3. Effects of post-partum BW, BCS, age and parity on ovarian steroids and metabolites during oestrus in *Bos indicus* cows in extensive production systems and the related effects on conception rates

Summary

The objectives of the present study were to test the effects and interactions between post-partum BW, post-partum BCS, age and parity number on plasma concentrations of estradiol, progesterone, creatinine, urea and cortisol around oestrus and the related conception rates in *Bos indicus* cows under extensive management conditions. The study also aimed at establishing the minimum BCS at the beginning of the breeding season in order to maximise the subsequent conception rates. Twenty-five peri-parturient cows on parity ≥ 2 were randomly selected to compose the experimental group. BW and BCS were measured around partum and thereafter at monthly intervals to the beginning of the breeding season, along with the reproductive tract monitored until the cows had shown a RTS≥4. The experimental animals were kept in the herd under extensive conditions.

Thirty-five days prior to the breeding season cows were divided into two groups and synchronised for oestrus using Crestar® (implant:3 mg Nergestomet + 2ml of crestar injection:5 mg oestradiol valerate; 3 mg Nergestomet and 10% Benzil alcohol as preservative) per group, three days apart. Blood samples for hormonal analysis were only collected during the second oestrus after synchronisation, from 24 hr pre-oestrus to 24 hr post-oestrus. A total of 18 samples were collected per animal: Sample 1 to 3 (collected at 4-hr intervals during the 24 to12 hr preceding oestrus); samples 4 to 15 (collected at 2-hr intervals during 12hr-0hr-12hr oestrus); and samples 16 to 18 (collected at 4-hr intervals during the 12 to 24 hr after oestrus). Blood samples were collected from the jugular vein into vacuum tubes containing EDTA, which were centrifuged immediately after collection and plasma stored at -20^{0}C. Estradiol, progesterone, cortisol and urea were assayed by ADVIA Centaur assay and SYNCRON LX® systems using Chemiluminescent technology while creatinine by Cobas Molecular P, based on the method of Jaffé reaction.

Data were analysed by means of ANOVA in SPSS. At the start of the breeding season the cows were in a positive energy balance and had a BCS of 2.8±0.3. The CR of cows was 90.5% and these

conceptions were concentrated in the first 21 days after the onset of the breeding season. BCS at the beginning of the breeding season correlated positively with estradiol (r=0.12), progesterone (r=0.2), createnine (r=0.3) at $p<0.05$. Negative correlations were observed between age of the cows and estradiol (r=-0.4) and cortisol (r=-0.2) and a similar trend with parity number at $p<0.05$. Creatinine and urea were correlated (r=0.5) and the values for both were within the normal range. The hormonal pattern of estradiol and progesterone around oestrus were similar to that observed in *Bos taurus* cows under intensive conditions. A relative increase in cortisol concentrations was observed at the beginning of the blood sampling and then declined. Better results on estradiol pattern and conception rates were related to a BCS of ≥ 2.5 and it was thus concluded that the post-partum management of extensive *Bos inducus* cows should be performed toward achieving at least a BCS of 2.5 at the beginning of the breeding season to maximise the re-conception rates.

3.1 Introduction

A better understanding of the hormonal mechanisms that occur during the oestrous cycle is undoubtedly becoming more important in a description of the outcome of any reproductive management strategy of extensive beef cows under tropical conditions (Machado *et al.,* 2008). However, to analyse and interpret a hormonal profile of beef cows under extensive conditions is a challenge. Previous reports indicate that to obtain the ovarian steroids and metabolite profile of post-partum cows involves monitoring the entire period over the oestrous cycle or longer (Evans *et al.,* 2003). In addition, the monitoring activity consists of an intensive blood-sampling schedule varying from once or twice daily during the first 19 days of the oestrous cycle to 15-minute intervals on the days around oestrus (Forde *et al.,* 2011). Following these methodologies requires the removal of the experimental animals from the herd and changes in handling facilities. Hence, the experiment is not performed under extensive conditions. On the other hand the relationships between post-partum BCS, BW, age, parity number and the related conception rates have been investigated in *Bos taurus* breeds under intensive conditions (Burns *et al.,* 2010) but have not been adequately investigated in *Bos indicus* breeds in extensive production systems.

This study was conducted to determine the effects of and interactions between post-partum BW, BCS, age and parity number on plasma concentrations of (1) estradiol, (2) progesterone, (3)

cortisol, (4) creatinine and (5) urea and the related conception rates of *Bos indicus* beef cows in extensive production systems under sub-tropical conditions. Since BCS is a good predictor of conception rates and can easily be estimated by farmers (Ayres *et al.,* 2009), the study also aimed at establishing minimum BCS values during the post-partum period in order to maximise post-partum re-conception rates in the subsequent breeding season.

3.2 Materials and methods

3.2.1 Study location

The experiment was carried out at the Inácio de Sousa extensive beef cattle farm located in the Manhiça district, approximately 100 km to the north of Maputo city, in Mozambique. The climate at this location is sub-tropical humid, with an average temperature of 28˚C and average annual rainfall of 950 mm. About 80% of the rainfall occurs during the normal rainy season of six months (October to March), of which about 50% occurs in December and January.

3.2.2 Animals

Twenty-five peri-parturient Brahman type cows on parity ≥2 were randomly selected to compose the experimental group. Body weight and BCS were measured around calving (November) and thereafter at monthly intervals until the beginning of the breeding season. The post-partum suckling cows were monitored monthly for uterine involution by rectal palpation and the reproductive tracts were scored using the reproductive tract score method as described by Schwalback e*t al.* (2000) until they had shown a RTS ≥ 4. The experimental cows were maintained in the herd under similar management conditions as compared to the rest of the herd, which was kept under extensive conditions.

3.2.3 Experimental design

3.2.3.1 Principle of blood-sample collection to monitor hormonal changes

A critical evaluation of hormonal changes in the peripheral plasma during the oestrous cycle of cows suggests that the endocrine changes that occur around oestrus are the most important during the oestrous cycle. Therefore, hormonal assays of blood samples around oestrus were obtained to provide the required information for analysis. Thus, in order to study the effects of BW, BCS, age and parity on ovarian steroids and metabolites in circulating blood, sampling procedures were scheduled for these hormones to cover the period of normal secretion of the reproductive hormones; e.g. before, during and after oestrus.

The efficiency of oestrus detection in *Bos indicus* cows is generally rather low (Landaeta-Hernandez *et al.,* 2002; Acevedo *et al.,* 2007; Galina and Orihuela, 2007; Portillo *et al.,* 2008) and a similar response was anticipated in the present study. For that reason, oestrus synchronisation was performed to concentrate oestrus within a short period of time during which blood samples were collected. The exogenous progesterone used in the present study was combined with estradiol, a well-known estrane with a demonstrated capacity to induce oestrus (Burke *et al.;* 2001; Bó *et al.,* 2003; Evans *et al.,* 2003; Macmillan *et al.,* 2003; Maneghetti *et al.,* 2009; Sá Filho *et al.,* 2011). To avoid possible effects of estradiol treatment on the hormonal profile, blood samples were only collected during the second oestrous cycle, after synchronisation.

3.2.3.2 Oestrus synchronization

Thirty-five days prior to the breeding season, cows incorporated in the study were divided into two groups and the synchronisation was performed per group three days apart. Cows were separated into two groups (group 1= 10 cows; group 2=11 cows) to facilitate the blood sampling and to ensure the sampling interval. Cows were synchronised for oestrus using a Crestar®1 (implant:3 mg Nergestomet + 2ml of crestar injection:5 mg oestradiol valerate; 3 mg Nergestomet and 10% Benzil alcohol as preservative). The synchronisation and blood sampling schedule are presented in Figure 3.1.

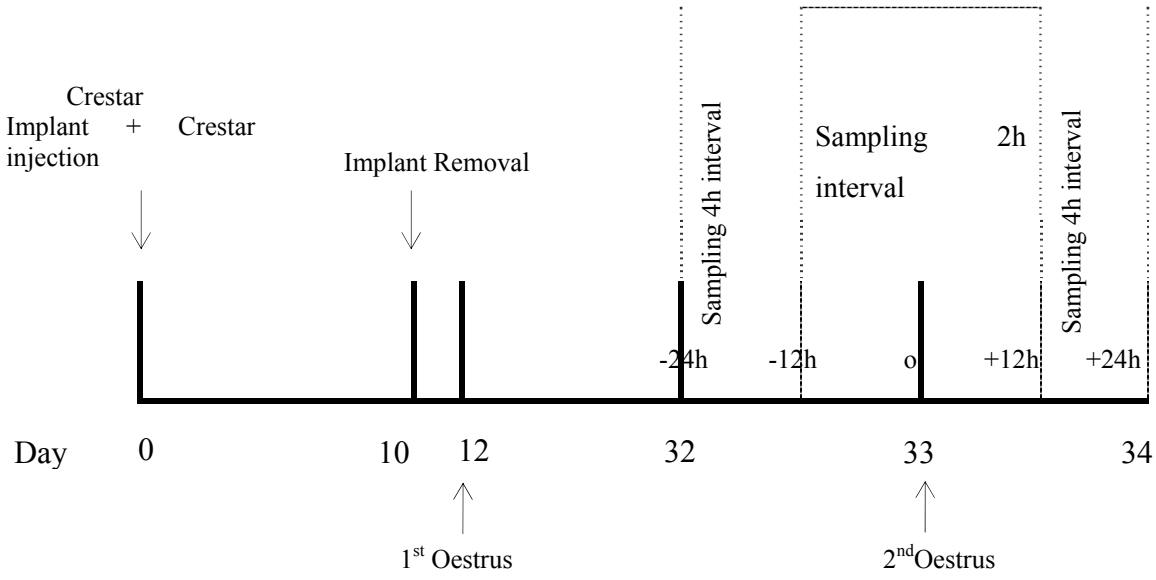

Figure 3.1.Diagram of oestrus synchronisation and blood sampling of *Bos indicus* cows for hormonal assay around oestrus

Blood samples were collected from the jugular vein into vacuum tubes containing EDTA and centrifuged immediately after collection at 3000 rpm. The plasma was stored at – 20°C. After blood sampling cows were returned to the herd. A total of 18 blood samples were collected from each animal: Sample 1 to 3 (collected at 4-hr intervals during the 24 to12 hr preceding oestrus); samples 4 to 15 (collected at 2-hr intervals during 12 hr-0 hr-12 hr oestrus); and samples 16 to 18 (collected at 4-hr intervals during the 12 to 24 hr after oestrus). Since the blood sampling was followed by the onset of the breeding season, satisfactory classified bulls (Hopkins and Spitzer 1997) were used at a ratio of 1:20 cows for a breeding season of 90 days, January to March (Schwalback *et al.,* 1997). Pregnancy diagnosis was done by rectal palpation 60 days after the breeding season ended and the non-pregnant cows were re-bred in the follow-up breeding season (June to July). Pregnant cows were monitored to the end of the gestation period and calving dates recorded. The calving season (October to December) was divided into early, mid and late, as described by *Escrivão et. al.* (2009).

3.2.4 Hormonal assay

Hormonal assay were performed at Department of Clinical Pathology in the Ampath Laboratory in Pretoria.

Estradiol: performed using a second generation kit as supplied by Roche, functional sensitivity 44 pmol/l, measuring range 44-15 781 pmol/l. Results above the analytical range were rerun after dilution to get an absolute result. The claimed total precision for the method is 2.3-6.2% (higher value applicable to lower results), and the Laboratory participate in external quality assurance scheme run by Thistle, to verify method performance.

Progesterone: performed using a second generation kit as supplied by Roche, functional sensitivity 0.5 nmol/l, measuring range 0.5-191 nmol/l. Results above the analytical range can be rerun after dilution to get an absolute result. The claimed total precision for the method is 2.0-4.8% (higher value applicable to lower results), and laboratory participate in external quality assurance scheme run by Thistle, to verify method performance.

3.2.4.1 Test validation

Validation of tests for bovine hormones and metabolites was done by collecting blood samples from cows confirmed pregnant (n=3) and in oestrus (n=3) via caudal venepuncture at the first blood sampling (t_0) and again at the second sampling 12-hr later (t_{12}). Samples were centrifuged immediately after collection and plasma was stored at -20ºC. Plasma samples were analysed for FSH, LH, progesterone, estradiol, cortisol, creatinine and urea. The ADVIA Centaur Assay and SYNCRON LX system automatically calculate the precision illustrated by intra- and interassay coefficient of variation, sensitivity and specificity as the main criteria for validation. The test was repeated twice and similar results were obtained. Based on these results, tests were validated for estradiol, progesterone, cortisol, urea and creatinine.

3.2.4.2 Estradiol assay

The estradiol ADVIA Centaur assay is a competitive assay using direct chemiluminescent technology that derives its name from coupling of the estradiol immunogen at the specificity-enhancing sixth position, allowing for the production of a highly specific antibody. This 17β-estradiol-6-antibody allows the ADVIA Centaur Estradiol-6 assay to be used across a wide range of applications. Estradiol in the sample competes with acridinium ester-labelled estradiol in the Lite Reagent for a limited amount of rabbit anti-estradiol antibody in the Antibody Reagent. Rabbit anti-estradiol is captured by mouse anti-rabbit IgG, which is coupled to paramagnetic particles in the solid phase.

The system automatically performs the following steps:

- Dispenses 50 µL of sample and 50 µL of Antibody Reagent into a cuvette and incubates for 5.5 minutes at 37 ^{0}C;
- Dispenses 50 µL of Lite Reagent and 250 µL of Solid Phase and incubates for 5.0 minutes at 37 ^{0}C;
- Separates, aspirates, and washes the cuvettes with reagent water;
- Dispenses 300 µL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction; and
- Reports results according to the selected option, or in the online help system;

An inverse relationship exists between the amount of estradiol present in the sample and the amount of relative light units (RLUs) detected by the system.

3.2.4.3 Progesterone assay

As with the estradiol assay, the ADVIA progesterone assay is a competitive immunoassay using direct chemiluminescent technology. Progesterone in the sample binds to an acridinium ester-labelled mouse monoclonal anti-progesterone antibody in the Lite Reagent. Unbound antibody binds to a progesterone derivative, covalently coupled to paramagnetic particles in the Solid Phase.

The system automatically performs the following steps:

- Dispenses 20 µL of sample and 90 µL of Releasing Agent into a cuvette;
- Dispenses 100 µL of Lite Reagent and incubates for 2.5 minutes at 37^0C;
- Dispenses 200 µL of Solid Phase and incubates for 5.0 minutes at 37^0C;
- Separates, aspirates, and washes the cuvettes with reagent water;
- Dispenses 300 µL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction; and
- Reports results according to the selected option, as described in the system operating instructions or in the online help system.

There is also an inverse relationship between the amount of progesterone present in the sample and the amount of relative light units (RLUs) detected by the system.

3.2.4.4 Cortisol assay

The ADVIA Centaur cortisol assay is also a competitive immunoassay using direct chemiluminescent technology. Cortisol in the sample competes with acridinium ester-labelled cortisol in the Lite Reagent for binding to polyclonal rabbit anti-cortisol antibody in the Solid Phase. The polyclonal rabbit anti-cortisol antibody is bound to monoclonal mouse anti-rabbit antibody, which is covalently coupled to paramagnetic particles in the Solid Phase.

The system automatically performs the following steps:

- Dispenses 20 µL of the sample into a cuvette;
- Dispenses 50 µL of Lite Reagent and 250 µL of Solid Phase and incubates for 5.0 minutes at 37 ^{0}C;
- Separates, aspirates, and washes the cuvettes with reagent water;
- Dispenses 300 µL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction; and
- Reports results according to the selected option, as described in the system operating instructions or in the online help system.

As with the previous assays there is an inverse relationship between the amount of cortisol present in the sample and the amount of relative light units (RLUs) detected by the system.

3.2.4.5 Urea Nitrogen (BUN) assay

BUN Reagent is used to measure urea nitrogen by an enzymatic rate method (. In the reaction, urea is hydrolysed by urease to ammonia and carbon dioxide. Glutamate dehydrogenase (GLDH) catalyses the condensation of ammonia and α-ketoglutarate to glutamate with concomitant oxidation of reduced β-nicotinamide adenine dinucleotide (NADH) to β-nicotinamide adenine dinucleotide (NAD) (Tietz, 1995).

The SYNCHRON LX System automatically dilutes samples and proportions to the appropriate sample reagent into the cuvette. The ratio used was one part sample to 100 parts reagent for plasma. The system monitors the change in absorbance at 340nm. This change in absorbance is directly proportional to the concentration of urea nitrogen in the sample and used by the SYNCHRON LX System to calculate and express the urea nitrogen concentration. The chemical reactions are as follows:

$$\text{Urea} + H_20 \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \alpha\text{-ketoglutarate} + NADH + H^+ \xrightarrow{\text{GLDH}} \text{Glutamate} + NAD^+ + H_2O$$

3.2.4.6 Creatinine assay

Creatinine in the plasma was assayed based on the method of Jaffé reaction as described by Pooper *et al.* (1937), Seeling and Wust (1969) and modified by Bartels *et al.* (1972). The modified version has a higher sensitivity and better precision than the original Jaffé method (Tietz, 1995). The

addition of sodium hydroxide and piric acid trigger kenetic colorimetric assay with the following reaction:

creatinine + piric acid $\xrightarrow{\text{Alkaline solution}}$ Creatinine-piric acid complex

In the alkaline solution, creatinine forms a yellow-orange-complex with picrate. The colour intensity is directly proportional to creatinine concentration and can be measured photometrically. The plasma samples contain proteins which react non-specifically in the Jaffé method. Therefore, plasma results were corrected by 26 µmol/L (0.3mg/dl) to obtain accurate values. The assay was performed in Cobas Modular P.

Estradiol, progesterone, cortisol and urea were measured using ADVIA Centaur Assay and SYNCRON LX Systems, while creatinine was assayed by Cobas Modular P.

3.2.5 Statistical analysis

Data were analysed by means of the analysis of variance (ANOVA) procedure in SPSS version 14.0 for Windows by including sampling time, BCS at the beginning of the breeding season, age, parity and pregnancy status as fixed factors as well as the corresponding interactions in the model. Estradiol, progesterone, cortisol, creatinine and urea were included as variables. Pearson product moment correlation coefficients were calculated between variables as well as the significance levels. Differences between factors were assessed at the level of $p< 0.05$ (95% accuracy). All results were expressed as least square means (LSmeans) ± standard deviation (SD) and multiple comparisons of means were done by means of the Bonferroni method in order to correct for unbalanced data, where the number of observations differed. Pregnancy status was analysed by Chi-square analysis (SPSS, 2005).

3.3 Results

From calving to the beginning of the breeding season all cows incorporated in the study had a positive energy balance and a relative increase in BCS as indicated in Table 3.2. Four cows were not synchronised for oestrus because the RTS were below 4 and, thus, excluded from the study. All cows showed oestrus signs between day 11 and 13 post-synchronisation. During the scheduled period for blood sampling cows also showed oestrus signs. The numbers of observations per factor are presented in Table 3.1

Table 3.1 Number of observations (n) for BCS, parity number and age of cows.

Characteristic		No of observations
Body Condition Score	2	4
	2.5	10
	3	7
Parity Number	2	5
	3	5
	4	5
	5	6
Age	4	4
	5	3
	6	3
	7	8
	8	3

The overall conception rate of cows in the study was 90.5%. The majority of conceptions occurred during the first 21 days after the onset of the breeding season. The two cows that did not conceive had a BCS of 2 and 3 but were relatively old cows of about 8 years of age.

The mean concentrations of steroids and metabolites around oestrus and the related reproduction data are presented in Table 3.2

Table 3.2 Serum concentration of steroids and metabolites around oestrus and the related reproduction data in Brahman type cows under extensive conditions

Characteristics	LSMeans±SD
Estradiol (pmol/l)	131.7±107.7
Progesterone (nmol/l)	6.3 ±10.9
Urea (mmol/l)	4.6 ±1.4
Creatinine (µmol/l)	96.9 ± 15.4
Cortisol (nmol/l)	21.1 ± 14.8
BCS Nov	2.4 ± .39
BCS Jan	2.8 ± .28
BW Nov	404 ± 50
BW Jan	418 ±51
Age (Years)	6.4 ±1.2
Parity_Number	3.8 ± 1.1
Early conceptions (%)	60
Mid conceptions (%)	20
Late conceptions (%)	10

BCSJan- BCS January (BCS at the start of the breeding season)
Early conception – Conceptions in the 1st 21 days after the onset of the breeding season
Mid conception - Conceptions in the 2nd 21 days after the onset of the breeding season
Late conceptions - Conceptions above 42 days after the onset of the breeding season

The blood concentrations of steroids and metabolites around oestrus and the productive and reproductive characteristics included in the present study were either correlated or not, as illustrated in Table 3.3.

Table 3.3 Pearson product moment correlation coefficients between BCS, age, parity and estradiol, progesterone, urea, criatenine and cortisol around oestrus in *Bos indicus* cows under extensive conditions

Control Variables			Estrad	Proge	Urea	Creat	cortisol	BCSJan	Age	P_No#
Sample_No	Estradiol	Correlation	1.000							
		Significance (2-tailed)	.							
		Df	0							
	Progesterone	Correlation	-.081	1.000						
		Significance (2-tailed)	.141	.						
		Df	334	0						
	Urea	Correlation	.202	.035	1.000					
		Significance (2-tailed)	**.000**	.519	.					
		Df	334	334	0					
	Creatinine	Correlation	.009	.378	.455	1.000				
		Significance (2-tailed)	.870	**.000**	**.000**	.				
		Df	334	334	334	0				
	Cortisol	Correlation	.079	-.006	-.094	-.026	1.000			
		Significance (2-tailed)	.146	.908	.085	.632	.			
		Df	334	334	334	334	0			
	BCSJan	Correlation	.115	.231	.104	.248	.022	1.000		
		Significance (2-tailed)	**.035**	**.000**	.057	**.000**	.684	.		
		Df	334	334	334	334	334	0		
	Age	Correlation	-.352	.064	.136	.094	-.171	-.101	1.000	
		Significance (2-tailed)	**.000**	.244	**.012**	.084	**.002**	.064	.	
		Df	334	334	334	334	334	334	0	
	Parity_No#	Correlation	-.289	.094	.114	.185	-.095	-.163	.904	1.000
		Significance (2-tailed)	**.000**	.084	**.037**	**.001**	.082	**.003**	**.000**	.
		Df	334	334	334	334	334	334	334	0

In **Bold** means differ significantly $p<0.05$

The effect of BCS at the onset of the breeding season on estradiol, progesterone, creatinine, urea and cortisol around oestrus are illustrated in figures 3.2 to 3.6 while the effect of age is presented in figures 3.7 to 3.11 and the effect of parity number in figures 3.12 to 3.16 (These effects will be discussed in Section 3.5).

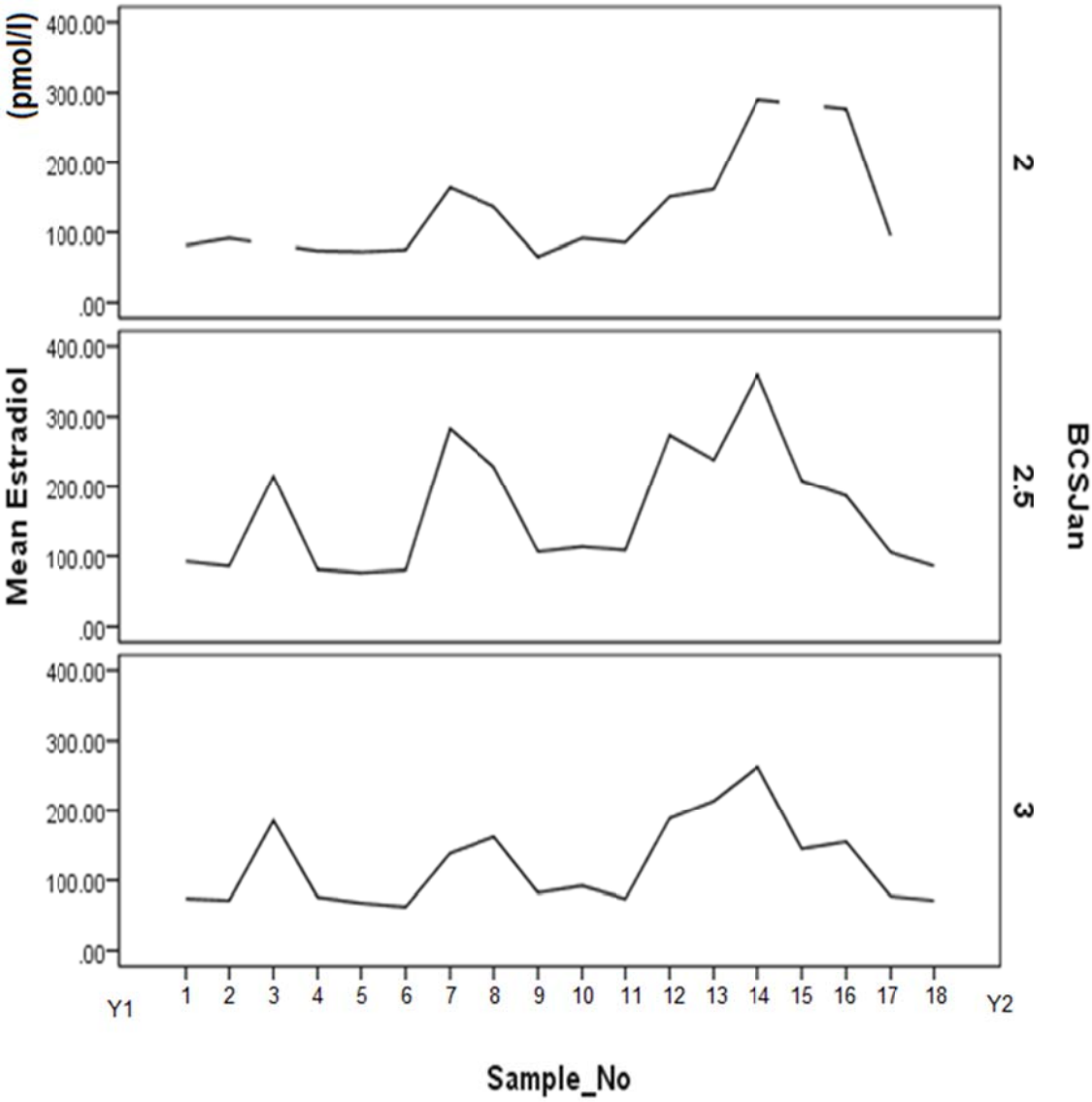

Figure 3.2 Effects of BCS on estradiol concentrations around oestrus

Estradiol at BCS 2; 2.5 and 3 (illustrated on axis $-Y_2$) differed significantly ($p<0.05$)

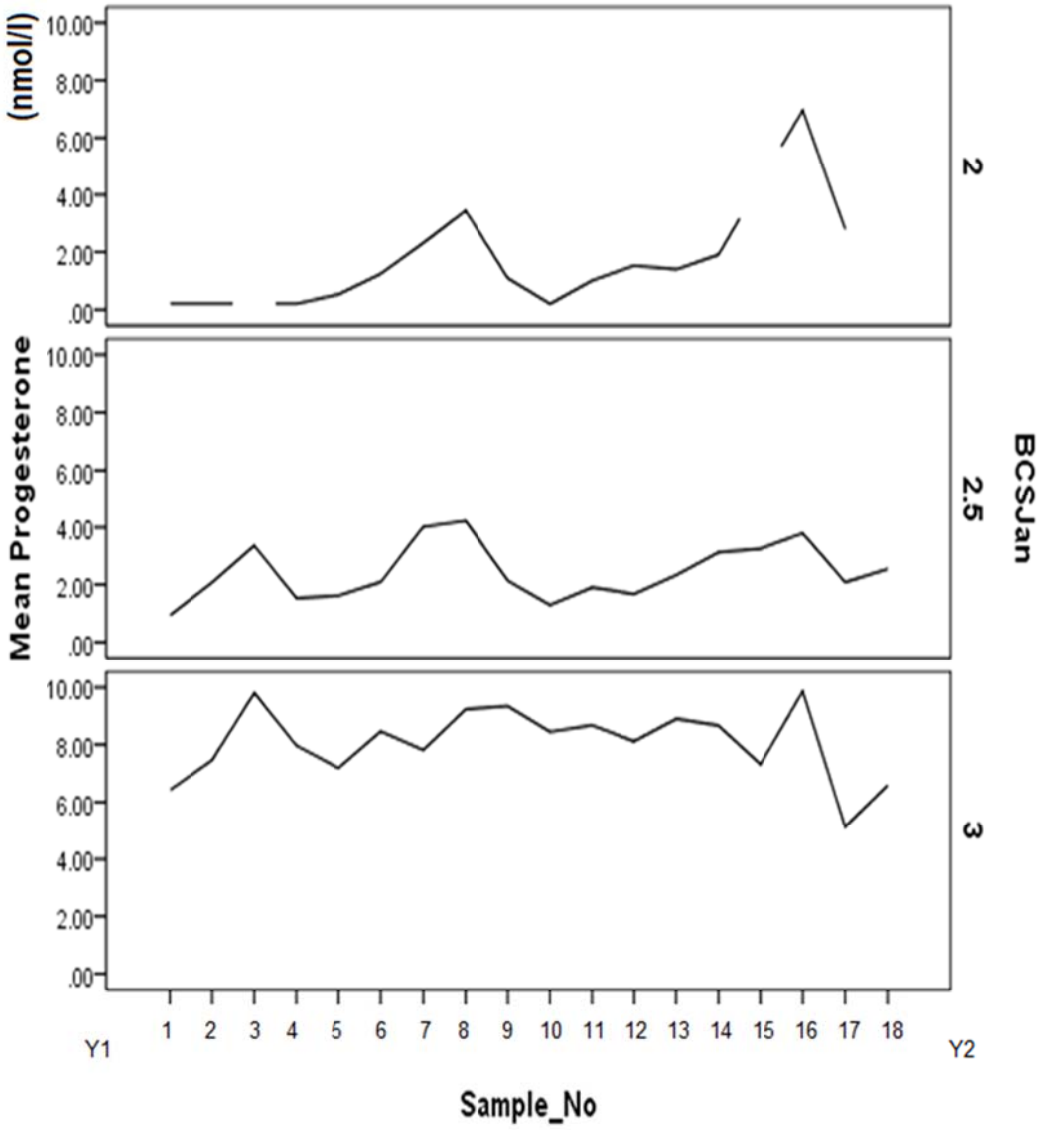

Figure 3.3 Effect of BCS on progesterone concentrations around oestrus
Progesterone at BCS 2; 2.5 and 3 (illustrated on axis $-Y_2$) differed significantly ($p<0.05$)

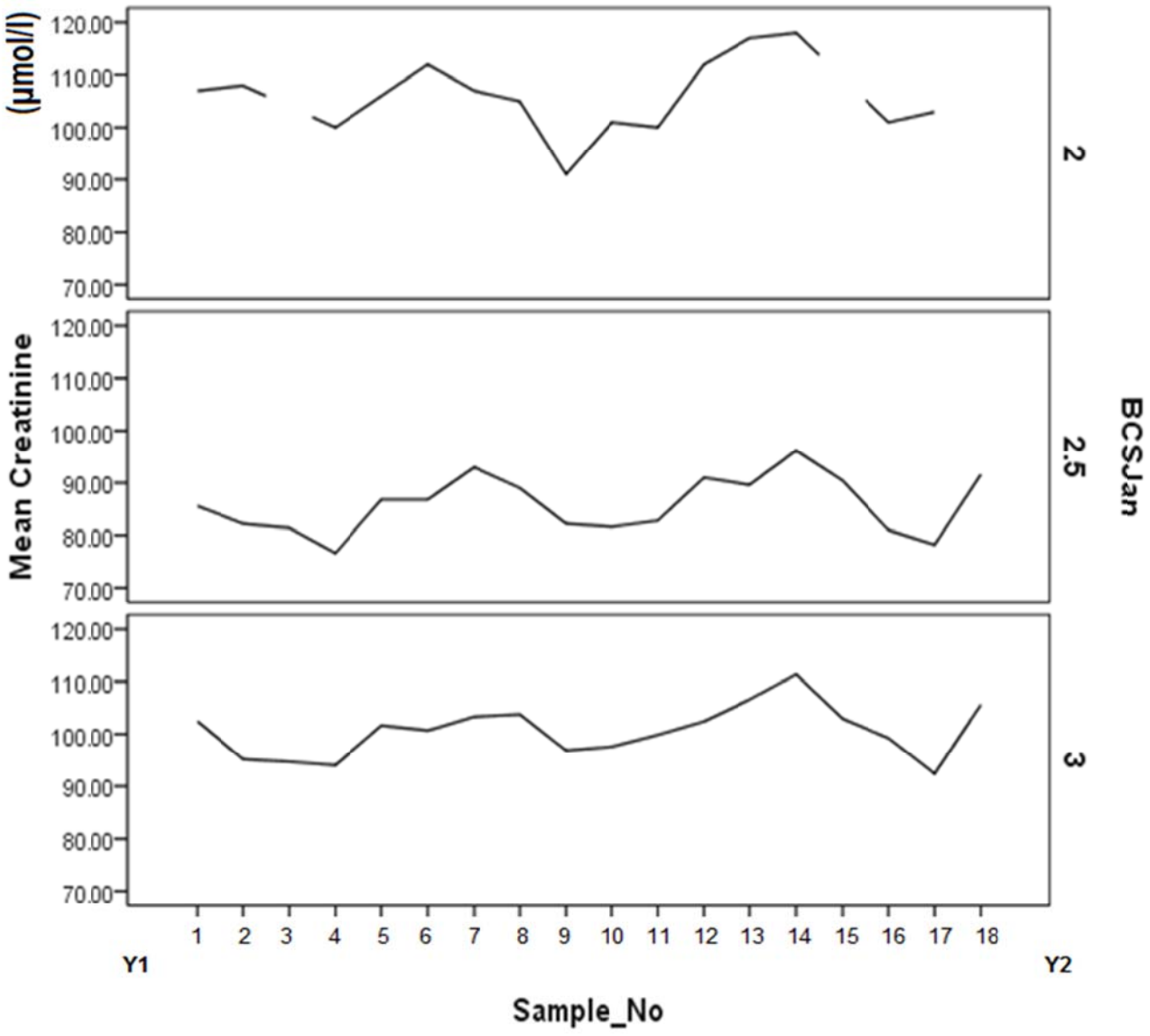

Figure 3.4 Effect of BCS on creatinine concentrations around oestrus

Creatinine at BCS 2; 2.5 and 3 (illustrated on axis $-Y_2$) differed significantly ($p<0.01$)

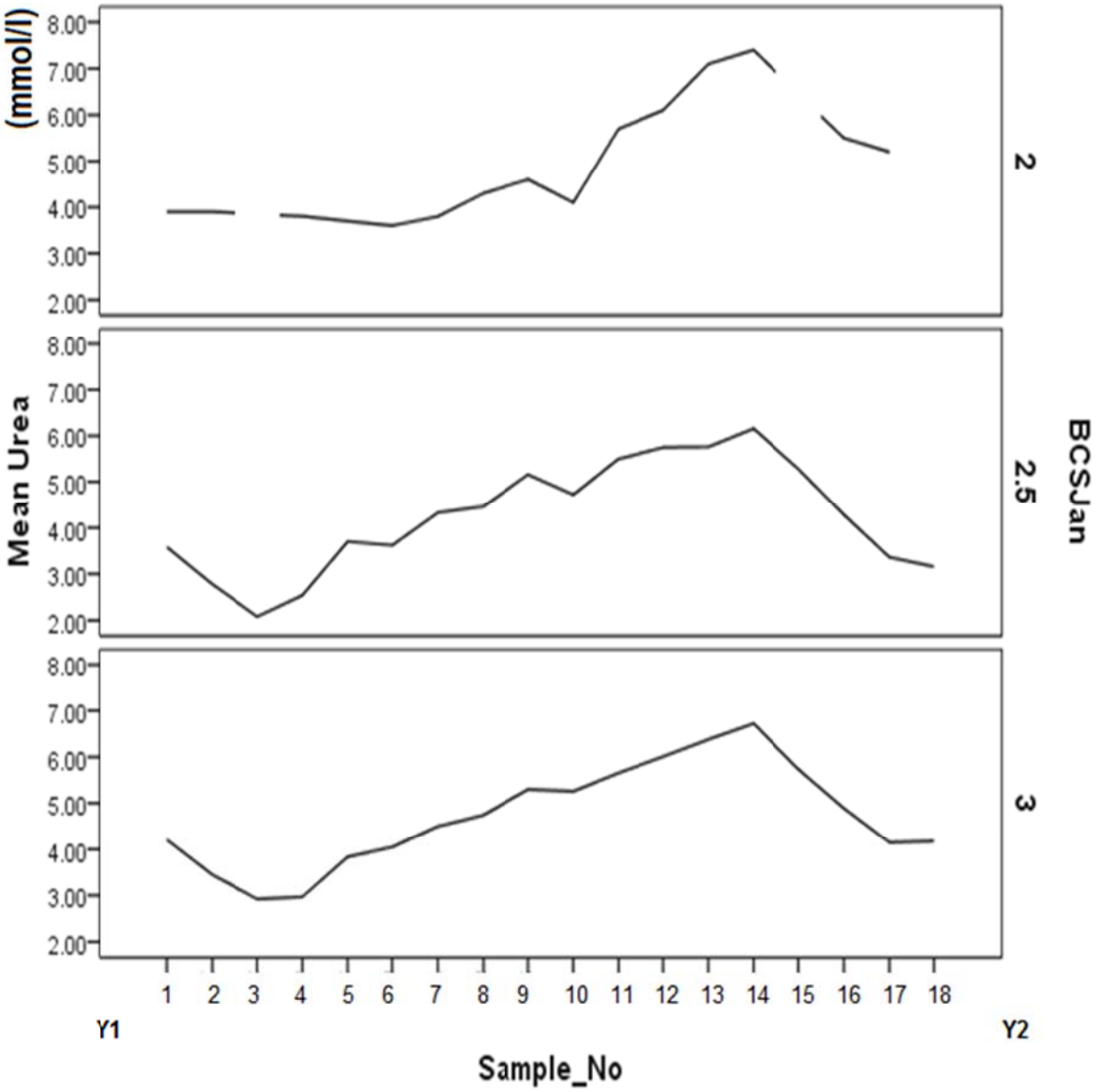

Figure 3.5 Relationship of BCS and urea concentrations around oestrus

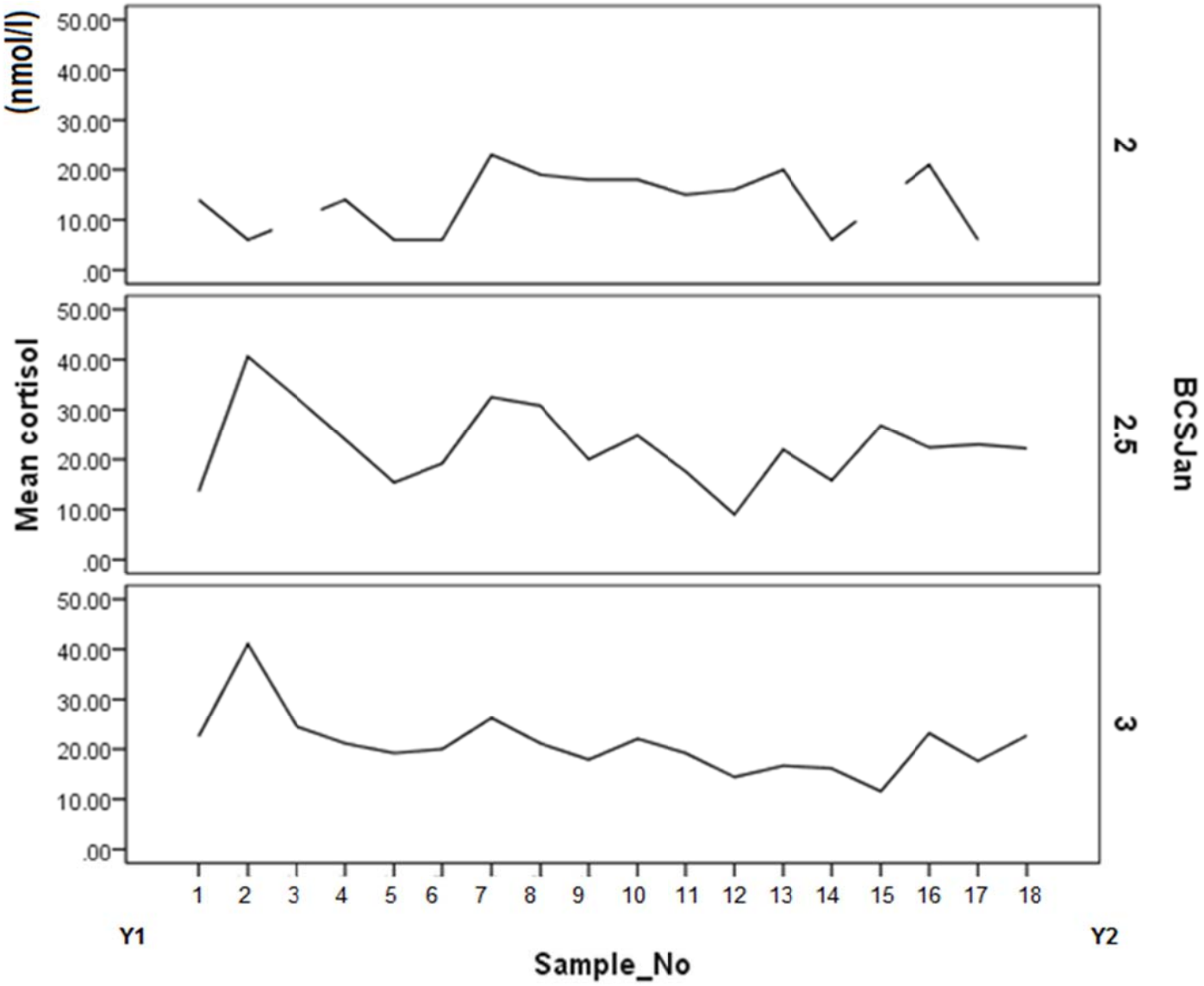

Figure 3.6 Relationship BCS and cortisol concentrations around oestrus

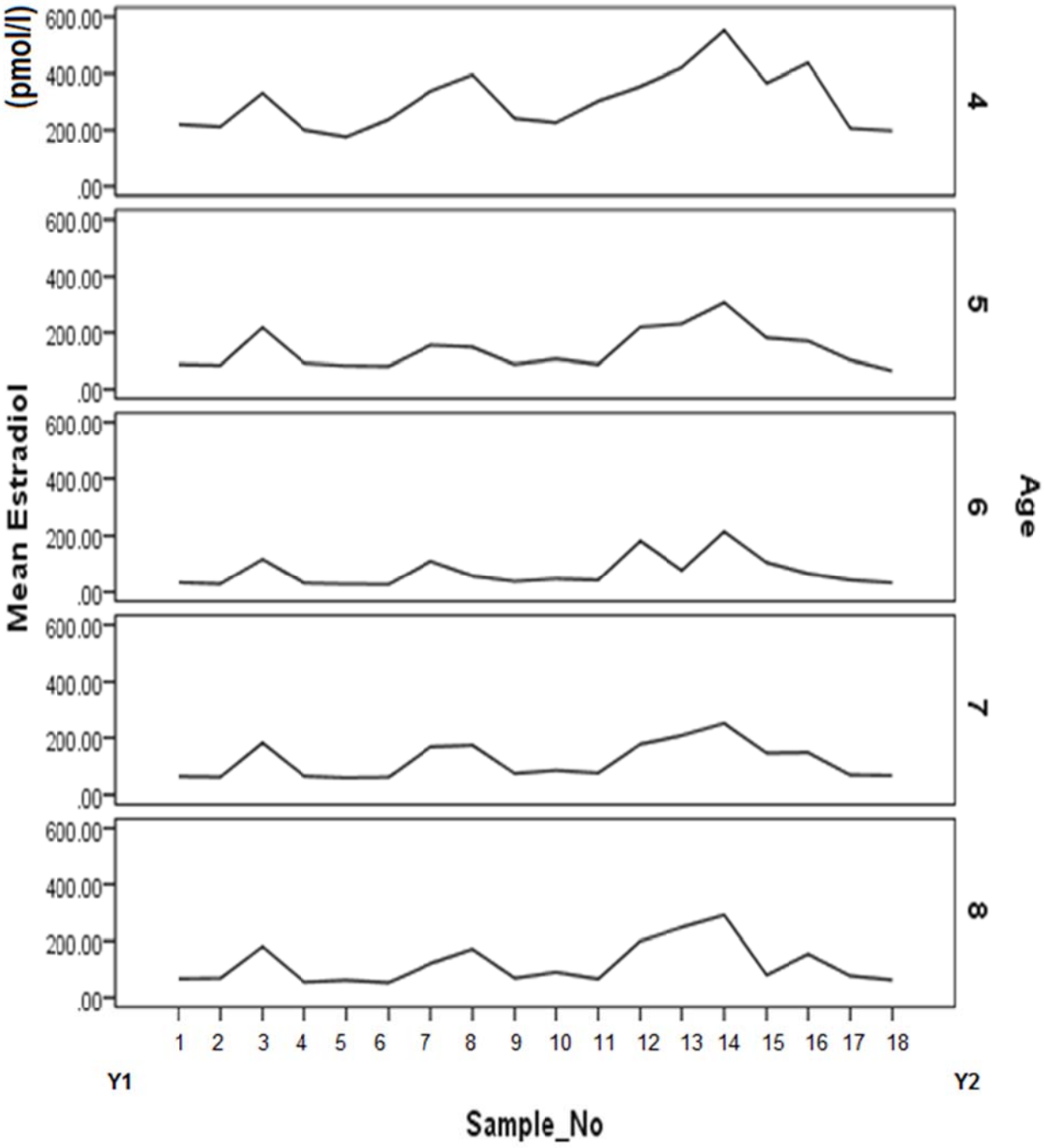

Figure 3.7 Effect of age of cows on estradiol concentrations around oestrus

Estradiol at age 4; 5; 6; 7 and 8 (illustrated on axis $-Y_2$) differed significantly ($p<0.05$)

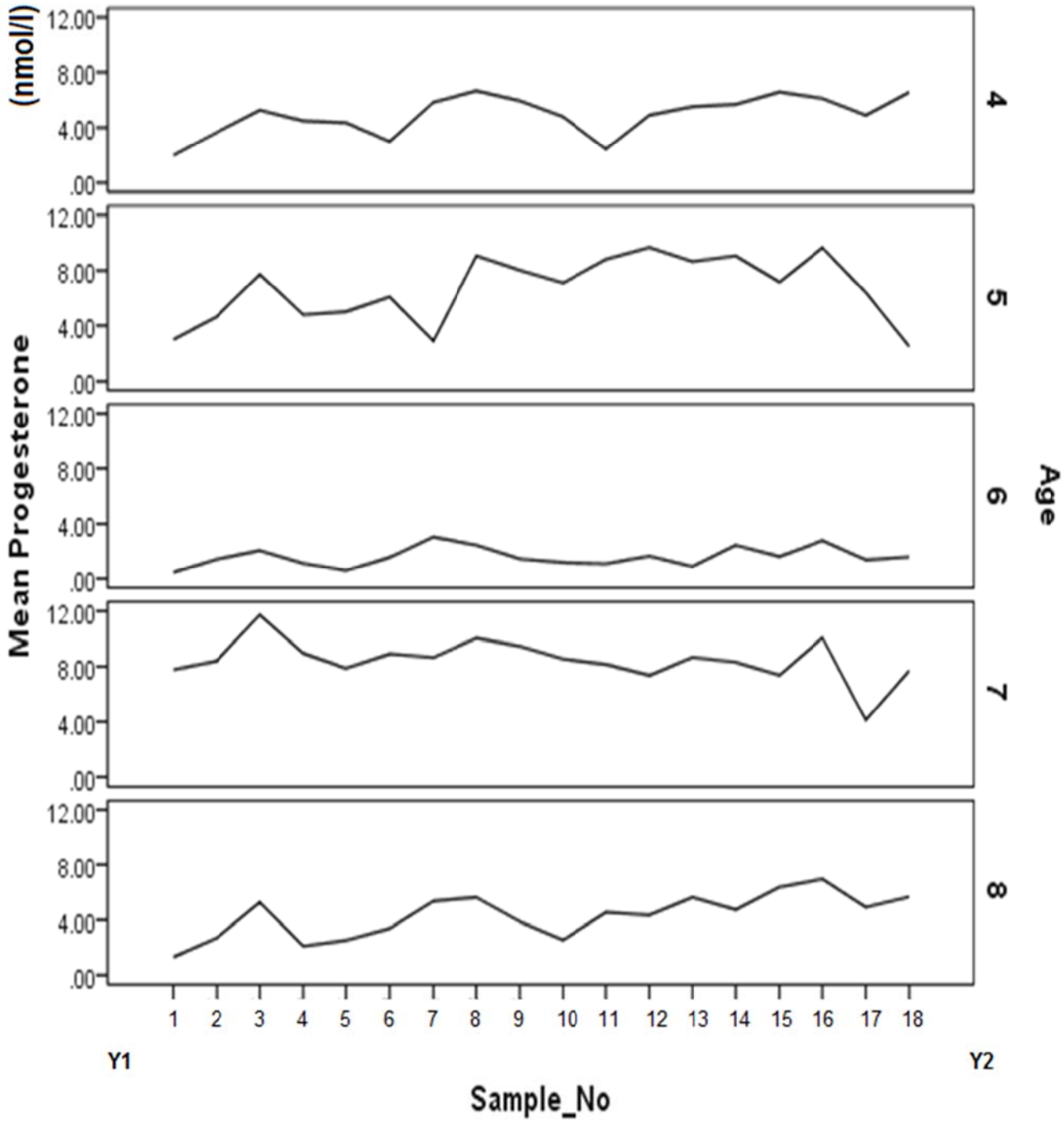

Figure 3.8 Effect of age of cows on progesterone concentrations around oestrus

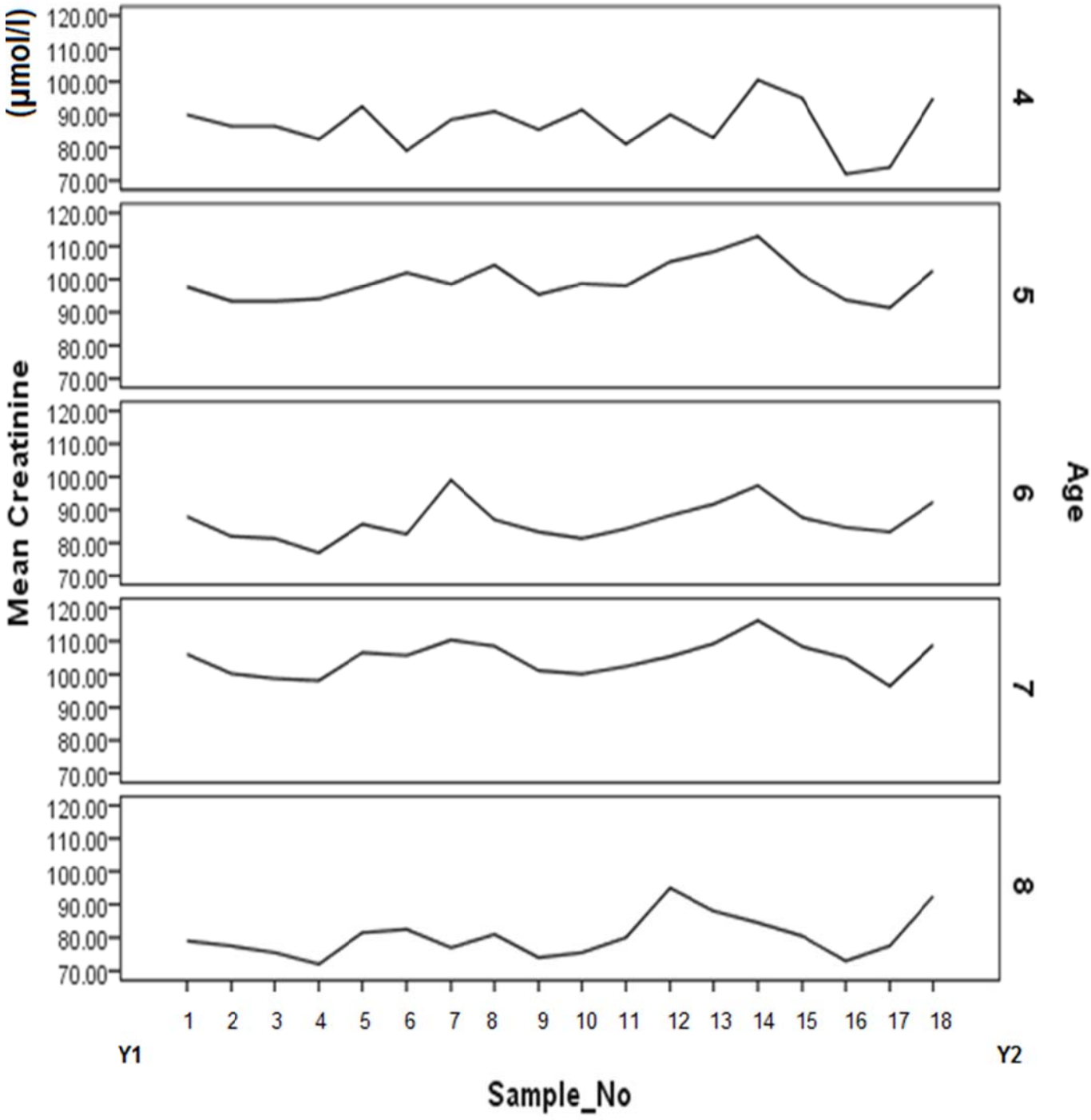

Figure 3.9 Effect of age of cows on creatinine concentrations around oestrus

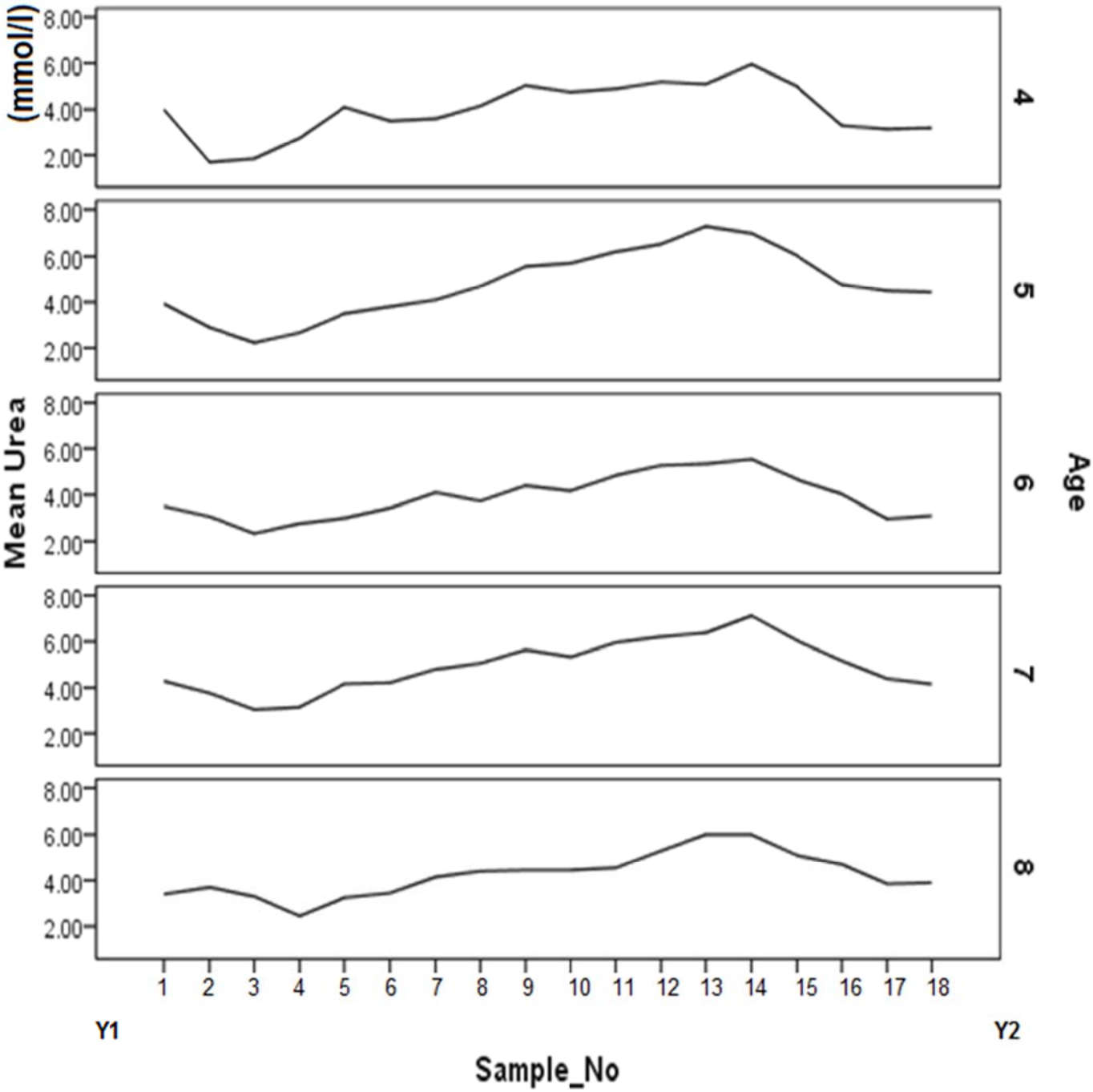

Figure 3.10 Relationship age of cows and urea concentrations around oestrus

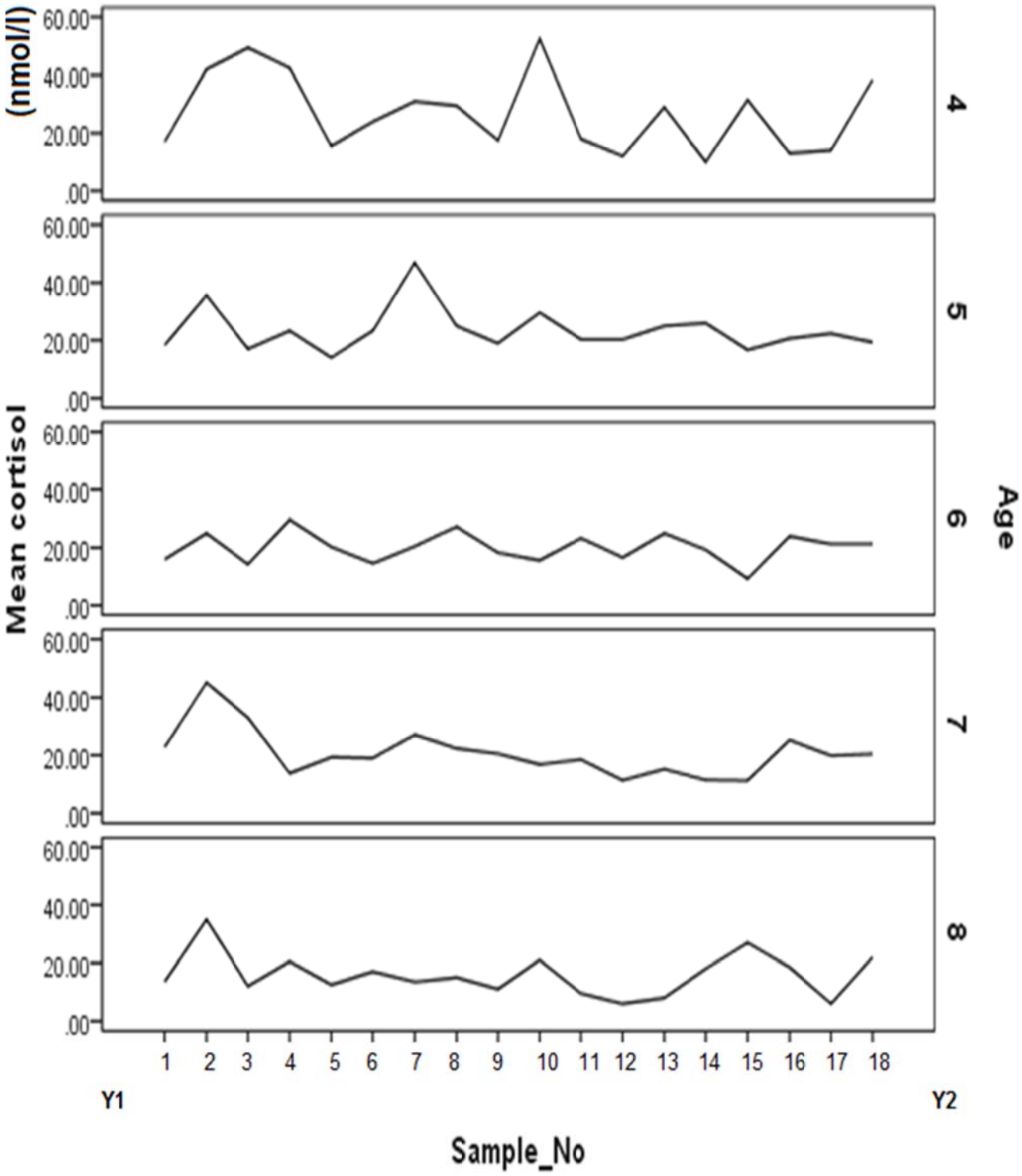

Figure 3.11 Relationship age of cows and cortisol concentrations around oestrus Cortisol at age 4; 5; 6; 7 and 8 (illustrated on axis $-Y_2$) differed significantly ($p<0.05$)

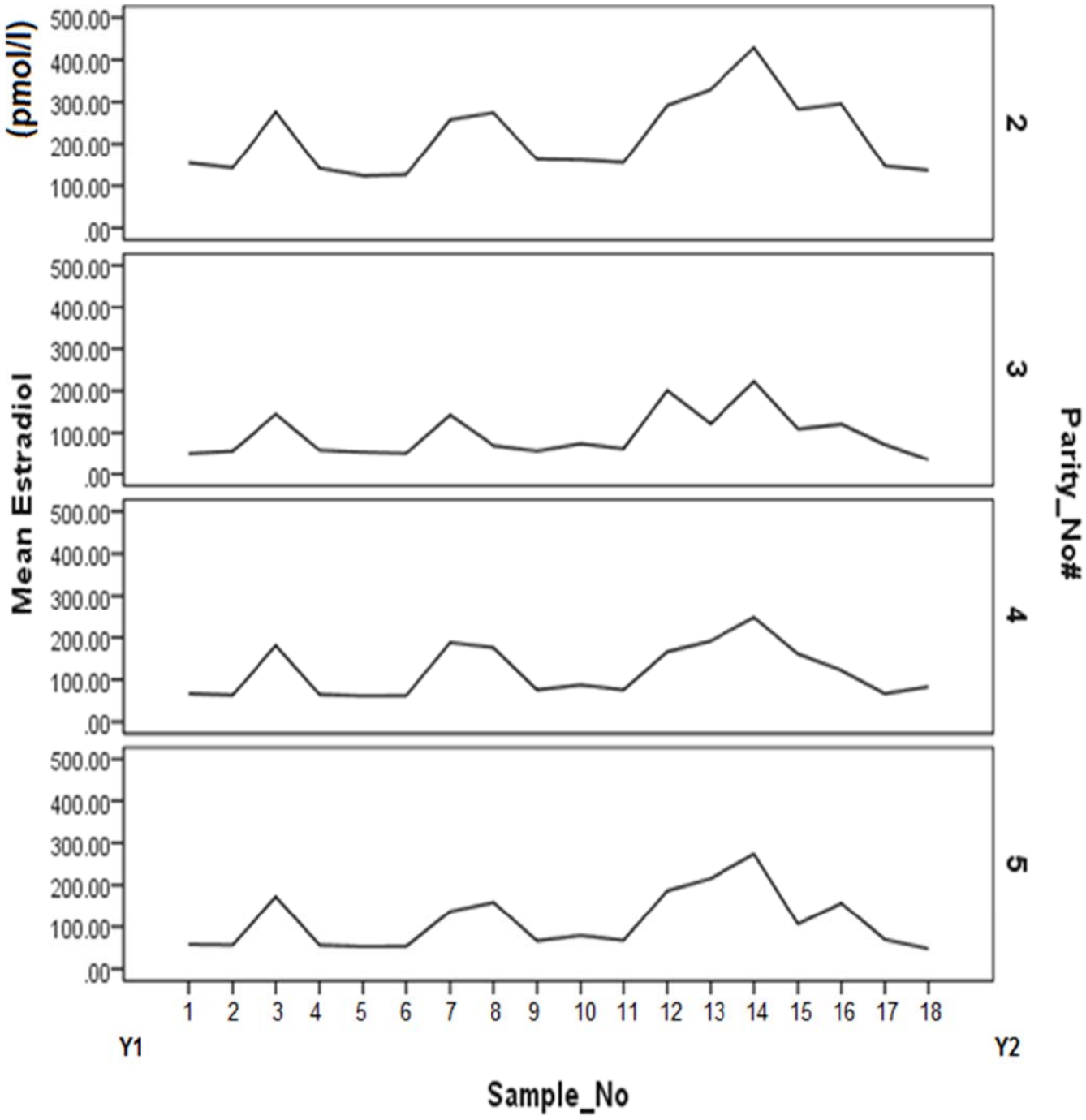

Figure 3.12 Effect of parity number on estradiol concentrations around oestrus
Estradiol at parity number 3; 4; 5 and 6 (illustrated on axis $-Y_2$) differed significantly ($p<0.05$)

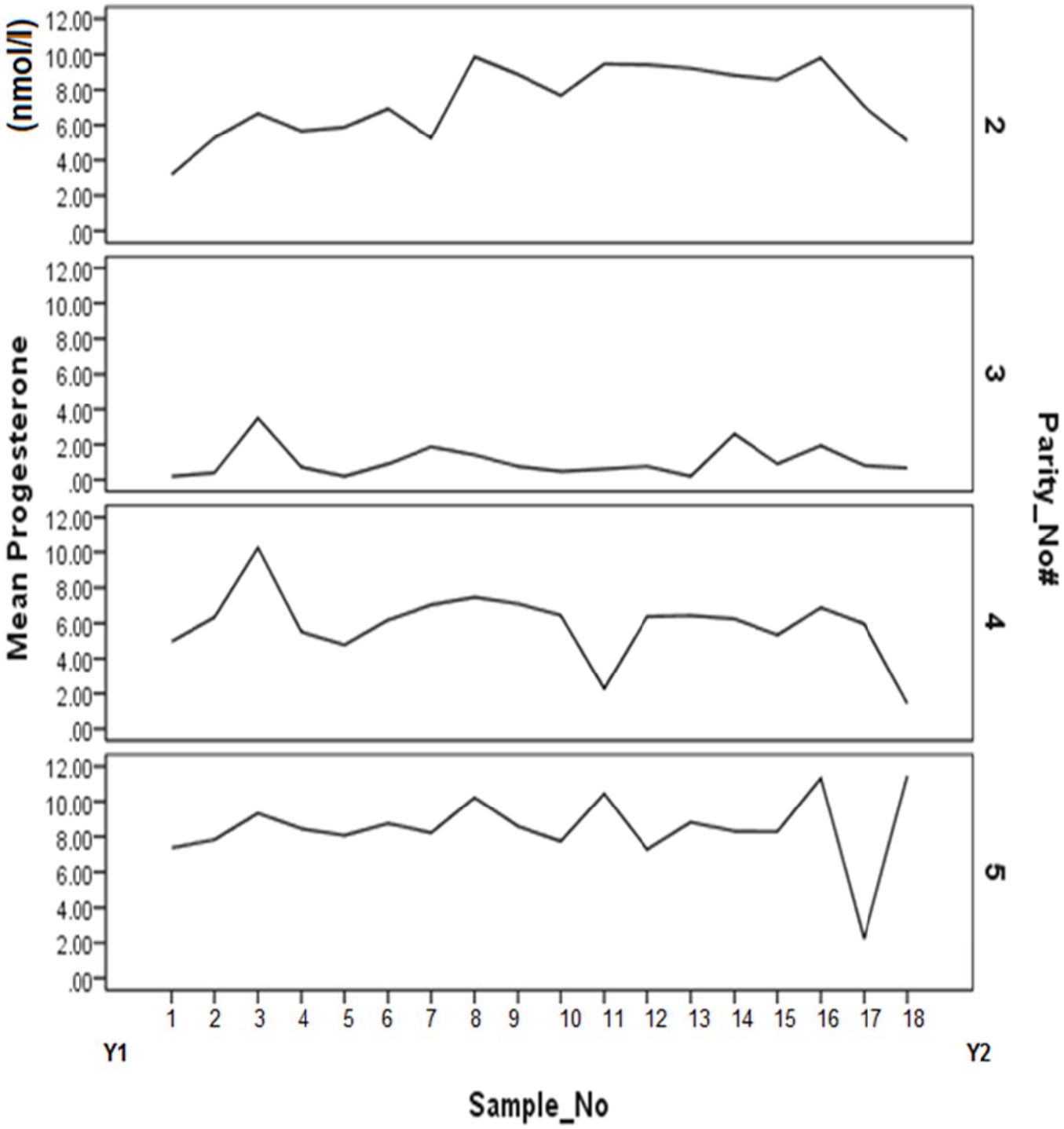

Figure 3.13 Effect of parity number on progesterone concentrations around oestrus

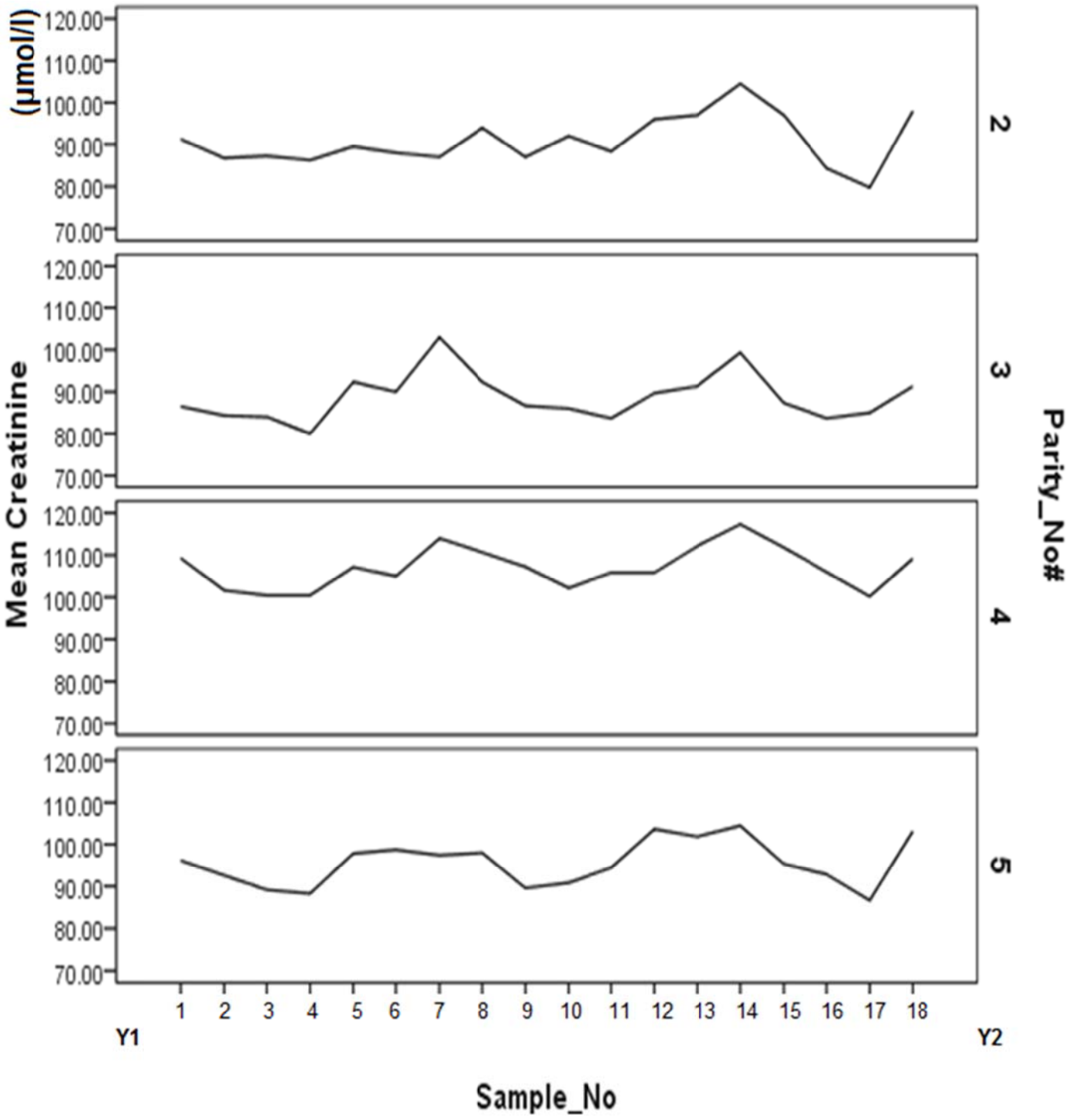

Figure 3.14 Relationship parity number on creatinine concentrations around oestrus

Creatinine at parity number 3; 4; 5 and 6 (illustrated on axis $-Y_2$) differed significantly ($p<0.05$)

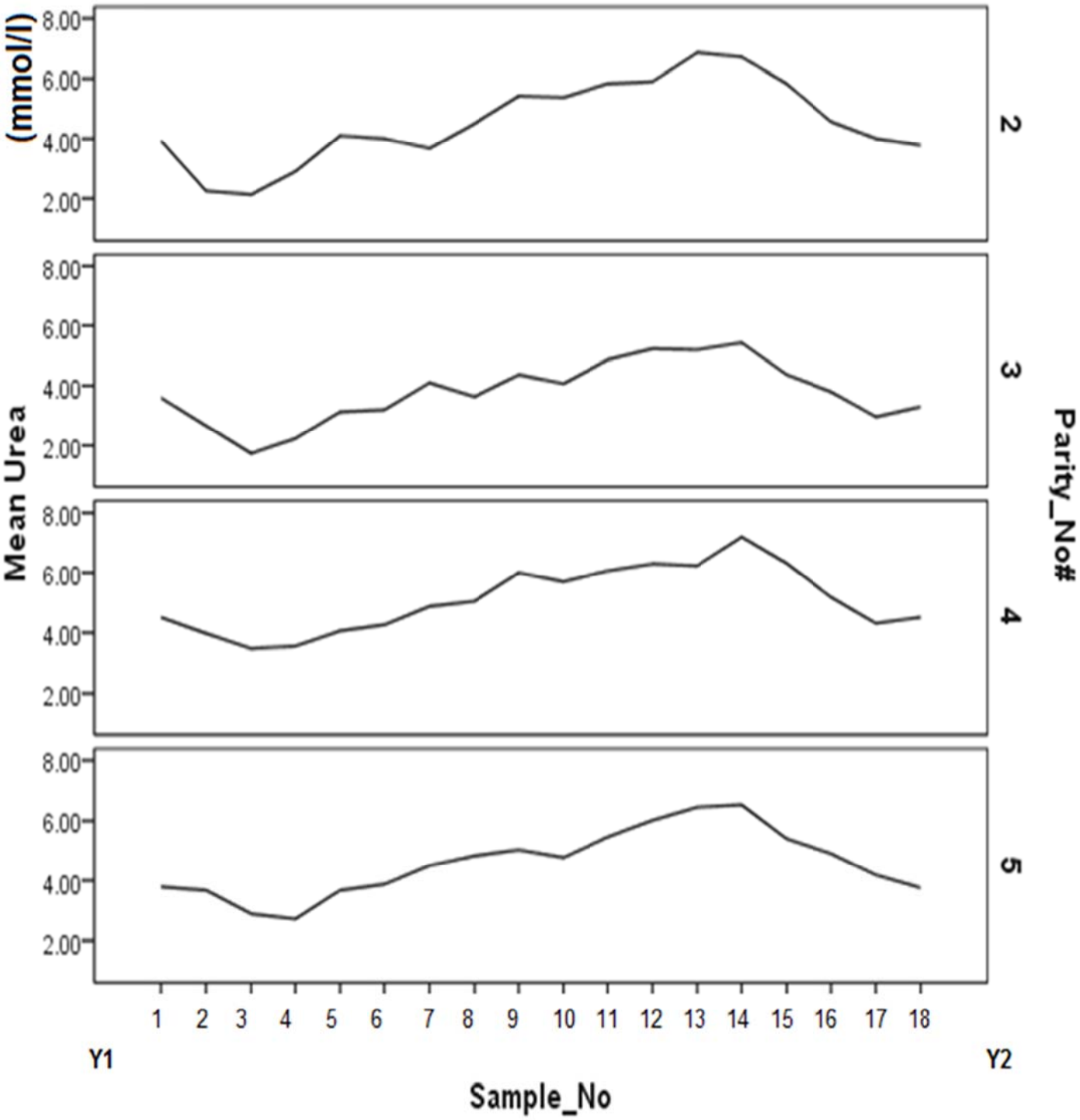

Figure 3.15 Relationship parity number and urea concentrations around oestrus

Urea at parity number 3; 4; 5 and 6 (illustrated on axis $-Y_2$) differed significantly ($p<0.05$)

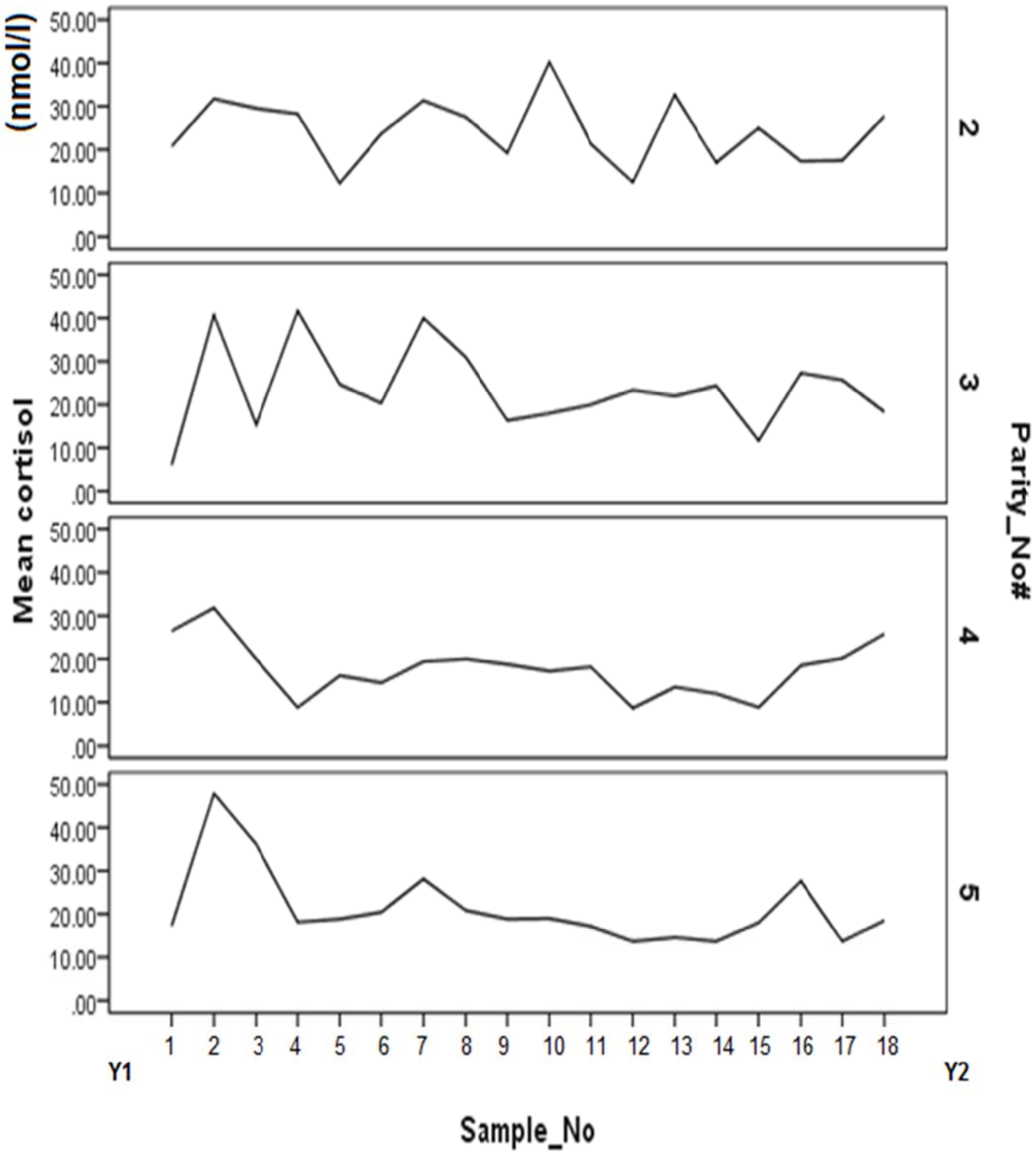

Figure 3.16 Relationship parity number and cortisol concentrations around oestrus

The mean concentrations of estradiol and progesterone around oestrus (from 24 hours before oestrus to 24 hours after oestrus) are presented in Figure 3.17

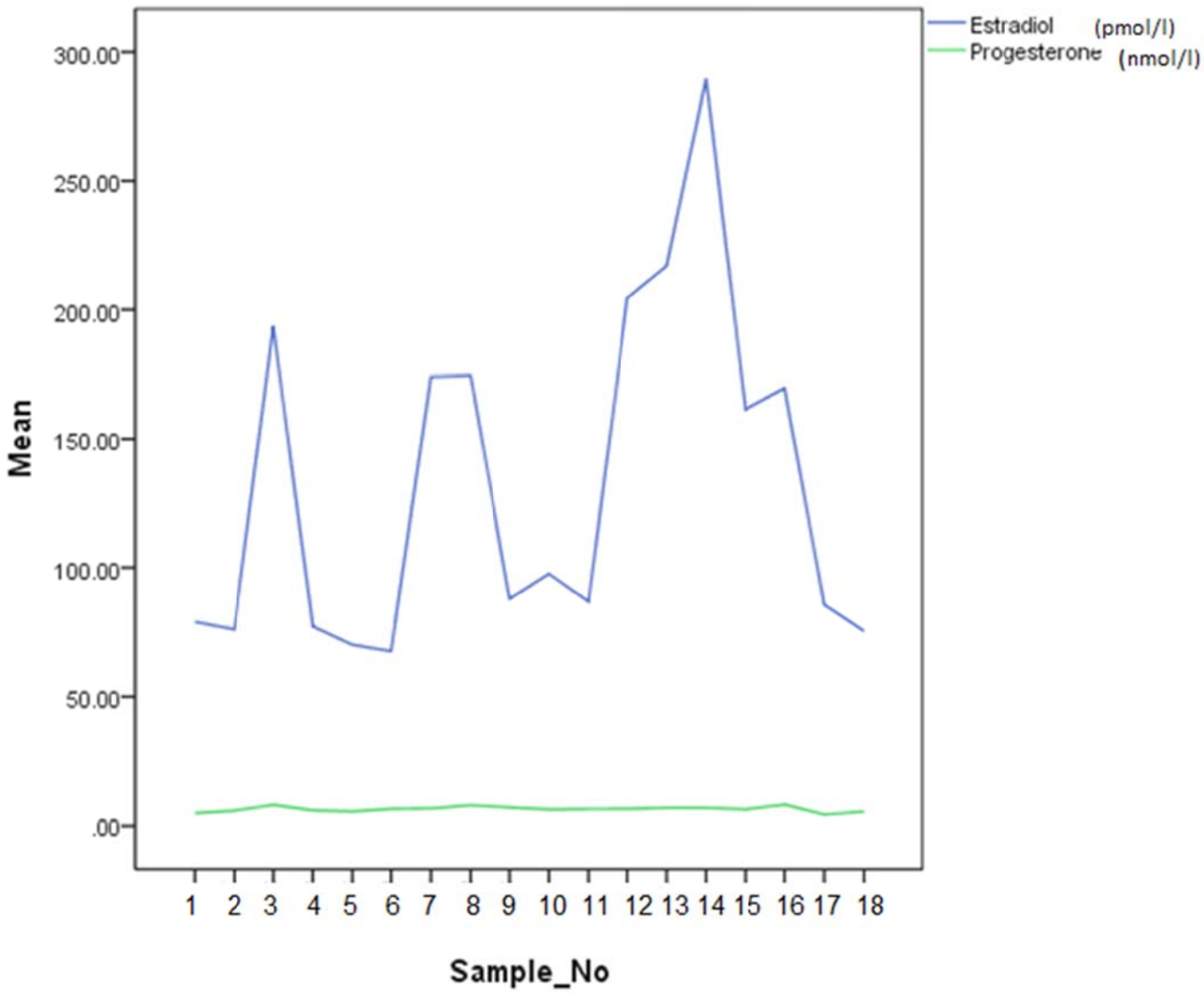

Figure 3.17 Pattern of plasma concentrations of estradiol and progesterone around oestrus in *Bos indicus* cows under extensive conditions

Mean concentrations of estradiol, progesterone, urea, creatinine and cortisol in plasma of *Bos indicus* cows around oestrus are illustrated in Figure 3.18.

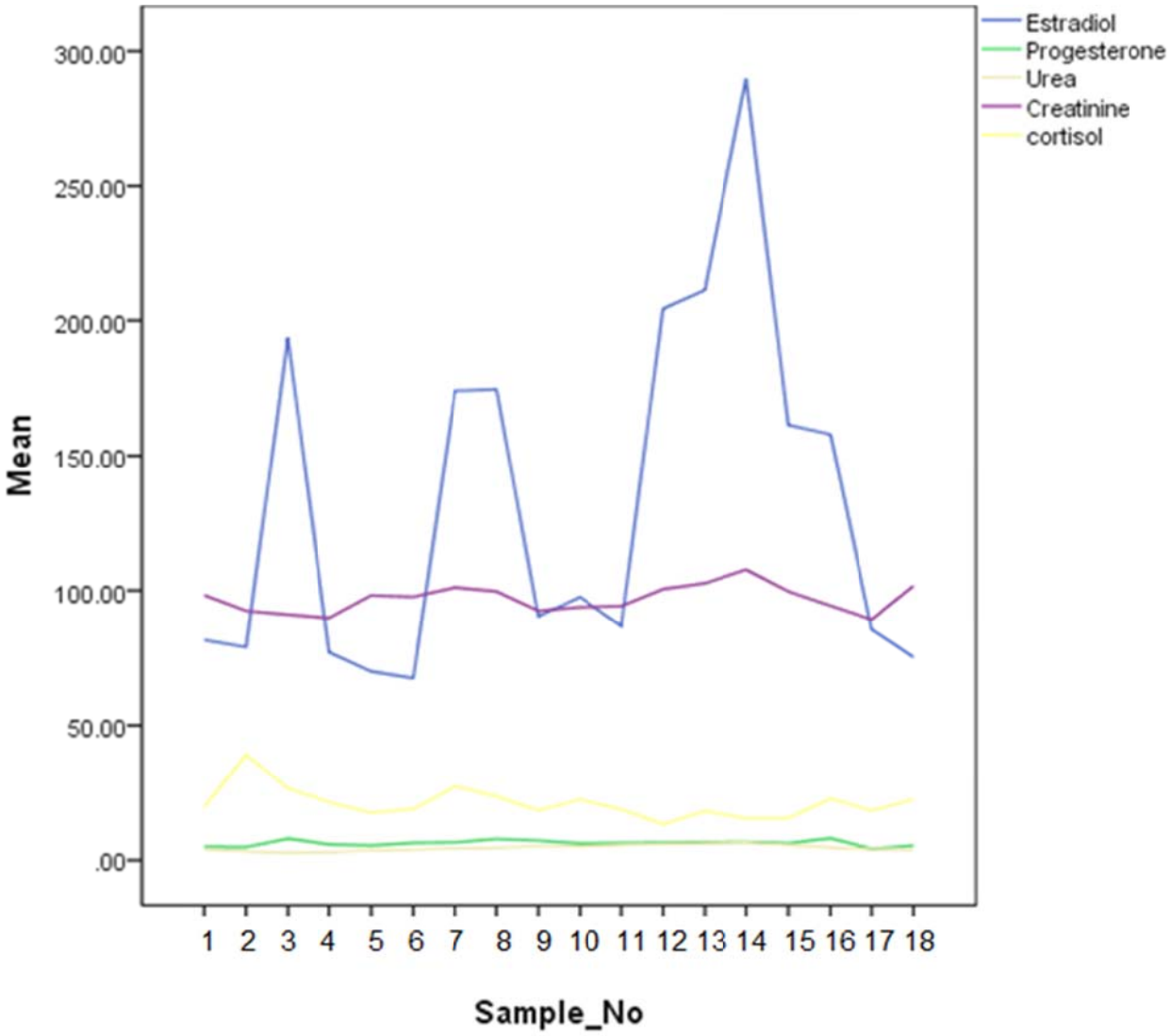

Figure 3.18 Pattern of steroids and other metabolites during oestrus in *Bos indicus* cows under extensive conditions

3.4 Discussion

The present study shows that the hormonal pattern of estradiol and progesterone during oestrus in *Bos indicus* cows under extensive management conditions is similar to that observed in *Bos taurus* cows under intensive management conditions (Forde *et al.,* 2011) as illustrated in Figure 3.17.

The great value of determining BCS prior to the breeding season in cows under extensive management to maximise conception rates is demonstrated in Figure 3.2 where estradiol concentrations tend to peak in cows with BCS 2.5. In addition, the conception rate of cows with BCS of 2.5 was 100%. Although the conception rate of cows included in this study was 90.5%, the two cows that did not conceive during the breeding season of 90 days were aged 8 years but with BCS of 2 and 3, respectively. Since cows were in positive energy balance at the start of the breeding season these results suggest the possibility of age-related sub fertility in *Bos indicus* cows under extensive conditions.

BCS correlates positively with estradiol ($r=0.12$; $p<0.05$) as well as with the conceptions early in the breeding season. This finding strongly suggest that in *Bos indicus* cows under extensive management conditions, a minimum BCS of 2.5 has to be achieved at the start of the breeding season in order to increase the conception rates. Similar results on the correlations between BCS and conception rates have been previously reported (Renquist *et al.,* 2006; Roche *et al.,* 2009).

The blood concentrations of estradiol correlate negatively with age and parity number ($r= -0.35$; $r= -0.29$ and $p<0.001$), respectively. Studies suggests that the threshold estradiol value to induce oestrus may differ between cows (Coe and Allrich, 1989; Forde *et al.,*2011) and it appears that in older *Bos indicus* cows under extensive management the estradiol peak is lower during oestrus (Figure 3.7) compared to young cows.

The effect of stress was more pronounced in young cows compared to older cows as cortisol correlated negatively with age ($r= -0.171$). In addition, cortisol did not interfere with reproduction in any of the cows in the present study, which agrees with the findings from other studies (Wikhund *et al.,* 1996; Butler, 2000; Eberhard *et al.,* 2007; Walker *et al.,* 2008). During the scheduled period of blood sampling, from 24 hours before oestrus to 24 hours after oestrus, cortisol tended to

decrease irrespective of BCS, age and parity number, indicating the probability of adaptability of cows to the stressor; i.e., blood sampling procedure.

Creatinine and urea concentrations were correlated, as both indicate the catabolism of protein (Wikhund *et al.,* 1996; Butler, 2000; Ndlovu *et al.,* 2007). In addition, a correlation between creatinine and BCS was observed although higher BCS is often accompanied with increased muscular mass from where creatinine originates. During oestrus in *Bos indicus* cows the magnitude of muscular protein catabolism was related to BCS (r=0.248), but serum creatinine concentrations around oestrus were within the normal range. Although serum creatinine is primarily linked to glomerular filtration rate, an increased concentration in circulating creatinine is mostly seen when breakdown of endogenous protein from the muscles occurs to compensate for energy deficiency or after a long walking distance or renal pathologies. In terms of reproduction, creatinine is important due to its impact on blood urea nitrogen, which has a positive influence on fertility (Butler, 2000) while very high levels may have a negative effect on fertility (Butler, 1998). Therefore, the maintenance of creatinine within the normal range in the cows included in the present study is accomplished due to nutritional status of the cows (positive energy balance) and further confirmed with the blood urea concentrations.

Blood urea concentrations were within the normal range (<3.6 mmol/l) and correlated positively with estradiol. This was expected to occur since cows included in the experiment were extensively managed based on natural pastures without any type of energy or protein supplementation. The present study indicated that post-partum cows managed at a BCS of 2; 2.5 and 3 at the beginning of the breeding season under extensive management should not be expected to reach the critical levels of urea (19 mmol/l) that impair reproduction (Betler *et al.,* 1998).

The conception rates of cows in the present experiment (90.5%) were elevated considerably as compared to those rates reported in commercial beef cattle farms in Mozambique (<60%) (Schwalback *et al.,* 1997; Escrivão *et al.,* 2009). Considering that cows that did not conceive were under the minimum BCS reported for oestrus to occur, BCS of 2 and 3 (Flores *et al.,* 2008; Quintans *et al.,* 2009) the present study lacks a complete retrospective post-partum reproductive history of every cow for a better understanding of the observed phenomenon.

3.5 Conclusions

The present study suggest that better BCS (≥2.5) at the onset of the breeding season improves the conception rates and concentrates conceptions during the first 21 days of the breeding season in Brahman type cows under extensive management conditions.