is no evidence to suggest that accumulation of triglycerides in hepatocytes directly decreases the rate of gluconeogenesis, however, accumulation of triglycerides has been shown to decrease ureagenesis (Strang *et al.*, 1998). Furthermore ammonia decreases the ability of hepatocytes to synthesise glucose (Overton *et al.*, 1999), thus accumulation of triglycerides in hepatocytes may indirectly impede on gluconeogenesis (Drackley, 1999).

$\beta$ -hydroxy butyrate (BHB) is the predominant ketone body produced by ketogenesis after the oxidation of NEFAs in hepatocytes. Serum concentrations of BHB provide an indication of oxidation of fatty acids in the liver (Wathes *et al.*, 2007a). Elevated concentrations of NEFA (>0.4mmol/l) and BHB (>1.4mmol/l) during the transition period are diagnostic indicators of NEB in cattle (Stokol & Nydam, 2005). However NEFAs are a more direct measure of fat mobilisation than BHBs (Drackley, 1999).

Serum urea concentrations provide an indication of available fermentable metabolisable energy (FME) i.e. energy available for microbial growth in the rumen. Urea concentrations are elevated when FME is low; inadequate levels of FME limit

microbial crude protein so elevated urea concentrations give an indication that there is not enough available energy from the rumen and that volatile fatty acid production may be low. Urea is also involved in the metabolism of nitrogen containing compounds, producing substrates for gluconeogenesis.

Cholesterol is a lipid soluble steroid metabolite, synthesised in, among other places, the liver. Cholesterol and triglycerides are assembled together to form very low density lipoproteins (VLDLs) in order to allow for transportation out of the liver through the blood. Serum measurement of cholesterol provides an indication of the liver's ability to produce VLDLs and compromised VLDL production results in hepatic infiltration (Van Saun, 2000). Hepatic lipidosis occurs when accumulation of triglycerides in hepatocytes occurs at a faster rate than the assembly of triglycerides and cholesterol into VLDLs (Grummer *et al.*, 1993).

Aspartate transaminase (AST) and  $\gamma$ -glutamyltranspeptidase (GGT) are enzymes found, among other places, within cell membranes of hepatocytes. They are involved in the degradation and transport of amino acids. Both AST and GGT activities are elevated during periods of liver damage or disease (Blackshaw, 1978; Itoh *et al.*, 1998). Elevated AST alone can also be indicative of muscle damage (Sattler & Fürll, 2004).

Total protein (TP) measures both albumin and globulin together in the blood and gives an indication of an animal's nutritional status. Elevated TP concentrations can indicate infection or inflammation of the liver, and suppressed concentrations may indicate liver disease. Albumin is a protein produced in the liver that binds to fatty acids. Cows with hepatic lipidosis have reduced albumin concentrations (Bogin *et al.*, 1988), reflecting poor liver health (Whitaker, 2000) and indicating that accumulated triglycerides in hepatocytes have negative implications for protein

synthesis in the liver. Globulin concentrations are elevated following an immune challenge and can be estimated by subtracting albumin from total protein.

#### 4.2 Body condition scoring

By palpating the transverse and spinous processes it is possible to estimate how much subcutaneous fat is being stored by a cow. The process of assigning a body condition score (BCS) is subjective and thus it can be difficult to draw relevant conclusions from the literature because of this. Most scales score on half or whole points from 0-5, when 0 represents an animal that is extremely malnourished and 5 represents an animal that is storing excessive body fat (Wildman *et al.*, 1982; Edmonson *et al.*, 1989).

#### 4.3 Materials and methods

One hundred multiparous Holstein-Friesian cows (range: parity 2-9) from one farm in the UK were recruited onto this trial. Maiden heifers were excluded from this trial as incidence of cysts is known to be lower in this group (Marcek *et al.*, 1985). Dry cows were housed in cubicles at a separate unit and transferred to the main facility roughly one week prior to expected calving where they were housed in straw yards. A total mixed ration of the following was fed per cow per day as follows; 19kg grass silage, 19kg maize silage, 8.5kg concentrate 'blend' at 24% crude protein, 6.5kg wheat gluten feed (Trafford Gold), and 5.0kg brewers grains (fresh weight). The ration was formulated to provide 11.9 MJME/kgDM and 16.3% crude protein for cows producing 40kg milk per day with no additional concentrate feed given during milking. From January 2009 to March 2009, the dry cows were housed indoors and fed *ad libitum* wheat straw and 3kg maize gluten daily (n=28). From early April

2009, the dry cows (n=6) were turned out to grass with no supplementary feeds. Cows on trial were separated from the herd at milking or immediately prior to sampling (dry cows) and accommodated together during the sampling process which typically lasted 1-2 hours.

When working closely with the herdsmen, it was revealed that they were not following the dietary formulation advised by the nutritionist (retrospectively worked out as maintenance + 32 rather than maintenance + 40 based on an 8 litre milking deficit per cow), they felt this was more appropriate based on their experience working with the herd. Unfortunately, it was not possible to get the details of this formulation.

Cows were followed from four weeks before expected parturition up to ten weeks post-partum between January and October 2009. All the animals were body condition scored by one person (JW) to ensure consistency, using a five point system with quarter-point divisions, developed specifically for Holstein dairy cattle by Edmondson *et al.*, (1989). Milk yield (305 day) records were retrospectively obtained from Interherd (InterAgri, Reading, UK).

From four weeks postpartum, the ovaries were examined once a week by transrectal ultrasonography to check their function and formation of any ovarian cysts. All follicles >5mm, characterised as anechoic structures with a clearly defined follicle wall and antrum were recorded. A thick grainy echogenic structure, distinguishable from the less echogenic ovarian stroma, was identified as a CL. Cysts were initially differentiated based on veterinarian diagnosis as described in Jackson *et al.*, (2011); follicular cysts (FC) were defined as non-echogenic structures  $\geq 20$ mm with a wall less than 3mm thick that persisted in the absence of a CL for seven days. While a thick walled structure  $\geq 25$ mm that persisted for seven days or

more was defined as a luteal cyst (LC). Any cysts detected before week eight postpartum were left untreated. From week eight postpartum (the time dairy cows are typically presented for a routine pre-breeding examination, and therefore a time when abnormalities are usually detected) any cystic structures were treated for ethical reasons, according to a veterinarian's diagnosis, as follows: An intravaginal progesterone releasing device (Eazi-breed CIDR; Pfizer Ltd., Kent, UK) containing 1.38g of progesterone for 14 days for a follicular cyst, or a single injection (2ml, im) of the prostaglandin  $F_{2\alpha}$  analogue, cloprostenol (Estrumate; Schering-Plough Animal Health, Milton Keynes, UK) for a luteal cyst. However, in this study, a more definitive cyst diagnosis was conducted using retrospective serum progesterone profiles (i.e. cows with elevated progesterone were confirmed to be luteal and *vice versa* for follicular cysts) and this was used in analysis of these data.

Weekly blood samples were collected every Thursday for fifteen weeks via coccygeal venepuncture into 4ml evacuated silicone spray coated, clot activating vacutainers (Becton, Dickson Ltd., Oxford, UK), under the appropriate Animals in Scientific Procedures Act licence. Blood samples were transported back to the laboratory, left to coagulate for 1 hour at room temperature, centrifuged, aliquoted and analysed for metabolites on the same afternoon following advice given in Stokol & Nydam (2005). While the remaining aliquots were frozen and stored at -20°C for hormone analysis.

#### 4.3.1 Metabolite analyses

Assays were performed using a Randox RX Imola clinical chemistry analyser (Randox Laboratories Ltd., Belfast, UK), in single replicate to the manufacturers specifications (unless otherwise stated) using the kits described in Table 4.1,

obtained from Randox (Randox Laboratories Ltd., Belfast, UK). Parameters measured were; BHB, NEFA, urea, cholesterol, triglycerides, AST, GGT, total protein and albumin (normal values are given in Table 4.2). Bovine serum supplied by Randox (Randox Laboratories Ltd., Belfast, UK), was used as a quality control and inter-assay % CV were calculated (Table 4.1). After 16/07/09 it was decided that total protein and albumin would be added to the analysis set, so retrospective analysis was done on all samples obtained before 16/07/09 following one freeze-thaw cycle, after which total protein and albumin concentrations were determined in weekly samples as normal.

**Table 4.1:** Individual kit codes for each metabolite plus inter-assay % coefficient of variation (CV) values for the 3 different lots of bovine sera analyte level II QC used (AM1026, Randox Laboratories Ltd., Belfast, UK).

Parameter	Manufacturer Code	Inter-assay %CV		
		Lot# 361SN	Lot# 367SN	Lot# 378SN
β-hydroxy butyrate	RB1007*	4.78	3.44	0.94
Non-esterified fatty acids	FA115*	5.42	4.82	1.80
Urea	UR3825	4.42	4.46	1.15
Albumin	AB3800	-	0.76	0.65
Total protein	TP3869	-	1.60	1.86
γ-glutamyl transpeptidase	GT3817	7.39	3.39	7.49
Aspartate transaminase	AS3804	5.56	2.16	2.67
Cholesterol	CH3810	6.92	3.95	2.86
Triglycerides	TP3822	8.12	3.61	0.01

\*Volumes reduced to 80% of manufacturers specifications

**Table 4.2:** Expected normal values of blood metabolites and implications of elevated/subnormal concentrations postpartum for Holstein-Friesian dairy cows during the transition period (where 0 is day of calving) Rukkwamsuk *et al.*, (1998)<sup>a</sup>; Tedesco *et al.*, (2004)<sup>b</sup>; Stokol & Nydam (2005)<sup>c</sup>; Constable *et al.*, (2007)<sup>d</sup>; Seifi *et al.*, (2007)<sup>e</sup>; Ospina *et al.*, (2010)<sup>f</sup>; Li *et al.*, (2011)<sup>g</sup>. Much of these data were compiled from non-UK cows and as such there may be subtle differences in the metabolite concentrations of the UK cows used in this study.

NEFA = Non-esterified fatty acids, BHB =  $\beta$ -hydroxy butyrate; AST = Aspartate transaminase; GGT =  $\gamma$ -Glutamyl transpeptidase; FME = Fermentable metabolisable energy; VLDL = Very low density lipoprotein; NEB = negative energy balance.

Metabolite	Days relative to Calving (0)				Implications
	-21	-7	+7	+21	
Non-esterified fatty acids mmol/l	0.27 <sup>c, e</sup>	0.26 <sup>a, c, e</sup>	0.61 - 0.91 <sup>a, e</sup>	0.40 - 0.60 <sup>a, e</sup>	↑ NEFA = fat mobilisation
$\beta$ -hydroxy butyrate mmol/l	0.60 <sup>c, e</sup>	0.60 <sup>c, e</sup>	0.65 <sup>c, e</sup>	0.70 - 1.40 <sup>c, d</sup>	↑BHB + ↑NEFA = NEB
Urea mmol/l	4-6 <sup>f</sup>	4-6 <sup>f</sup>	4-6 <sup>f</sup>	3.91 <sup>g</sup>	↑ urea = ↓FME
Triglyceride mmol/l	0.20 <sup>e</sup>	0.18 <sup>e</sup>	0.08 <sup>e</sup>	0.06 <sup>d, e</sup>	↑ triglyceride = ↓ ureagenesis
Cholesterol mmol/l	3.34 <sup>e</sup>	2.39 <sup>e</sup>	1.89 <sup>e</sup>	3.20 <sup>d, e</sup>	↓cholesterol = compromised VLDL production
Total protein g/l	80 <sup>e</sup>	72 <sup>e</sup>	62 <sup>e</sup>	73 <sup>d, e</sup>	↑total protein = liver infection or inflammation
Albumin g/l	38 <sup>e</sup>	36 <sup>e</sup>	29 <sup>e</sup>	35 <sup>e</sup>	↓albumin = hepatic lipidosis
Aspartate transaminase IU/l	46 <sup>e</sup>	50 <sup>e</sup>	72 <sup>e</sup>	63 <sup>e</sup>	↑ AST = muscle damage or liver disease
$\gamma$ -Glutamyl transpeptidase IU/l	-	12 <sup>b</sup>	14 <sup>b</sup>	17 <sup>b</sup>	↑GGT = liver disease



#### 4.3.2 Progesterone concentration

Progesterone concentrations were measured once weekly as previously described in Chapter 2 from two weeks postpartum until the end of the trial using the Ridgeway plasma progesterone kits (Ridgeway Science, Gloucestershire, UK). Intra-assay % CV was calculated at <10% and inter-assay % CV were 17.2% at 2 ng/ml and 19.2% at 4 ng/ml.

#### 4.3.3 Statistical analysis

Statistical difference in metabolite concentrations were examined by ANOVA for repeated measurements (Genstat 12, VSN International, Hemstead, UK). The main effects tested included treatment, time and interaction of treatment x time. A one way ANOVA was used to determine significant differences between CCI or milk yield between the groups over a range of pre-determined periods of time. Initially the groups were analysed from the start to the end of the trial (weeks -4 to +10). After this the observation period was separated into different physiologically based time windows, namely the pre-calving period (weeks -4 to -1), the early post-calving period (weeks +1 to +5), the late post-calving period (weeks +6 to +10), and the transition period (weeks -3 to -1 and +1 to +3) (NB: this period overlaps the pre-calving and the early post-calving period). Week 0, the week in which cows calved, was omitted from the analysis as cows calved on various days during this week and it was felt that including these data into the specific time points might inadvertently influence analysis of these data due to its high variability. Residuals were tested for normality and no transformations were required. Probability values  $P < 0.05$  were considered to be significant, while values  $P < 0.1$  were considered to show a trend. Animals cycling normally with no aberrant follicular structures were classified as the

control no-cyst group. All data were corrected for actual calving dates before analysis.

#### 4.4 Results

Of the 100 cows that started the trial, data from 15 cows were excluded from analysis for unrelated health reasons. Of the remaining cows, 6/85 cows gave birth to twin offspring and 79/85 gave birth to single offspring. In these singleton pregnancies, 21 cows were diagnosed with a follicular cyst (non-progestagenic) and 10 with a luteal cyst (progestagenic). The remaining 48 singleton pregnancies served as the control (no-cyst) group. Cows with cysts were also subdivided based on whether the cyst was identified before or after the 8 week post-partum pre-breeding exam. The 6 cows bearing twins (3/6 mixed sex and 3/6 same sex) were excluded from cyst analysis however, a comparison of metabolic parameters between singles and twins was undertaken. From this point onwards cows calving single offspring shall be referred to as ‘singles’ and cows calving twins as ‘twins’.

##### 4.4.1 Results from cows bearing single offspring

Concentrations of NEFA, triglycerides and AST activity were all elevated above the normal expected values (Table 4.2), while concentrations of BHB, urea, total protein, albumin, cholesterol and GGT activity were all within the expected range across the transition period in cows with a follicular or luteal cyst and the control no-cyst group.

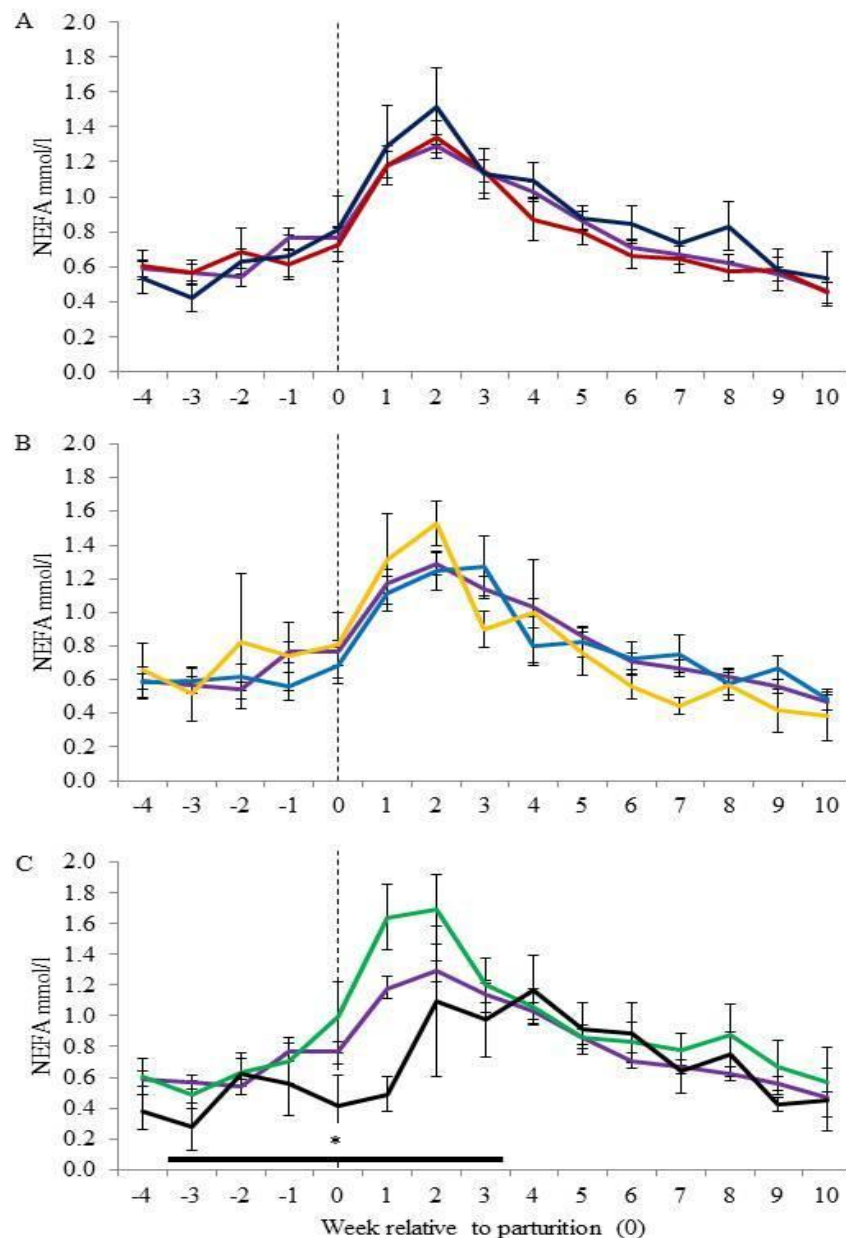
##### 4.4.2 Energy parameters (singletons)

In the control group, NEFA (Figure 4.1) and BHB (Figure 4.2) concentrations steadily rose in the 4 weeks prior to calving, with the largest increases observed in

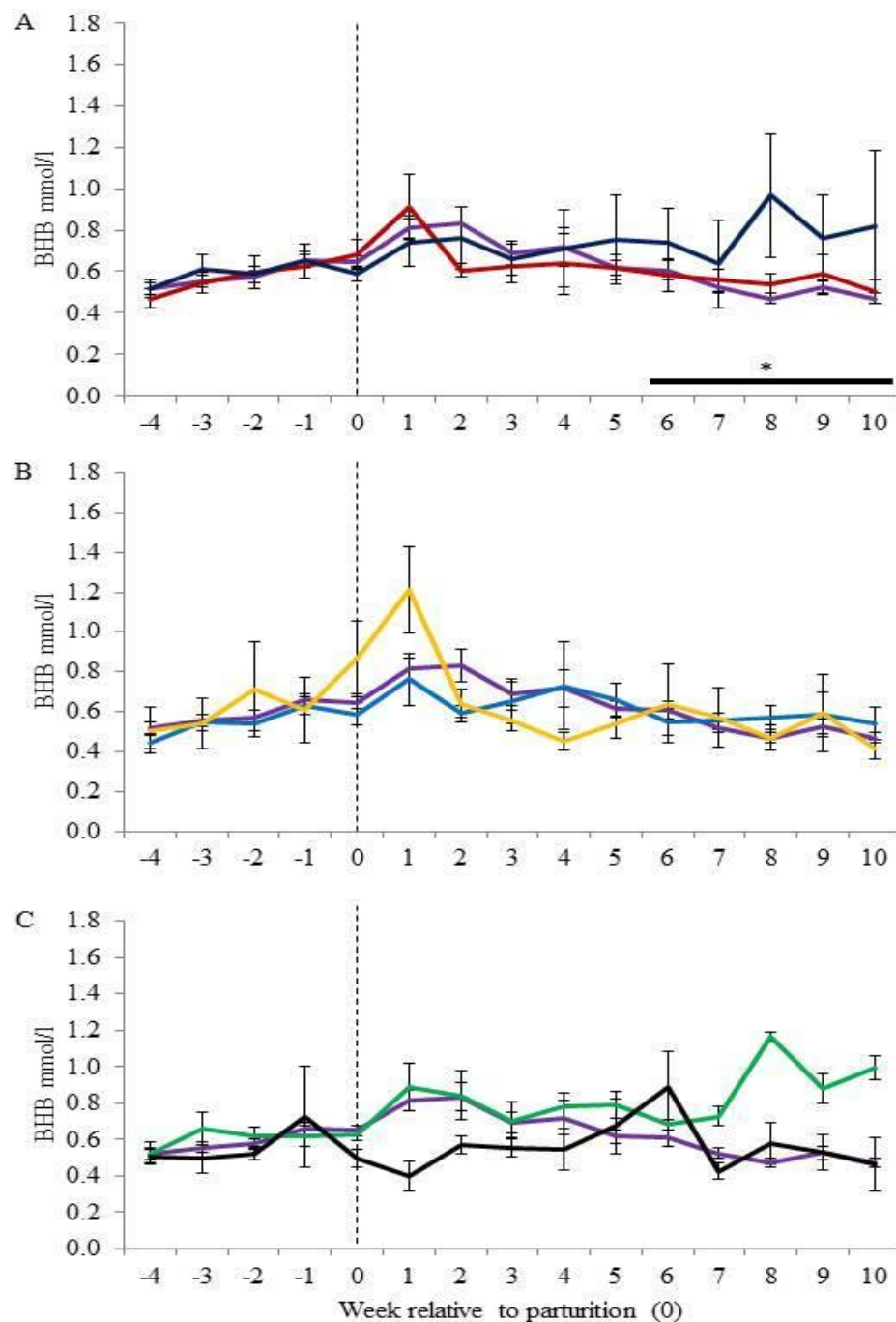
the 2 weeks after calving, after which they declined continuously for the remainder of the trial ( $P<0.05$ ). Urea concentrations (Figure 4.3) steadily increased in the late gestation period and generally peaked at calving. From calving onwards urea concentrations fluctuated for the remainder of the trial with no particular pattern being observed ( $P<0.05$ , time effect). The BCS (Figure 4.4) remained constant prior to calving in all groups but after calving it decreased steadily throughout the trial window ( $P<0.05$ ).

Across the 15 weeks, NEFA concentrations (Figure 4.1) initially increased and then decreased ( $P<0.05$ ) irrespective of cysts over time. BHB (Figure 4.2) and urea (Figure 4.3) concentrations also changed significantly over time ( $P<0.05$ ), irrespective of cysts, increasing prior to calving and then decreasing. Cows exhibiting a follicular cyst demonstrated no significant deviation in BHB concentrations from the control no-cyst group ( $P>0.1$ ) (Figure 4.2). However, BHB concentrations in cows exhibiting a luteal cyst significantly diverged ( $P<0.05$ ) and were elevated ( $P<0.05$ ) above those of the no-cyst group during the late post-calving period (Figure 4.2). When comparing cows exhibiting follicular or luteal cysts to cows within the no-cyst group, there were no significant differences in NEFA concentrations for either comparison across the 15 weeks or within individual time frame periods. However, there was a significant group x time interaction ( $P<0.05$ ) as NEFA concentrations in the pre-calving period of the no-cyst group were elevated above those of the follicular cyst group (Figure 4.1). Elevated urea concentrations ( $P<0.05$ ) were observed in cows exhibiting a follicular cyst compared to those within the no-cyst group but only during the early postpartum period (Figure 4.3). There was a significant loss of BCS over time ( $P<0.05$ ) for both cows with and without cysts across the 15 weeks (Figure 4.4). This was particularly apparent during the

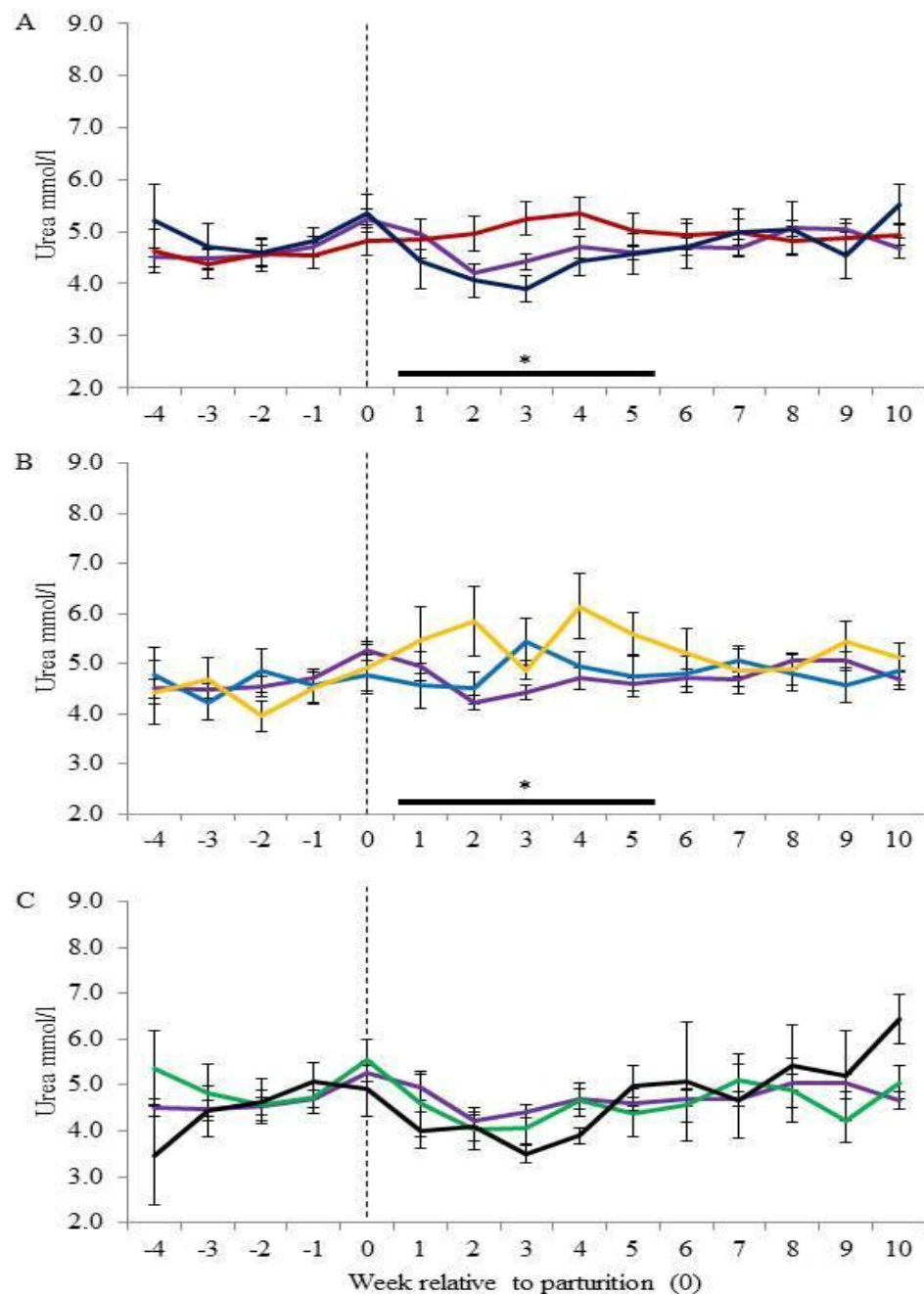
early post-calving ( $P<0.001$ ) and transition ( $P<0.001$ ) periods, but not in the pre-calving or late post-calving periods (Figure 4.4). Interestingly, the loss of BCS was similar between cows identified as having had a follicular or luteal cyst or no-cysts across the 15 weeks and within specific time frame periods (Figure 4.4).



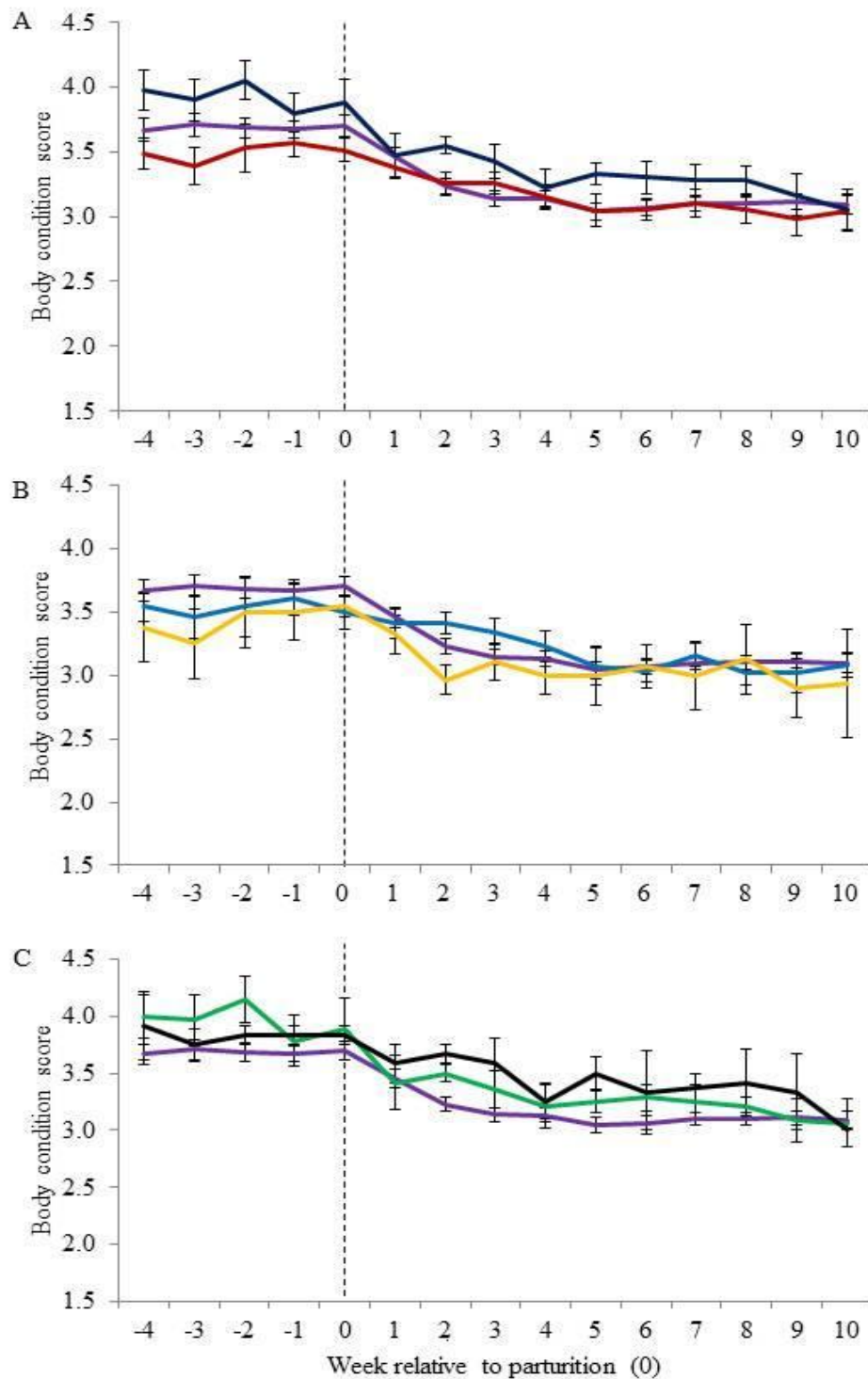
**Figure 4.1:** Non-esterified fatty acid (NEFA) concentrations (mmol/l) ( $\pm$ SEM) in pre- and post-calving cows over a 15 week period: **A)** Singles control no-cyst group (purple, n=48) vs. cows with a follicular cyst (red, n=21) or luteal cyst (dark blue, n=10). **B)** Singles control no-cyst group (purple, n=48) vs. cows with follicular cysts formed early (light blue, n=14) or late (orange, n=7) postpartum. **C)** Singles control no-cyst group (purple, n=48), vs. cows with luteal cysts formed early (green, n=7), or late (black, n=3) postpartum. NEFA concentrations were significantly higher ( $P<0.05$ ) during the transition period in cows with a luteal cyst that formed early compared to those that formed late.



**Figure 4.2:**  $\beta$ -hydroxy butyrate (BHB) concentrations (mmol/l) ( $\pm$ SEM) in pre- and post-calving cows over a 15 week period **A)** Singles control no-cyst group (purple, n=48) vs. cows with a follicular cyst (red, n=21) or luteal cyst (dark blue, n=10). **B)** Singles control no-cyst group (purple, n=48) vs. cows with follicular cysts formed early (light blue, n=14) or late (orange, n=7) postpartum. **C)** Singles control no-cyst group (purple, n=48), vs. cows with luteal cysts formed early (green, n=7), or late (black, n=3) postpartum. BHB concentrations were significantly higher (P<0.05) in the late post-calving period in cows with a luteal cyst compared to the control no-cyst group.



**Figure 4.3:** Urea concentrations (mmol/l) ( $\pm$ SEM) in pre- and post-calving cows over a 15 week period: **A)** Singles control no-cyst group (purple, n=48) vs. cows with a follicular cyst (red, n=21) or luteal cyst (dark blue, n=10). **B)** Singles control no-cyst group (purple, n=48) vs. cows with follicular cysts formed early (light blue, n=14) or late (orange, n=7) postpartum. **C)** Singles control no-cyst group (purple, n=48), vs. cows with luteal cysts formed early (green, n=7), or late (black, n=3) postpartum. Urea concentrations were higher in cows with a follicular cyst ( $P < 0.05$ ) compared to the control no-cyst group during the early post-calving period (A), as well as in cows with a follicular cyst that formed early ( $P < 0.05$ ) compared to the control no-cyst group in the early post-calving period (B).



**Figure 4.4:** Body condition score ( $\pm$ SEM) in pre- and post-calving cows over a 15 week period: **A)** Singles control no-cyst group (purple, n=48) vs. cows with a follicular cyst (red, n=21) or luteal cyst (dark blue, n=10). **B)** Singles control no-cyst group (purple, n=48) vs. cows with follicular cysts formed early (light blue, n=14) or late (orange, n=7) postpartum. **C)** Singles control no-cyst group (purple, n=48), vs. cows with luteal cysts formed early (green, n=7), or late (black, n=3) postpartum.

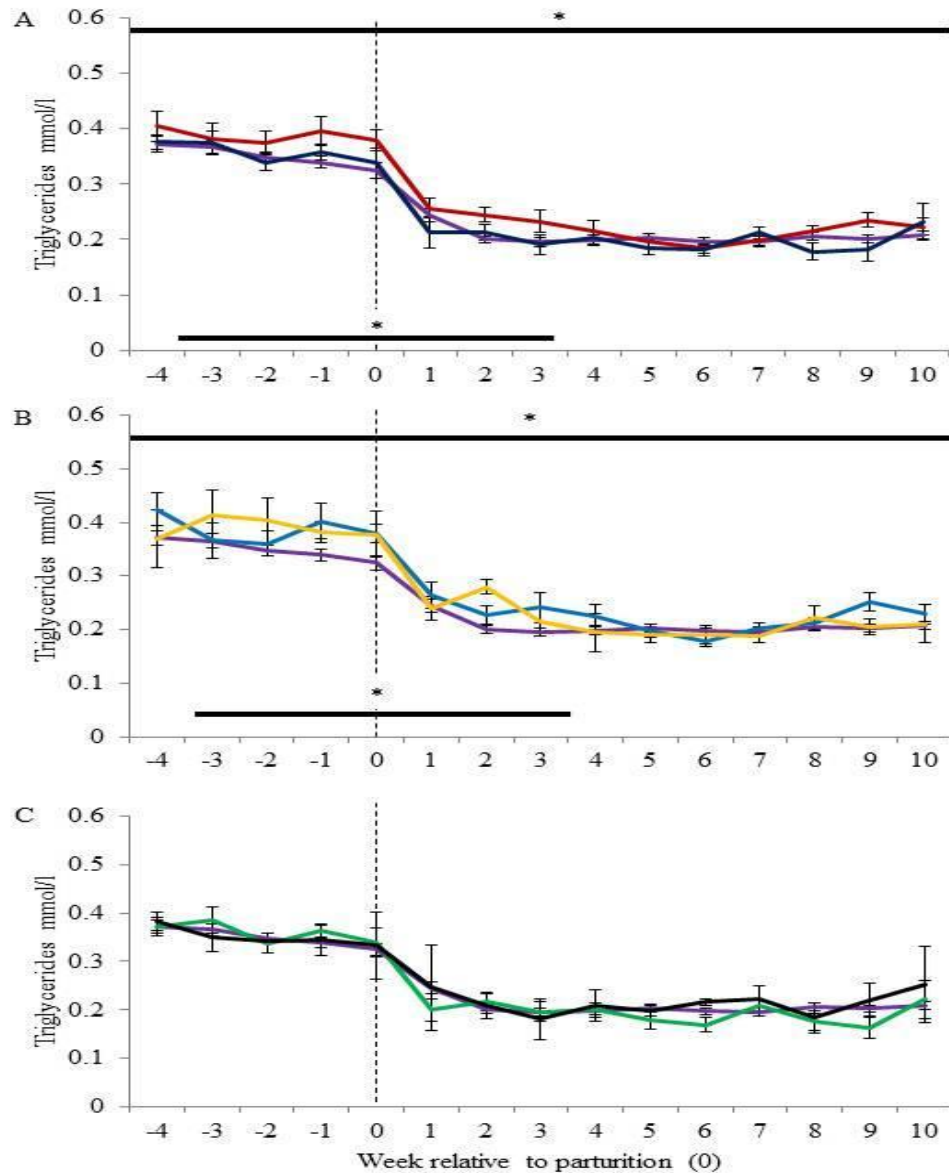


#### 4.4.3 Liver parameters (singletons)

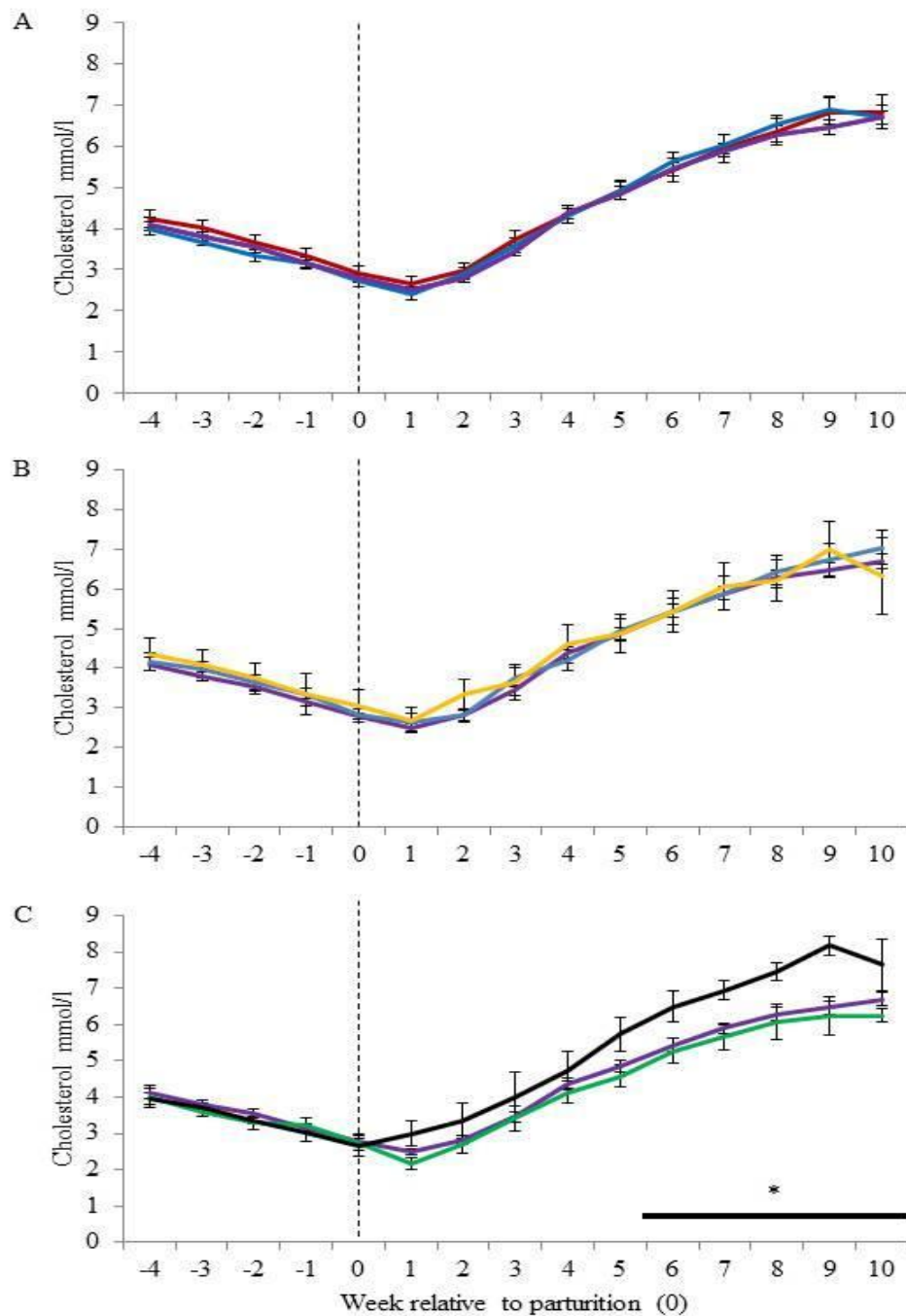
In the control group, plasma triglyceride concentrations (Figure 4.5) declined prior to calving and then substantially declined in all groups but then remained constant until the end of the trial. Cholesterol concentrations (Figure 4.6) declined until one week post-calving after which they steadily increased until the end of the trial ( $P<0.001$ ). GGT activity (Figure 4.7) remained constant until calving but then increased ( $P<0.001$ ) while AST activity (Figure 4.8) increased slightly prior to calving after which it declined ( $P<0.05$ ). Total protein (Figure 4.9), albumin (Figure 4.10) and globulin (Figure 4.11) concentrations remained constant until calving but then increased post-calving ( $P<0.001$ ).

Triglyceride concentrations (Figure 4.5) significantly decreased over the 15 weeks ( $P<0.001$ ) irrespective of the presence of an ovarian cyst. Initially, triglyceride concentrations decreased steadily in all groups, followed by a substantial decrease at calving after which they remained constant. Cows identified with a follicular cyst had triglyceride concentrations that were, with the exception of one time point, consistently ( $P<0.05$ ) higher than the control no-cyst group across the 15 weeks, this was particularly noticeable during the transition period ( $P<0.05$ ).

Plasma cholesterol concentrations (Figure 4.6) showed a significant temporal pattern ( $P<0.001$ ), which was similar in all groups. Namely, concentrations initially decreased during the late gestation period but then increased from weeks 1-2 post parturition. There was no significant difference in cholesterol concentrations between cows with and without follicular or luteal cysts when compared to the control no-cyst group.



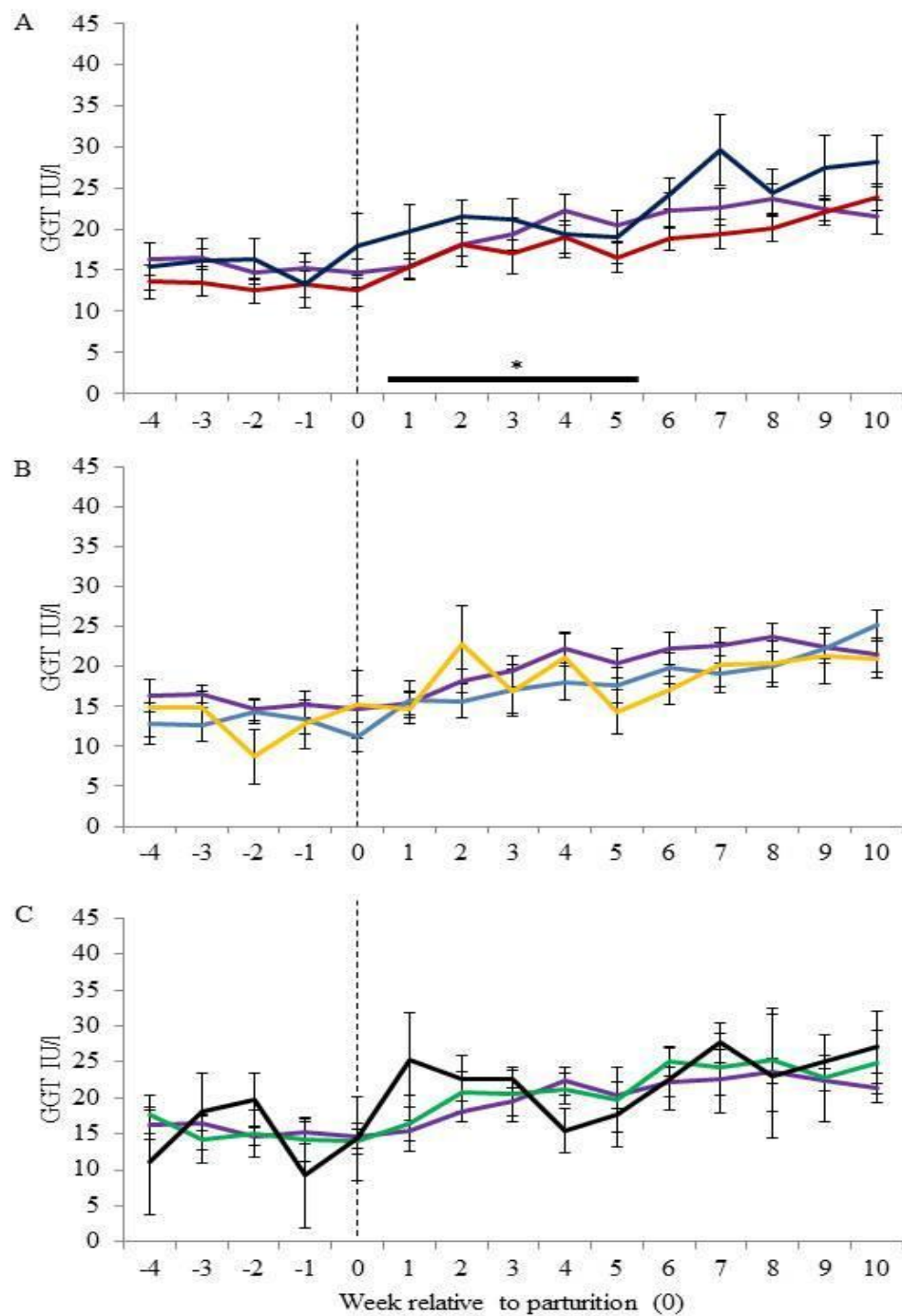
**Figure 4.5:** Triglyceride concentrations (mmol/l) ( $\pm$ SEM) in pre- and post-calving cows over a 15 week period: **A)** Singles control no-cyst group (purple, n=48) vs. cows with a follicular cyst (red, n=21) or luteal cyst (dark blue, n=10). **B)** Singles control no-cyst group (purple, n=48) vs. cows with follicular cysts formed early (light blue, n=14) or late (orange, n=7) postpartum. **C)** Singles control no-cyst group (purple, n=48), vs. cows with luteal cysts formed early (green, n=7), or late (black, n=3) postpartum. Cows with a follicular cyst had significantly higher ( $P < 0.05$ ) triglyceride concentrations over the whole 15 weeks, particularly in transition period ( $P < 0.05$ ) when compared to the no-cyst group (A). Triglycerides concentrations of cows with a follicular cyst that formed early were significantly higher ( $P < 0.05$ ) across the 15 weeks, particularly in the transition period when compared to the no-cyst group (B). Cows with a follicular cyst that formed late had significantly higher ( $P < 0.05$ ) triglyceride concentrations in the transition period when compared to the no-cyst group (B).



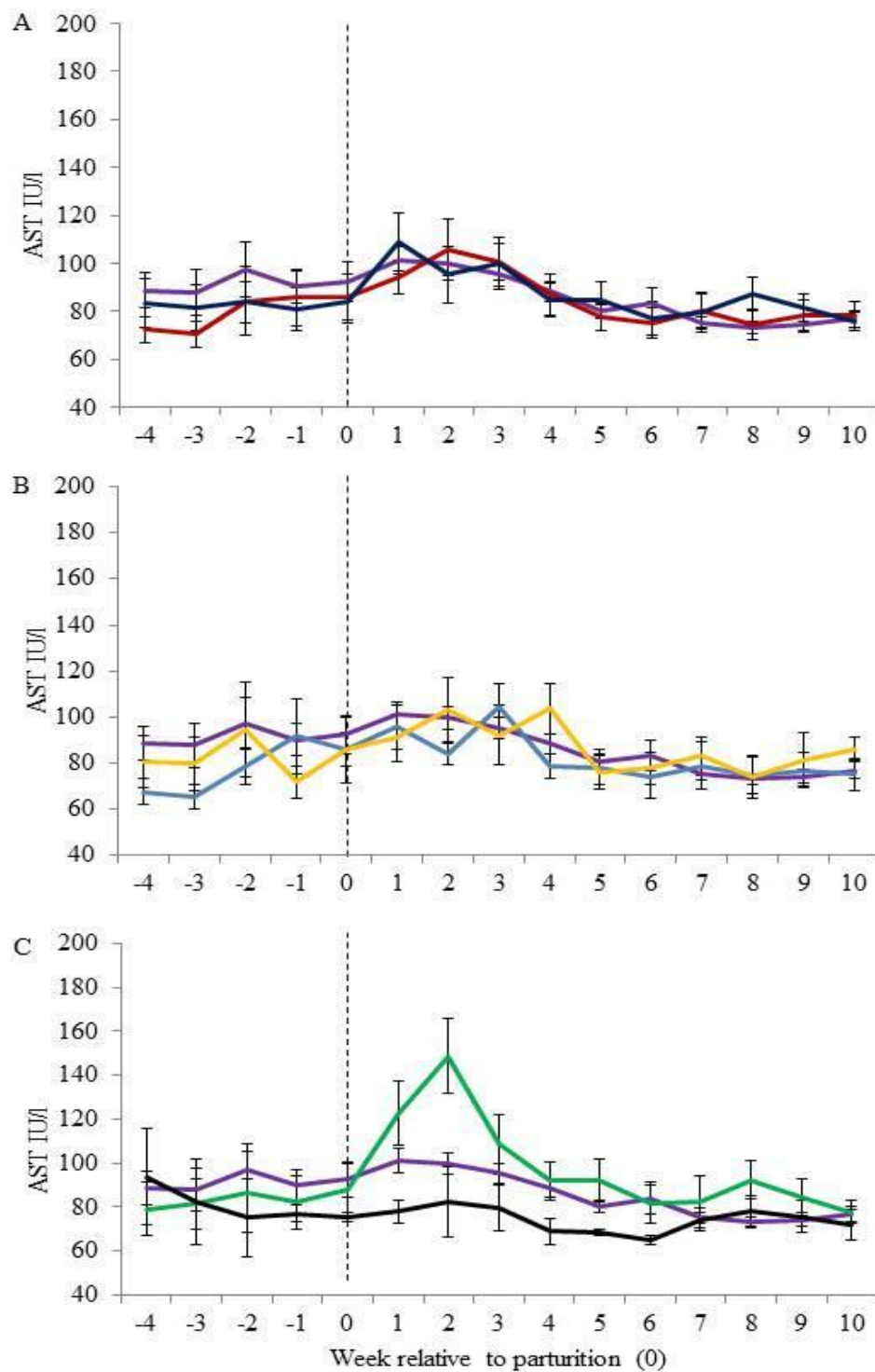
**Figure 4.6:** Cholesterol concentrations (mmol/l) ( $\pm$ SEM) in pre- and post-calving cows over a 15 week period: **A)** Singles control no-cyst group (purple, n=48) vs. cows with a follicular cyst (red, n=21) or luteal cyst (dark blue, n=10). **B)** Singles control no-cyst group (purple, n=48) vs. cows with follicular cysts formed early (light blue, n=14) or late (orange, n=7) postpartum. **C)** Singles control no-cyst group (purple, n=48), vs. cows with luteal cysts formed early (green, n=7), or late (black, n=3) postpartum. In the late post-calving period, cholesterol concentrations were significantly higher ( $P < 0.05$ ) in cows with a luteal cyst that formed late when compared to those that formed early (C).

The total activity of GGT in plasma (Figure 4.7) increased ( $P<0.01$ ) across the 15 weeks irrespective of cyst, GGT activities also increased over time, irrespective of cyst, during the early post-calving ( $P<0.001$ ) and transition ( $P<0.05$ ) periods. GGT activities were lower during the early post-calving ( $P<0.05$ ) and lower, but not significantly, during the late post-calving ( $P<0.1$ ) period in cows that had a follicular cyst compared to the no-cyst group. Cows identified to have had a luteal cyst had GGT activities that did not differ significantly from those of the control no-cyst group.

The total activity of AST in plasma (Figure 4.8) increased in the late gestation period and then decreased ( $P<0.05$ ) from two week post parturition over the 15 weeks, irrespective of cysts. There was no significant deviation in AST activities in either the follicular or luteal cyst when compared to the control no-cyst group.



**Figure 4.7:**  $\gamma$ -Glutamyl transpeptidase (GGT) activities (IU/l) ( $\pm$ SEM) in pre- and post-calving cows over a 15 week period: **A)** Singles control no-cyst group (purple, n=48) vs. cows with a follicular cyst (red, n=21) or luteal cyst (dark blue, n=10). **B)** Singles control no-cyst group (purple, n=48) vs. cows with follicular cysts formed early (light blue, n=14) or late (orange, n=7) postpartum. **C)** Singles control no-cyst group (purple, n=48), vs. cows with luteal cysts formed early (green, n=7), or late (black, n=3) postpartum. GGT activity in cows with a follicular cyst was significantly lower ( $P<0.05$ ) in the early post-calving period when compared to the control no-cyst group.

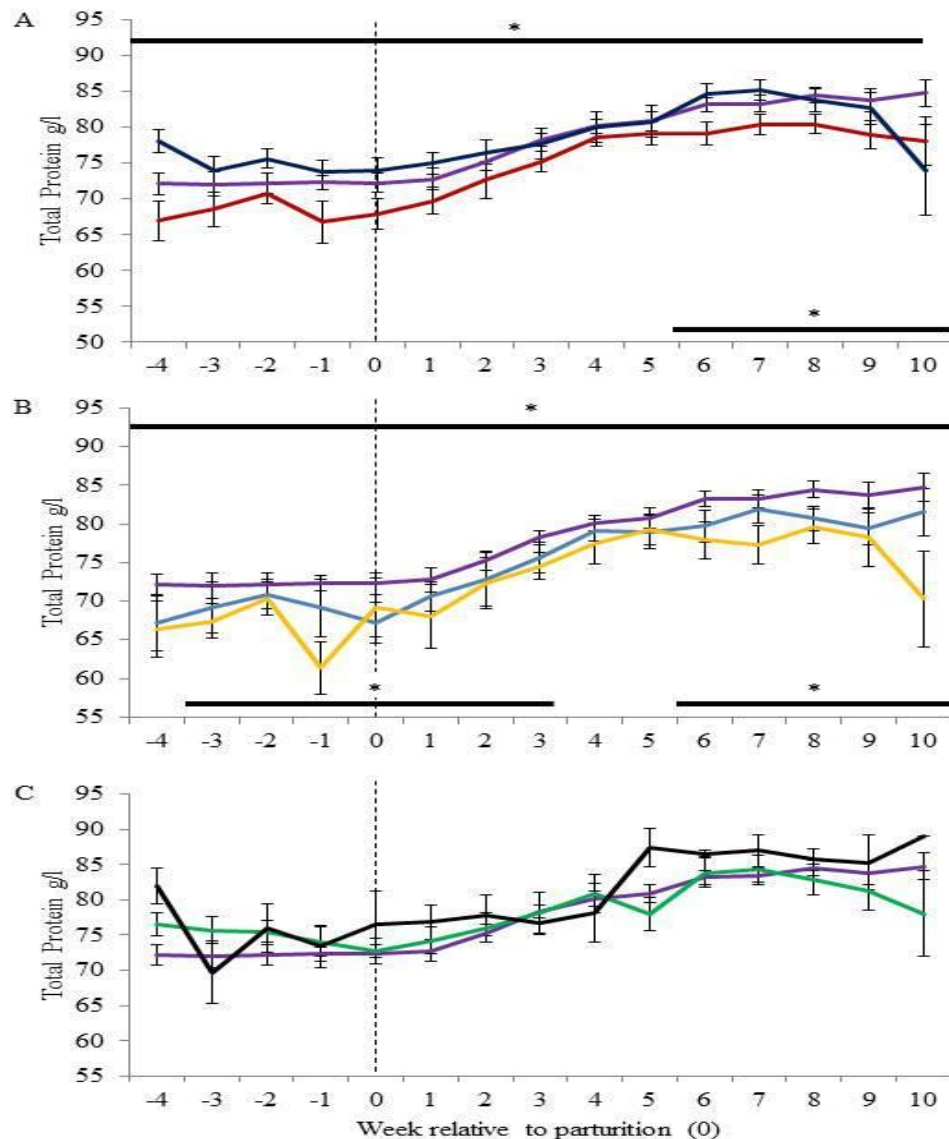


**Figure 4.8:** Aspartate transaminase (AST) activities (IU/l) ( $\pm$ SEM) in pre- and post-calving cows over a 15 week period: **A)** Singles control no-cyst group (purple, n=48) vs. cows with a follicular cyst (red, n=21) or luteal cyst (dark blue, n=10). **B)** Singles control no-cyst group (purple, n=48) vs. cows with follicular cysts formed early (light blue, n=14) or late (orange, n=7) postpartum. **C)** Singles control no-cyst group (purple, n=48), vs. cows with luteal cysts formed early (green, n=7), or late (black, n=3) postpartum.

Total protein concentrations (Figure 4.9) increased over time ( $P<0.001$ ) irrespective of the presence of an ovarian cyst, particularly during the early post-calving ( $P<0.001$ ) and transition periods ( $P<0.001$ ). Cows identified as having had a follicular cyst had consistently lower ( $P<0.05$ ) total protein concentrations compared with the control no-cyst group. Conversely, total protein concentrations were unaffected in cows having been identified with a luteal cyst across the 15 week observation period.

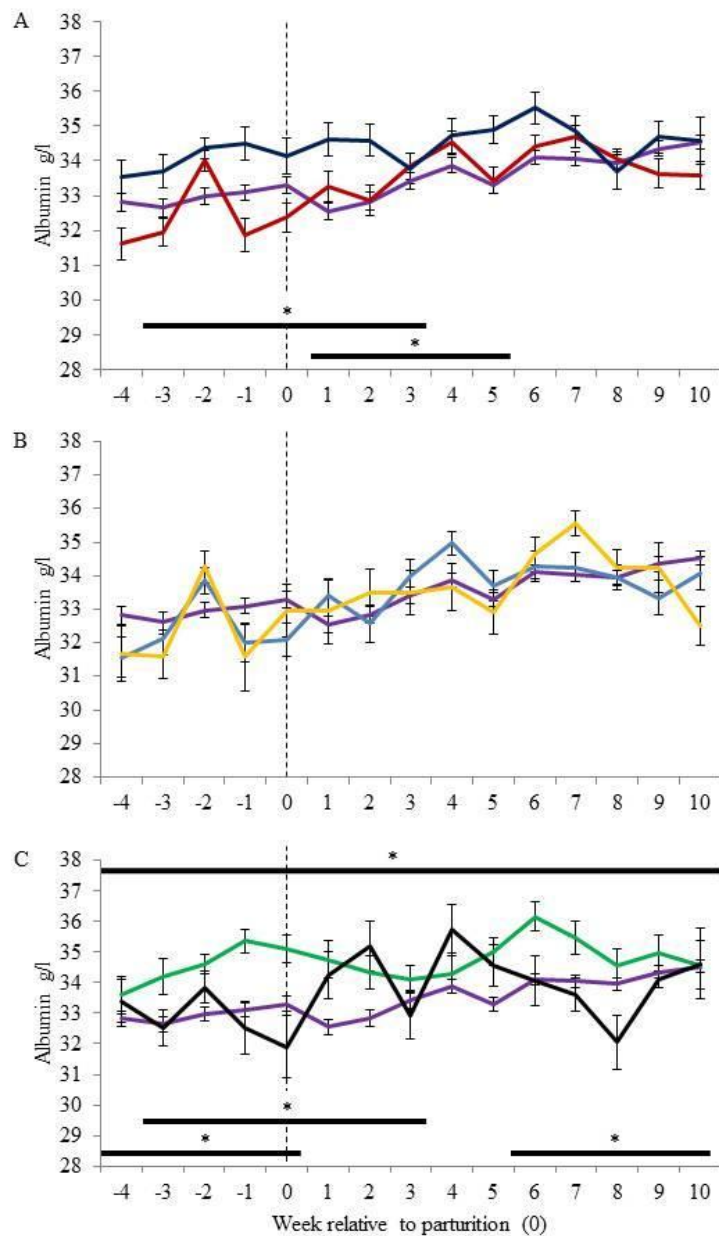
Albumin concentrations (Figure 4.10) increased ( $P<0.001$ ) across the 15 weeks irrespective of the presence of an ovarian cyst. Albumin concentrations were similar in cows identified to have had a follicular cyst to the no-cyst group. However, cows with a luteal cyst had elevated ( $P<0.05$ ) albumin concentrations across the transition period and the early post-calving period compared to the control cows.

Across the 15 weeks, globulin concentrations (Figure 4.11) increased ( $P<0.001$ ), irrespective of the presence of an ovarian cyst, and this was particularly noticeable during the early post-calving ( $P<0.001$ ) and transition ( $P<0.001$ ) periods. Throughout the trial, globulin concentrations were consistently lower ( $P<0.05$ ) in cows having been identified to have had a follicular cyst compared to control cows. Globulin concentrations in cows having been identified with a luteal cyst did not differ significantly to those in the control no-cyst group.

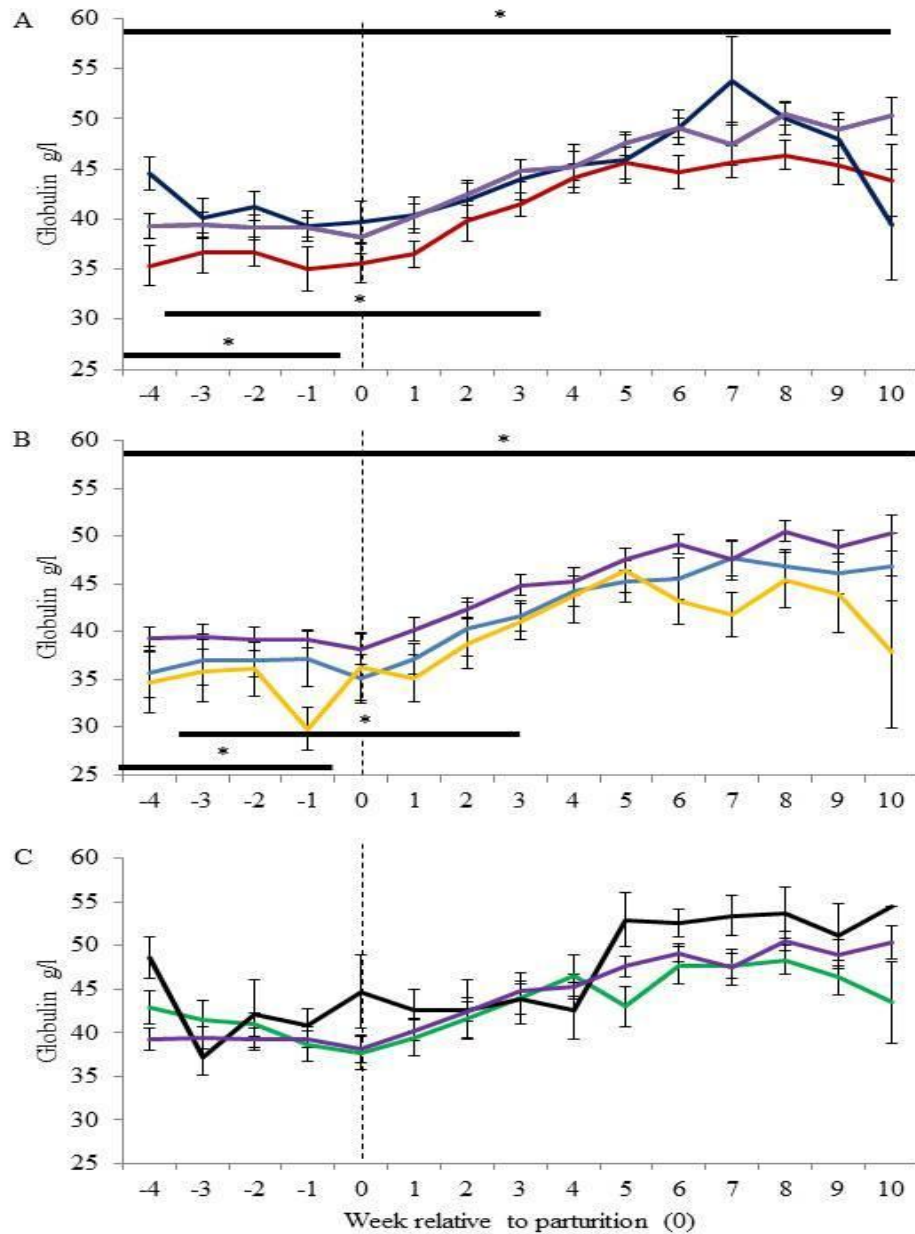


**Figure 4.9:** Total protein concentrations (g/l) ( $\pm$ SEM) in pre- and post-calving cows over a 15 week period: **A)** Singles control no-cyst group (purple, n=48) vs. cows with a follicular cyst (red, n=21) or luteal cyst (dark blue, n=10). **B)** Singles control no-cyst group (purple, n=48) vs. cows with follicular cysts formed early (light blue, n=14) or late (orange, n=7) postpartum. **C)** Singles control no-cyst group (purple, n=48), vs. cows with luteal cysts formed early (green, n=7), or late (black, n=3) postpartum. Cows with a follicular cyst had significantly lower ( $P < 0.05$ ) total protein concentrations across the 15 weeks, particularly in the late post-calving period ( $P < 0.05$ ) compared to the no-cyst group (A). Total protein concentrations were consistently lower in cows with a follicular cyst that developed late ( $P < 0.05$ ) compared to the no-cyst group across the 15 weeks, particularly in the transition ( $P < 0.05$ ) and late post-calving periods ( $P < 0.05$ ) (B).





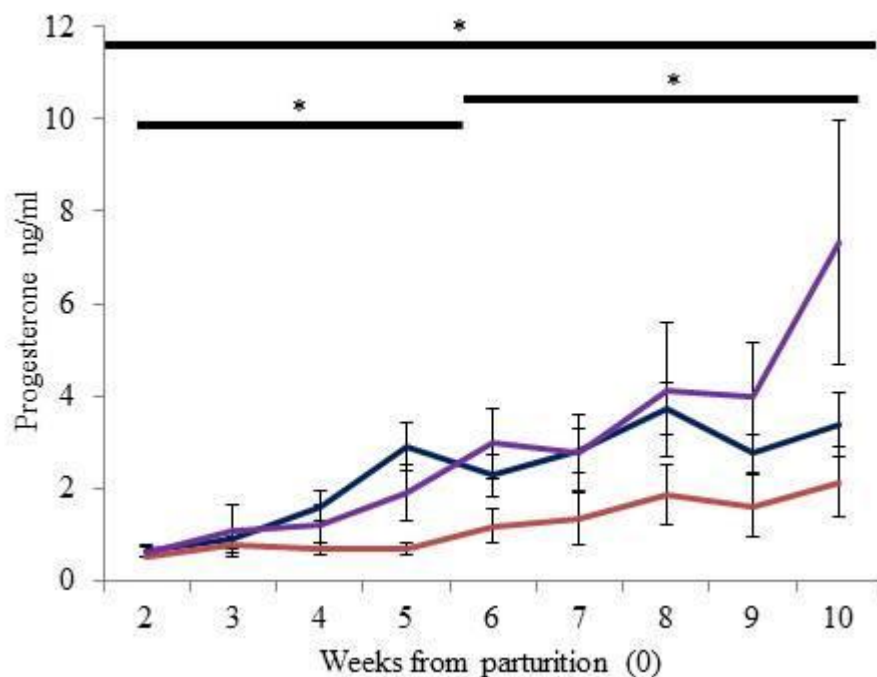
**Figure 4.10:** Albumin concentrations (g/l) ( $\pm$ SEM) in pre- and post-calving cows over a 15 week period: **A)** Singles control no-cyst group (purple, n=48) vs. cows with a follicular cyst (red, n=21) or luteal cyst (dark blue, n=10). **B)** Singles control no-cyst group (purple, n=48) vs. cows with follicular cysts formed early (light blue, n=14) or late (orange, n=7) postpartum. **C)** Singles control no-cyst group (purple, n=48), vs. cows with luteal cysts formed early (green, n=7), or late (black, n=3) postpartum. Cows with a luteal cyst had significantly elevated albumin concentrations ( $P < 0.05$ ) during the transition and early post-calving periods compared to the control group. (A). Albumin concentrations in cows with a luteal cyst that developed early were higher ( $P < 0.05$ ) than the no-cyst group over the 15 weeks, particularly in the pre-calving ( $P < 0.05$ ), transition ( $P < 0.05$ ) and late post-calving ( $P < 0.05$ ) periods (C).



**Figure 4.11:** Globulin concentrations (g/l) ( $\pm$ SEM) in pre- and post-calving cows over a 15 week period: **A)** Singles control no-cyst group (purple, n=48) vs. cows with a follicular cyst (red, n=21) or luteal cyst (dark blue, n=10). **B)** Singles control no-cyst group (purple, n=48) vs. cows with follicular cysts formed early (light blue, n=14) or late (orange, n=7) postpartum. **C)** Singles control no-cyst group (purple, n=48), vs. cows with luteal cysts formed early (green, n=7), or late (black, n=3) postpartum. Globulin concentrations were lower ( $P<0.05$ ) in cows with a follicular cyst across the 15 week period, particularly in the pre-calving ( $P<0.05$ ) and transition ( $P<0.05$ ) periods compared to the control group (A). Globulin concentrations of cows that developed a follicular cyst late were lower ( $P<0.05$ ) over the whole 15 week period, particularly in the pre-calving ( $P<0.05$ ) and transition ( $P<0.05$ ) periods when compared to the control group (B).

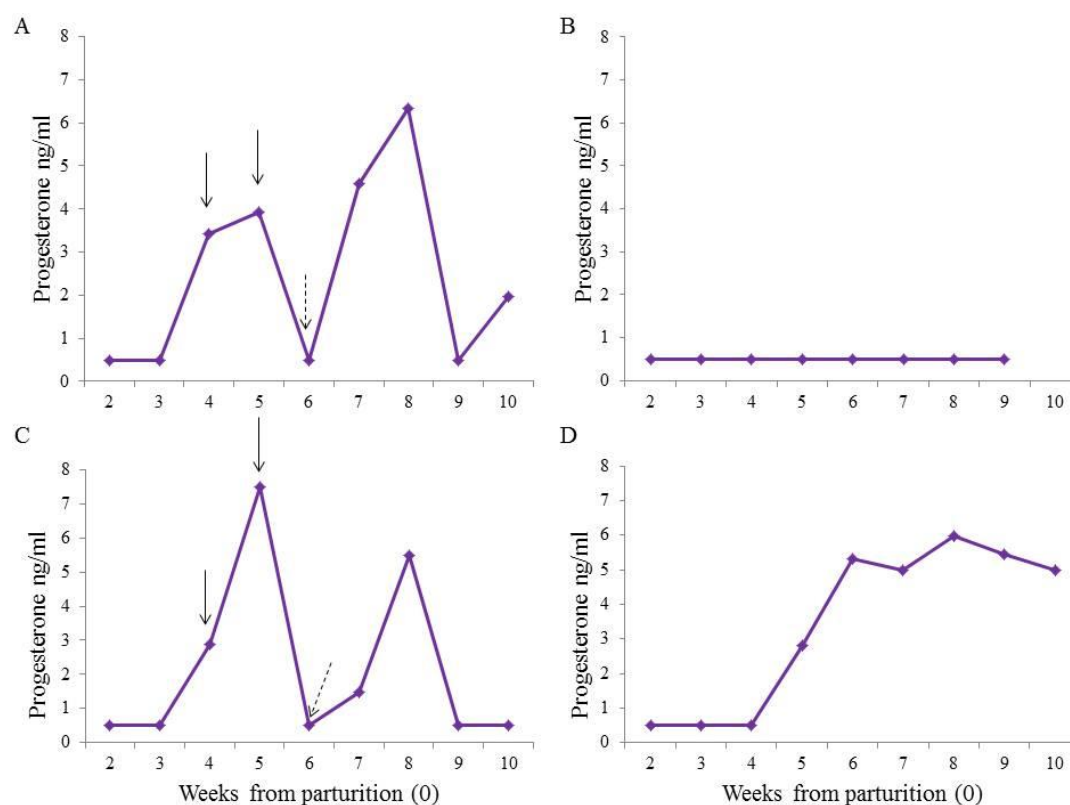
#### 4.4.4 Progesterone profiles (singletons)

Mean progesterone concentrations (Figure 4.12) were increased ( $P<0.001$ ) over the 15 weeks irrespective of cyst. There was a significant time x group interaction in which concentrations in cows with a luteal cyst were elevated compared to the control group, but only in the late post-calving period. Conversely, progesterone concentrations in cows with a follicular cyst were significantly lower than the control group from +2 to +10 weeks post-calving ( $P<0.05$ ).



**Figure 4.12:** Mean progesterone concentrations (ng/ml) ( $\pm$ SEM) in pre- and post-calving cows from two weeks post-partum. Singles control no-cyst (purple,  $n=48$ ) vs. cows with a follicular cyst (red,  $n=21$ ) or luteal cyst (dark blue,  $n=10$ ). Cows with a follicular cyst had lower concentrations of progesterone ( $P<0.05$ ) in weeks +2 to +5, +6 to +10 as well as from weeks +2 to +10 compared to the no-cyst cows.

The pattern of progesterone production in the control no-cyst group demonstrated that the cows were cycling normally and synchronously (i.e. two high and one low progesterone over three weeks (Bajema *et al.*, 1994)) (Figure 4.13).



**Figure 4.13:** Progesterone profiles (ng/ml) of two of the cows that did not develop an ovarian cyst from 2 weeks post-partum (**A** & **C**). Solid arrows indicate two measurements of high progesterone, and dashed arrows indicate the subsequent measurement of low progesterone that indicates a cow has successfully resumed cyclicity (Bajema *et al.*, 1994). Example progesterone profiles representative of cows that developed a follicular cyst (**B**) or a luteal cyst (**D**)

**NB:** the profile in D may also have resulted from a persistent CL rather than a luteal cyst, differentiation between the two at diagnosis can be difficult.

**Table 4.3:** Summary of key parameters that differed ( $P < 0.05$ ) from the control cows during specific time windows for cows bearing single offspring. NB: Progesterone was not measured in the pre-calving or transition period or for the first week post-calving.

	All	Pre-Calving	Early Post-Calving	Late Post-Calving	Transition
FC	↓ Total Protein ↓ Globulin ↓ GGT ↑ Triglyceride ↓ Progesterone	↓ Globulin ↑ Triglyceride	↑ Urea ↓ Globulin ↑ Triglyceride ↓ GGT ↓ Progesterone	↓ Total Protein ↓ Progesterone	↓ Globulin ↑ Triglyceride
LC			↑ Albumin	↑ BHB	↑ Albumin

#### 4.4.5 Calving to conception intervals and 305d milk yield data for cows bearing single offspring

Cows that developed a follicular cyst had a longer CCI than those that developed no cyst. Conversely, cows that developed a luteal cyst had a numerically shorter CCI than those that did not. Furthermore at least 10% more cows with a luteal cyst became pregnant within the timeframe of the trial than those of the control group. However, the sample size of cows with a luteal cyst was low and there was no significant difference in length of CCI for cows with a cyst vs. cows without (Table 4.4).

Table 4.4: The effect of the presence of a follicular or luteal cyst on the calving to conception interval (CCI). n indicates the number of animals that conceived out of the total number in that group. These data were analysed by one-way ANOVA.

SEM = standard error of the mean; NS = not significant.

Group	n	%	CCI (days)	±SEM	P
Control	38/48	79	162	12	
Follicular cyst	20/21	95	178	18	NS
Luteal cyst	9/10	90	137	23	NS

Cows with an ovarian cyst (either follicular or luteal) had a lower 305d milk yield than the no-cyst group (Table 4.5); however this difference was small and not significant.

Table 4.5: Summary of 305 day (d) milk yield for the previous lactation in each group. These data were analysed by one-way ANOVA.

SEM = standard error of the mean; NS = not significant.

Group	n	305d Milk Yield	±SEM	P
Control	48	8333	307	
Follicular cyst	21	8229	564	NS
Luteal cyst	10	8181	561	NS

#### 4.4.6 Early versus late ovarian pathology

Comparison of follicular or luteal cysts that form early or late demonstrated no discernible pattern. In cows that developed an early cyst (irrespective of follicular or luteal) urea, triglyceride and albumin concentrations were all elevated ( $P < 0.05$ ) above those of the control group. In cows that developed a late cyst (irrespective of follicular or luteal) triglyceride concentrations were elevated ( $P < 0.05$ ) above those of the control group. While total protein and globulin concentrations were lower ( $P < 0.05$ ) than the control group.

#### 4.4.7 Effect of cows bearing twin offspring on metabolite parameters

Concentrations of NEFA, BHB, triglycerides as well as AST activity were all elevated above normal expected values while concentrations of BHB, urea, total protein, albumin, cholesterol as well as GGT activity were within the expected range across the transition period (Table 4.2).

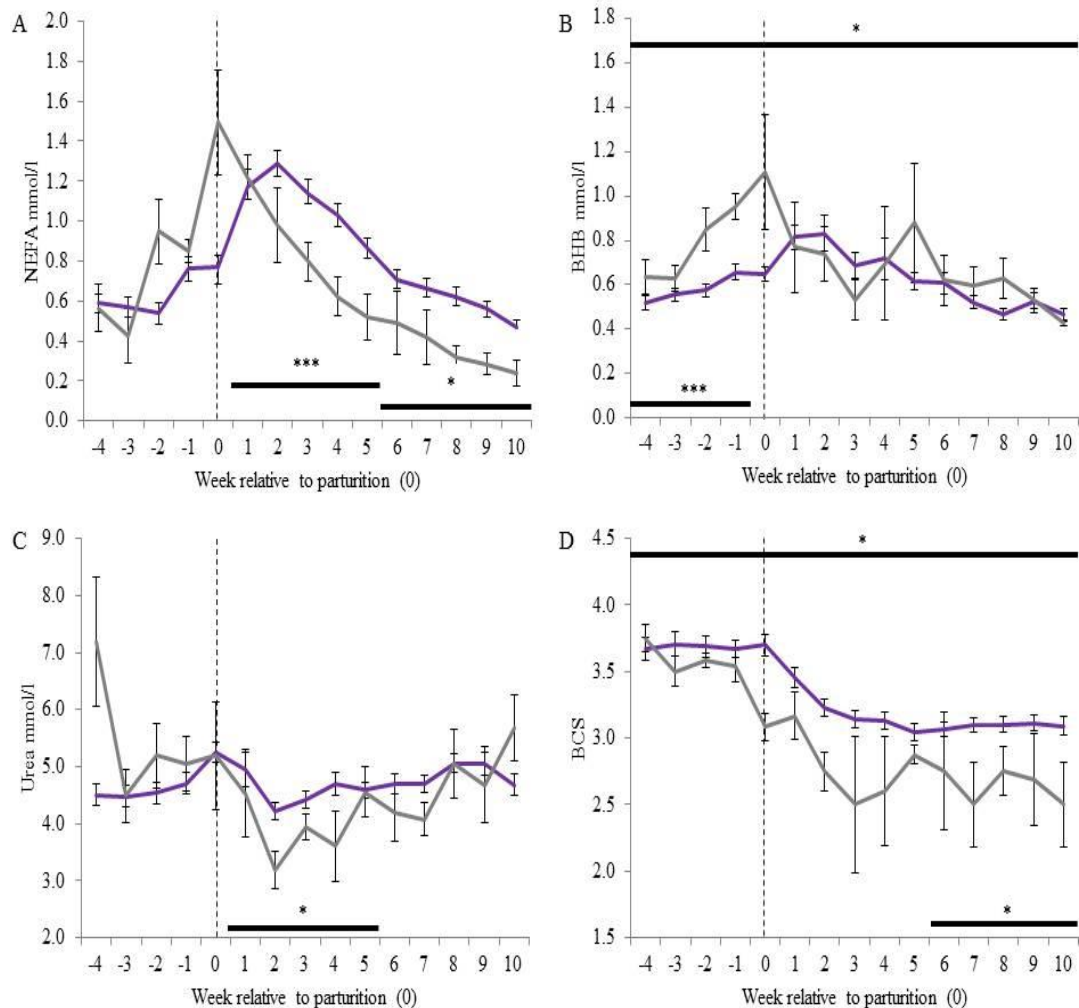
##### 4.4.7.1 Energy parameters (twins)

The temporal profiles of NEFA, BHB and urea concentrations, as well as loss of BCS were similar across the whole 15 weeks (Figure 4.14) in twins compared to singles. However, there were some important differences, these were as follows;

- NEFA and BHB concentrations in twins peaked one week earlier, at calving, compared to singles but decreased steadily from calving onwards. At week 3 post parturition, BHB concentrations increased in twins for two weeks before decreasing again.
- There was a greater fluctuation in urea concentrations in twins compared to singles.
- Loss of BCS was more acute from the start of the trial in twins up until week 3 post parturition compared to singles.

There was no significant difference between NEFA and urea concentrations nor was there a significant difference in BCS in twins compared to singles in the pre-calving period. During the transition period, there was no significant difference in concentrations of NEFA, BHB, urea or loss of BCS in twins compared to singles. In the early post-calving period, NEFA and urea concentrations were significantly lower ( $P < 0.001$  and  $P < 0.05$ , respectively) in twins compared to singles. During the

late post-calving period NEFA concentrations and BCS were lower ( $P<0.01$ ) in twins compared to singles, and there was a time x group interaction ( $P<0.05$ ) as urea concentrations of twins diverged above those of singles.



**Figure 4.14:** Concentrations of (A) NEFA (mmol/l) ( $\pm$ SEM), (B) BHB (mmol/l) ( $\pm$ SEM), (C) urea (mmol/l) ( $\pm$ SEM), and (D) body condition score ( $\pm$ SEM) in twin bearing (grey,  $n=6$ ) vs. single bearing (purple,  $n=48$ ) cows. NEFA concentrations were significantly lower ( $P<0.05$ ) in the early and late post calving periods in twins compared to singles (A). Cows bearing twins had elevated BHB concentrations ( $P<0.05$ ) over the whole 15 weeks, but this was particularly noticeable in the pre-calving period ( $P<0.001$ ) (B). Urea concentrations were higher in cows bearing twins ( $P<0.05$ ) compared to singles in the early post-calving period (C). Loss of BCS in twins was significantly greater ( $P<0.05$ ) than singles across the 15 week period (D).



#### 4.4.7.2 Liver parameters (twins)

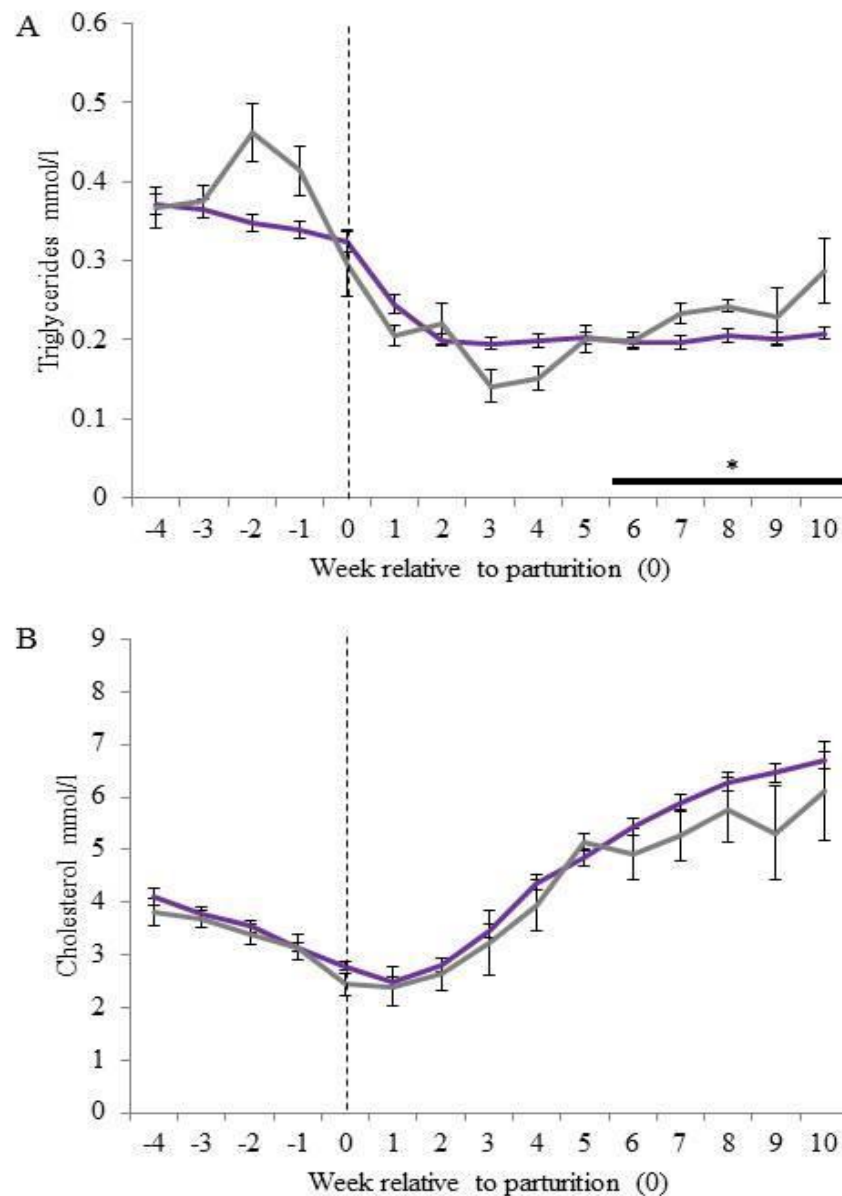
In the pre-calving period, there was no significant difference in concentrations or activity of any parameters when comparing twins with singles (Figures, 4.15, 4.16 and 4.17).

During the early post-calving period concentrations of cholesterol, total protein and globulin increased significantly ( $P<0.001$ ) while concentrations of triglycerides decreased significantly ( $P<0.001$ ) over time. AST activity also decreased significantly ( $P<0.001$ ) over time. There was a significant group x time interaction, in that concentrations of globulin ( $P<0.05$ ) and AST activity ( $P<0.05$ ) in twins and singles eventually converged and albumin concentrations were significantly lower ( $P<0.05$ ) in twins compared to singles in the early post-calving period.

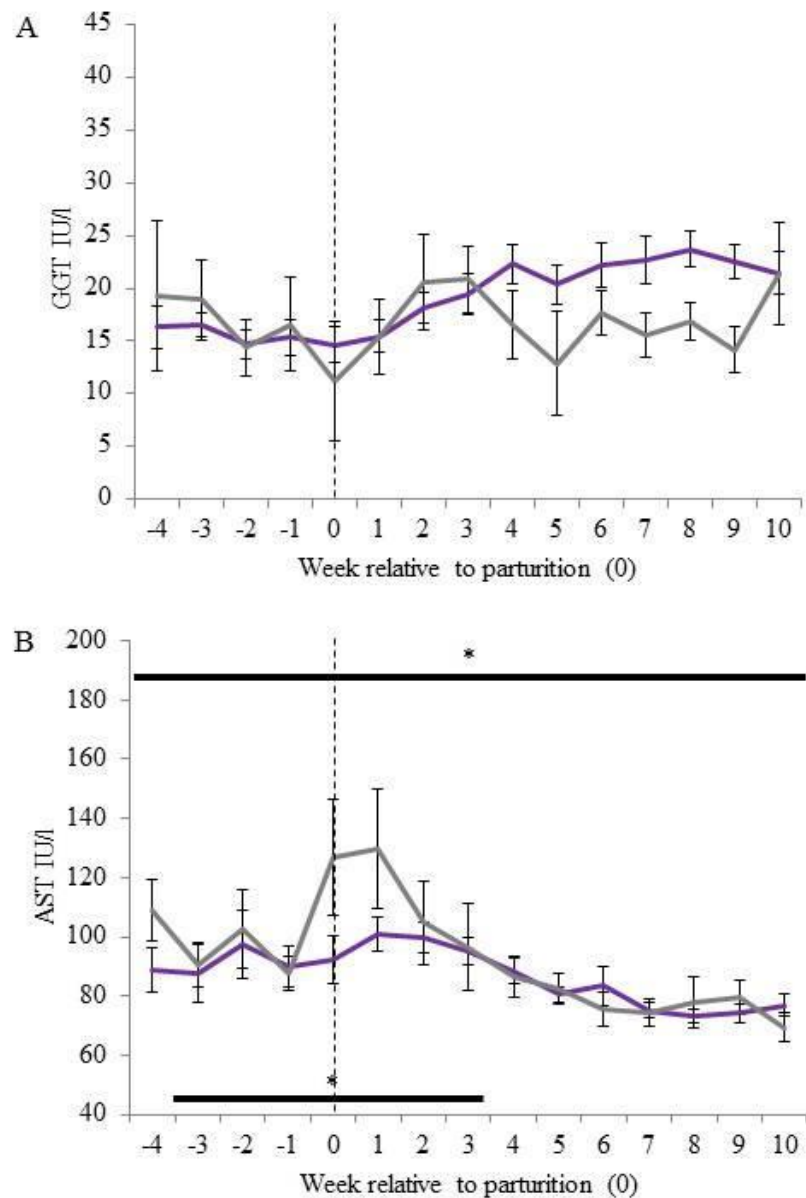
In the late post-calving period, concentrations of triglycerides and cholesterol significantly increased ( $P<0.05$ ) over time and there was a significant group x time interaction when albumin concentrations ( $P<0.05$ ) in twins diverged above those of singles. Triglyceride concentrations in twins also significantly diverged ( $P<0.05$ ) and elevated ( $P<0.05$ ) above those of singles. There was also a significant group x time interaction when concentrations of total protein ( $P<0.05$ ) and globulin ( $P<0.05$ ) in twins diverged below those of singles.

During the transition period, triglyceride concentrations significantly decreased ( $P<0.001$ ) and concentrations of cholesterol decreased then increased significantly ( $P<0.001$ ). Conversely, AST activities increased then decreased, and total protein concentrations ( $P<0.05$ ) and GGT activities ( $P<0.001$ ) significantly increased over time. AST activity was significantly higher ( $P<0.05$ ) in twins compared to singles, and there was a significant group x time interaction when a)

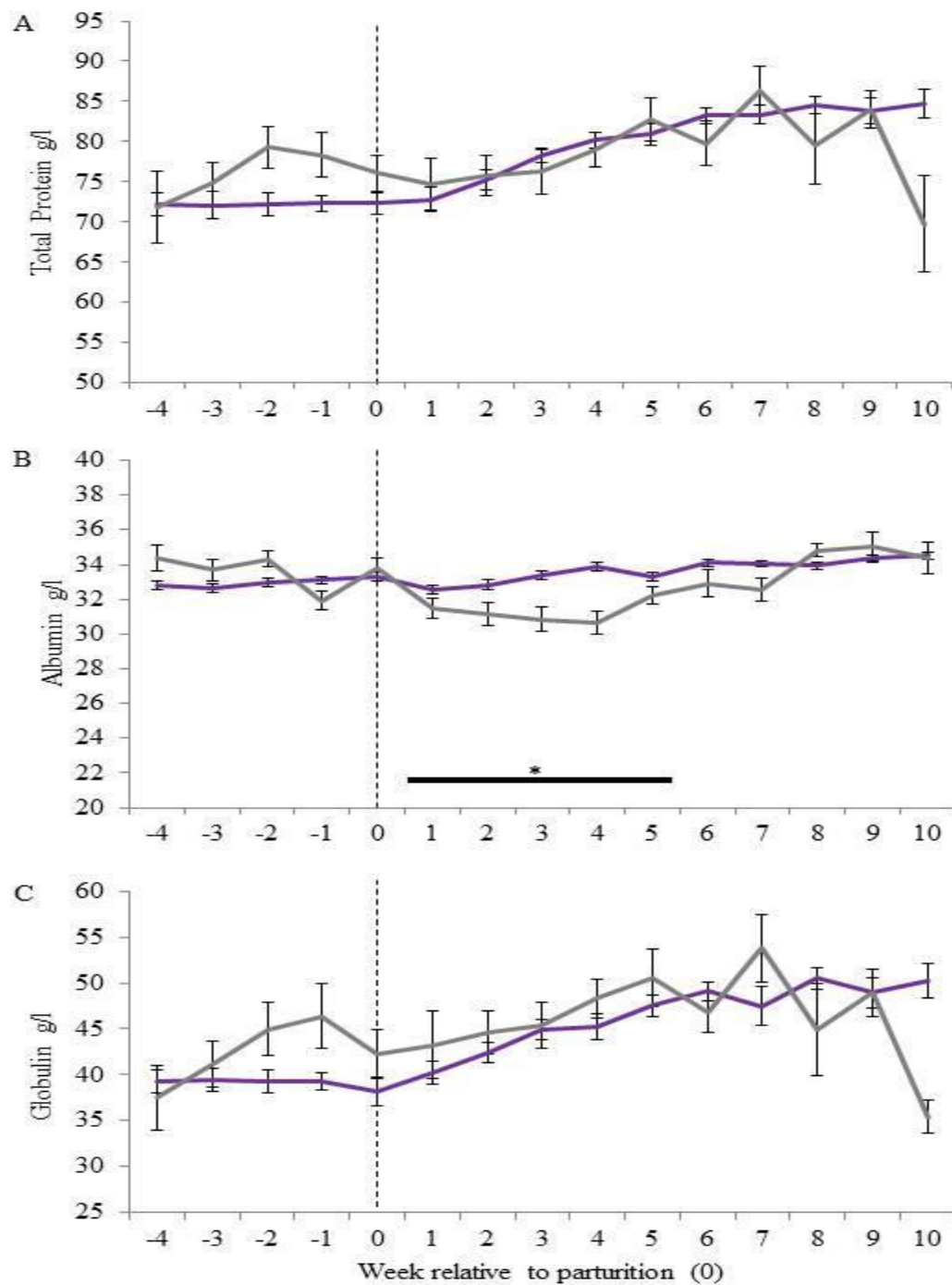
triglyceride concentrations in twins significantly diverged below those of singles ( $P<0.001$ ), and b) when globulin concentrations in twins significantly converged towards those of singles ( $P<0.001$ ).



**Figure 4.15:** Concentrations of **(A)** triglycerides (mmol/l) ( $\pm$ SEM) and, **(B)** cholesterol (mmol/l) ( $\pm$ SEM) in twin bearing (grey,  $n=6$ ) vs. single bearing (purple,  $n=48$ ) cows. Triglyceride concentrations were significantly higher ( $P<0.05$ ) in twins compared to singles in the late post-calving period (A).



**Figure 4.16:** Total activity of **(A)**  $\gamma$ -Glutamyl transpeptidase (GGT) (IU/l) ( $\pm$ SEM) and, **(B)** Aspartate transaminase (AST) (IU/l) ( $\pm$ SEM) in plasma of twin bearing (grey, n=6) vs. single bearing (purple, n=48) cows. AST activities were significantly higher ( $P<0.05$ ) in twins when compared to singles across the 15 weeks ( $P<0.05$ ), particularly during the pre-calving ( $P<0.05$ ) and transition ( $P<0.05$ ) periods (B).



**Figure 4.17:** Concentrations of **(A)** total protein (mmol/l) ( $\pm$ SEM), **(B)** albumin (mmol/l) ( $\pm$ SEM) and, **(C)** globulin (mmol/l) ( $\pm$ SEM), in twin bearing (grey, n=6) vs. single bearing (purple, n=48) cows. Albumin concentrations were lower ( $P<0.05$ ) in twins compared to singles in the early post-calving period (B).

**Table 4.5:** Summary of parameters in cows bearing twins that significantly differed (P<0.05) from cows bearing singles at each time point.

All	Pre-Calving	Early Post-Calving	Late Post-Calving	Transition
↑BHB ↓BCS ↑AST	↑BHB ↑AST	↓NEFA ↓Urea ↓Albumin ↓BCS	↓NEFA ↑Triglycerides ↓BCS	↑AST

#### 4.4.10 Calving to conception intervals and 305d milk yield data for cows bearing twin offspring

Twin bearing cows had a numerically shorter CCI than single bearing cows, and 13% more cows became pregnant from the single bearing group than from the twins bearing group, although this difference was not significant (Table 4.6).

**Table 4.6:** Effect of calving twins on calving to conception interval (CCI). These data were analysed by one-way ANOVA.

n indicates the number of animals that conceived out of the total number in that group. SEM = standard error of the mean; NS = not significant.

Group	n	%	CCI (days)	±SEM	P
Single	38/48	79	162	12	NS
Twin	4/6	66	147	30	

Twin bearing cows had a marginally higher 305d milk yield than single bearing cows (Table 4.7); however this difference was not significant.

Table 4.7: Summary of 305d milk yield data in twins and singles prior to starting the trial.

P values denote any significant difference in a group from the control no-cyst group. NS= not significant.

Group	n	305d Milk Yield	±SEM	P
Single	48	8333	307	NS
Twin	6	8771	915	

## 4.5 Discussion

### 4.5.1 Cyst versus no cyst comparisons in cows bearing single offspring

During the transition period, all cows had elevated NEFA concentrations (>0.4mmol/l) but not BHB concentrations (>1.4mmol/l) and therefore do not fit the classic criteria for NEB in cattle as defined by Stokol & Nydam (2005). However elevated NEFA concentrations combined with the loss of BCS experienced by these cows postpartum suggests that all cows were in a state of mild negative energy balance for at least the first 5 weeks postpartum.

Cows affected by a follicular cyst (n=21) had lower concentrations of total protein, globulin, as well as increased concentrations of triglyceride, compared to the control no-cyst group. Cows affected by a luteal cyst had elevated concentrations of albumin and BHB. As would be expected, cows with a follicular cyst had lower concentrations of progesterone, most likely resulting from a long period of low progesterone at the time a follicular cyst was present, and cows with a luteal cyst had high progesterone.

Decreased concentrations of total protein and globulin as well as increased triglycerides are indicative of impaired liver function; this may suggest that a negative energy balance could lead to impaired liver function that in turn may affect steroid metabolism in the liver (i.e. increased NEB = increased steroid metabolism), leading to interference in the HPO axis that may result in ovarian cysts. In sheep farming, ewes may be placed on a superior quality pasture in the weeks prior to exposure to the ram, a practice known as ‘flushing’ as it has been shown to increase lambing percentage by 15-20% (Coops, 1966) potentially as a result of decreased steroid metabolism (Payne *et al.*, 1991). Although this thesis does not examine mechanisms of superovulation, the principle remains that a change in diet can affect ovarian function. In the case of flushing, increased nutrition results in increased ovarian performance, is it possible that the opposite, NEB, may cause ovarian dysfunction?

Altering the plane of nutrition fed to lactating dairy cows has been shown to increase blood flow to the liver resulting in increased steroid metabolism (oestrogen and progesterone) (Sangsritavong *et al.*, 2002). This may, in turn, affect ovarian function by perturbation of the HPO axis; for example, if oestrogen secreted by the growing antral follicles was excessively metabolised (i.e. above that metabolised when an animal is fed to maintenance) by the liver then this may not feedback on hypothalamic-pituitary function to down regulate FSH secretion or stimulate an LH surge. This could lead to multiple dominant follicles or failure of ovulation. Furthermore, McCarthy *et al.*, (2010) demonstrated that a number of hepatic genes are both up- and down-regulated in NEB cows postpartum. Namely, the expression of genes involved in lipid transport, liver metabolism and catabolism were all up regulated in NEB cows (McCarthy *et al.*, 2010). This could explain why in this

thesis NEB cows may have lower plasma progesterone concentrations. This thesis proposes that ovarian cysts are not a reproductive disorder; but are instead a manifestation of postpartum liver dysfunction, arising from the high energy prepartum lactation diet and the physiological changes undergone by the liver in the postpartum period to facilitate lactogenesis.

The comparison between cysts that were identified before or after the 8 week postpartum pre-breeding exam identified some significant difference in concentrations of some, but not all, parameters at varying time points, but demonstrated no specific patterns. The time frame within which 'early' cysts developed encompassed 4 weeks, including the time at which the cows were most likely to reach peak lactation (week +6), whereas the time frame within which 'late' luteal cysts developed included only 2 weeks, therefore it is probable that more occurrences of difference were likely to have been seen. Furthermore, after week 8 postpartum any cysts confirmed or newly identified were treated immediately and that animal was removed from the trial, preventing the acquisition of a full metabolite profile. Moreover, the data shown for luteal cysts comes from a small sample group (n=10) and small differences may have been exaggerated by this sample size and could be misleading, therefore there is little benefit in pursuing this theory further.

Data from the previous lactation failed to identify a relationship between milk yield and the occurrence of ovarian cysts. Ideally, information from the subsequent lactation would have been investigated for a connection to ovarian cysts; however this information was not available for analysis.



The results from this study showed no relationship between CCI and occurrence of ovarian cysts, although the average CCI for cows with a luteal cyst was shorter than that of the control group, this difference was not significant.

#### 4.5.2 Twin vs. single comparisons

Twinning occurs at a frequency of 4% in UK dairy herds (Esslemont & Kossaibati, 2002) with multiple ovulations occurring more than split embryos (Wiltbank *et al.*, 2000). Risk factors for twins include parity, milk yield, season and previous incidence (Cady & Van Vleck, 1978; Nielen *et al.*, 1989; Ryan & Boyland, 1991). When comparing loss of BCS between the singles and the twins it is evident that cows calving twins mobilise more fat than those calving singles (Figure 4.14) in the pre-calving period which then plateaus post-calving. This pattern is reflected in circulating NEFA and BHB ( $P<0.05$ ) concentrations which, from weeks -4 up to -1 were also consistently higher in cows calving twins than those calving singles. Cows calving twins will have a greater requirement in late gestation to support the development of twin fetuses. This was indicated by the elevated total AST activity ( $P<0.05$ ) in the transition period which most likely resulted from muscle damage sustained during the late gestation and delivery of twins. Twin pregnancies can present a number of problems at calving and also during fetal growth when chances of fetal loss are increased in cows calving twins (López-Gatius & Hunter, 2005). This study demonstrates through early elevation of NEFA and BHB as well as early loss of BCS that cows calving twins developed NEB one week earlier than cows calving singles. Furthermore, circulating concentrations of NEFA and urea were significantly lower ( $P<0.05$ ) in twins compared to singles after calving which may indicate that they were beginning to recover from early onset NEB ahead of singles.

Knowing that twins can cause issues for both dairy cow management and health, this asks the question; why are twins not identified routinely during pregnancy diagnosis? While determination of twins is common place in valuable breeding cows at PD (from 25 days post insemination) as well as ewes, it is not routinely checked in most dairy cows. Differentiation between cows calving singles and cows calving twins would require no more than a thorough check of both uterine horns at the time of PD (Wapenaar W, personal communication).

Inadequate intake of energy during late pregnancy resulting in ketosis has been characterised as pregnancy toxaemia, or ‘twin lamb’ disease, in sheep (Sargison *et al.*, 1994). Separation of ewes carrying singles, twins and triplets is standard practice in sheep farming, allowing for each group to receive appropriate nutrition in order to support fetal growth (Russel *et al.*, 1967). There is no reason that this separation, or alteration in cow management, could not be applied to dairy cows in order to support the higher requirements for twin fetuses, as well as avoiding postpartum ketosis. Separating twins from singles at drying off could also help stockmen identify cows that may require more attention during calving. However, cows carrying twin offspring also have a higher incidence of calf mortality as well as the risk of freemartinism (Nielen *et al.*, 1989; Fricke 2001) and are thus not usually desired in dairy farming.

#### 4.6 Strengths and limitations

The control no-cyst group in this study were in a state of NEB throughout the duration of the trial. Although this does not give the more traditional ‘no-treatment’ control group it does provide a more accurate benchmark, in this instance, as it is likely that most UK dairy cows are unable to maintain a positive energy balance at and around calving, due to the intensive nature of British dairy farming.

Furthermore, the effects identified within this chapter can be attributed to the development of cysts, rather than any additional effects of NEB.

It is almost impossible to predict the proportion of cysts that will be diagnosed as follicular or luteal, making sample numbers to study specific cyst types unpredictable, despite a large herd size. Furthermore, as cows were inseminated and calved all year round it took longer to recruit the desired number of animals onto this trial; recruitment would have been easier if we had chosen a number of farms with a larger number of seasonally bred cows for this research.

Cows that were positively identified as having a cyst were treated at week +8 post-calving for ethical reasons; this may have affected metabolite readings in the final weeks of observation and given a slightly altered representation of what the metabolite concentrations would have reached if the cows were left untreated. It appears there may have been an effect of group on metabolite concentrations/activities in luteal cysts more so potentially than follicular cysts (based on similarities of cyst profiles compared to control no-cyst group). However this difference is very slight and the author is compelled to reiterate that n=10 for luteal cysts compared to n=21 for follicular cysts so it is likely that this slight difference is exaggerated by a smaller sample size.

#### 4.7 Conclusion

In conclusion, the results from this trial indicate that parameters traditionally measured to determine the health of the liver may also provide risk factors associated with the development of ovarian cysts. These cysts were associated with a perturbation of liver metabolism. Indeed, these factors may be better than currently used parameters which are indicative of the energy status of the animal. Furthermore, differentiation of follicular cysts may be achieved using biological markers identified from this work, specifically total protein, globulin and triglyceride concentrations, although these results warrant further investigation before this can be confirmed. If ovarian cysts are reclassified as a manifestation of liver dysfunction rather than a reproductive disease then this may ameliorate cow management through dietary rather than medicinal treatments.

The opportunistic comparison between cows bearing singles and cows bearing twins has identified an opportunity for improved cow management. Potentially, through the early identification of cows bearing twins to allow them to be separated pre-partum and also in the development of a more specific high energy diet that will facilitate the metabolic challenges encountered in the transition period by cows bearing twins.