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Abstract

The objectives of the present research were to study the factors that influence post-partum reproductive characteristics of suckling beef cows in extensive production systems in Mozambique and to develop new management strategies to improve their reproductive efficiency. The effects and interactions between post-partum BW, BCS, age and parity number on plasma concentrations of estradiol, progesterone, creatinine, urea and cortisol around oestrus and the related conception rates of *Bos indicus* cows in extensive production system were analysed as well as the minimum BCS at the beginning of breeding to maximise the subsequent conception rates. Thirty-five days prior to the breeding season cows were synchronised using Crestar. During the second oestrus after synchronisation, 18 blood samples were collected per animal for hormonal analysis, from 24 hr before oestrus to 24 hr after oestrus. The hormonal pattern of estradiol and progesterone around oestrus were similar to that observed in *Bos taurus* cows under intensive conditions. Conception rates of cows in the experimental group were 90.5%. 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 aim to achieve at least a BCS of 2.5 at the beginning of the breeding season.

Twelve-hour and 48-hr calf removal were conducted separately to evaluate their effects on conception rates of *Bos indicus* beef cows in extensive production systems and to quantify the related effects on calf-weaning weights. The 12-hr calf removal was performed from 45 days post-partum to the beginning of the breeding season, and the 48-hr calf removal was performed preceding the onset of the breeding season. It was concluded that 12-hr calf separation at night enhance the energy balance (3%), increases the conception rates (80%) and improves the calf-weaning weights, whereas 48-hr calf removal increases conception rates (76%) and does not affect calf weaning weights. Both calf removal management strategies concentrate conceptions in the early part of the breeding season and stress the importance of the effect of BCS and estradiol on conception rates in *Bos indicus* beef cows in extensive production systems.

Summary

The objectives of the present research were to study the factors that influence post-partum reproductive characteristics of suckling beef cows in extensive production systems in Mozambique and to develop new management strategies to improve their reproductive efficiency. This study was performed in three different experiments in which specific hypotheses were tested.

In Experiment 1 the objectives were: (1) to test the effects and interactions between post-partum body weight, post-partum body condition score, 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; and (2) to establishing the minimum BCS at the beginning of the breeding season in order to maximise the subsequent conception rates.

Twenty-five peri-parturient Brahman type 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 Nergetomet + 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 before oestrus to 24 hr after oestrus. A total of 18 samples were collected per animal at 4-hr intervals before 12 hr preceding oestrus and 12 hr after oestrus and at 2-hr intervals during the 12 hr preceding oestrus to 12 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 was analysed 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 2.5 at the beginning of the breeding season to maximise the re-conception rates.

Experiment 2 was conducted to evaluate if restricted suckling at night from 45 days post-partum increases the conception rates of *Bos indicus* beef cows in extensive production systems in sub-tropical conditions and to quantify the related effects on calf-weaning weights. Fifty-two multiparous Brahman type cows with reproductive tract scoring (RTS) ≥ 4 at 45 days post-partum were randomly assigned to two groups of 26 cows each separated into an *ad libitum* suckling group or calf no-removal group (NRG) and treatment group or calf removal group (RG). Calves in the treatment group were separated for 12 hr during the night from 45 days post-partum to the onset of the breeding season. Satisfactory classified bulls were used at the ratio of 1:20 cows for a breeding season of 90 days.

Body condition score and BW were recorded 45 days post-partum, at the start of the breeding season, and at pregnancy diagnosis that took place 60 days after the end of the breeding season. Pregnant cows were monitored throughout the gestation period. Calves were weighed at calving and weaning. Weaning weights were corrected to 205 days. BW and BCS at the onset of the breeding season was 395.8 ± 50 kg and 2.5 ± 0.3 for the RG and 410.5 ± 40 kg and 2.6 ± 0.3 for the NRG. Calving to breeding intervals were 93 ± 17.5 days for RG group and 99 ± 22.1 days for NRG group, respectively. Calving to conception intervals differed significantly between the experimental groups (110.9 ± 10 days for RG and 132.8 ± 19 days for NRG) and a similar result was obtained for the breeding to conception intervals (17.8 ± 15 days for RG and 31.1 ± 18.9 days for NRG). Conception rates were 80% for the RG group and 59% for the NRG group, which correlated better with BW than BCS at the onset of the breeding season. Weaning weights differed significantly between control and treatment groups (149.3 ± 18 kg for RG and 134.5 ± 20 kg for NRG). From 45 days post-partum to the onset of the breeding season, cows in the RG group experienced a positive energy balance (3%) while those in the NRG had a negative energy balance (-0.1%) . It was concluded that 12-hr calf separation at night increases the conception rates and improves the calf-weaning weights of *Bos indicus* beef cattle in extensive production systems under sub-tropical conditions.

The aim **of Experiment 3** was to determine if 48-hr calf removal prior to the breeding season affects (1) ovarian steroids, cortisol, urea and creatinine; (2) improves the conception rates; and (3) influence the calf-weaning weights of *Bos indicus* cattle in extensive production systems. Sixty multiparous Brahman-type cows were randomly selected in the early post-partum period and equally allocated into a calf removal group (RG) and a non-removal group (NRG). Calves from cows in the RG were removed for 48 hr prior to the breeding season and returned afterwards, whereas in the NRG the calves remained with their dams until weaning. BW and BCS of cows were recorded at the beginning of the breeding season, mid-breeding season and just after pregnancy diagnosis.

Pregnant cows were monitored throughout the gestation period and calving dates were accurately recorded. The calving season was divided into early, mid and late, corresponding conceptions occurred in the early, mid and late part of the breeding season, respectively. Calves were weighed at birth and at weaning. Weaning weights were corrected to 205 days. BW and BCS were similar throughout the experimental period. Conception rates (CR) were 76% for RG and 55% for NRG but did not differ significantly between the groups. However, differences ($p<0.05$) between the groups were observed for conception rates in the early and late part of the breeding season. CR was correlated with CBI and BCS at the onset of the breeding season. Product-

limit survival curves Vs CCI differed significantly ($p<0.05$) between treatment groups. It was estimated with 95% certainty that 50% of the cows in the RG would conceive within the first 19 days of the breeding season while for the NRG within the first 38 days of the breeding season. Weaning weights were 135.2± 22 kg for the RG and 135.5 ± 19 kg for the NRG. In the RG estradiol concentrations increased with sampling time, contrary to progesterone. Cortisol decreased with sampling time for both groups but with higher concentration in the RG. It was concluded that 48-hr calf removal prior to breeding enhances the conception rates with the majority of cows conceiving in the early part of the breeding season. It was also concluded that 48-hr calf removal increases plasma concentration of cortisol without adversely affecting reproduction and does not affect calf weaning weights of *Bos indicus* beef cattle in extensive production systems.

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List of Abbreviations

ANOVA – Analysis of Variance

BCI – Breeding to Conception Interval

BCS – Body Condition Score

Bi – *Bos indicus*

Bt – *Bos Taurus*

B_w – Birth weight

BW – Body weight

CBI – Calving to Breeding Interval

CCI – Calving to Conception Interval

CI – Calving Interval

CR – Conception Rates

C_{ww} – Corrected weaning weight

dl – Decilitres

DNSV- National Directorate of Veterinary Services

FAO - Food and Agriculture Organization of the United Nations

FSH – Follicle stimulanting hormone

GDP – Gross Domestic Product

GnRH – Gonodatropin Releasing Hormone

HPO – Hypothalamus Pituitary Ovarian Axis

IgF – Immunoglobulin F

LH – Luteinizing Hormone

LHRH- Luteinic Hormone Releasing Hormone

LSmeans – Least square means

LSU – Large Stock Unit

mg – Milligrams

NRG – Non-Removal Group

PPI – Post-partum Interval

RFAT – Rump Fat Thickness

RG – Removal Group

RH – Releasing Hormone

RLUs – Relative Light Units

RTS – Reproductive Tract Score

SD – Standard Deviation

SPSS – Statistical Package for Social Science

UFAT – Subcutaneous Fat Thickness at the *longissimus dorsi*

W_a – Weaning age

W_w – Weaning weight

µl – Micro litres

µmol – Micro mole

1. Introduction

1.1 Extensive beef cattle production

Beef cattle production plays an important role in the agricultural sector in Mozambique, contributing 14.8 % to agriculture GDP and 4.4% to the total GDP (FAO, 2005).

Extensive beef cattle provides farmers with the most efficient method of utilising forages grown from pasture, rangelands, forests and crop residues, which are converted into animal protein of high biologic value. In Mozambique, besides meat production, extensive beef cattle also contribute to the family livelihood through milk, ploughing, transport and family social standing. However, farming beef cattle is a complex activity consisting of various production factors that require appropriate and sustainable management interventions to ensure productivity and environmental sustainability.

The efficiency of a beef cattle production system depends on the reproductive rate of the cows, the growth rate of the calf to weaning, and the overall efficiency of feed utilisation (Duarte-Ortuno *et al.,* 1988; Pieris *et al.,* 1995; Naazie *et al.,* 1999; Morrison *et al.,* 1999; Kanuya *et al.,* 2006; Nqeno *et al.,* 2010). The age at which heifers calve for the first time and their lifetime production has an important effect on productivity (Nunez-Dominguez *et al.,* 1991; DeRouen *et al.,* 1994; Oliveira *et al.,* 2009). Both qualitative and quantitative aspects of animal nutrition influence the reproduction, growth and, therefore, the efficiency of beef cattle production systems.

Pastures are the main source of nutrition for extensive beef cattle, but the quantity or availability of pastures in the tropical and sub-tropical regions is influenced by the rainfall patterns, which results in seasonal variation in quantity and quality of nutrition throughout the year. Unfortunately, changes in nutritional requirements of extensive beef cattle during the reproduction and production cycle do not coincide with the seasonal changes in pasture availability. Consequently, when nutrient intake is inadequate and body energy reserves are depleted, the interval from calving to first oestrus is extended (Rutter and Randel, 1984; DeRouen *et al.,* 1994; Grimard *et al.,* 1995; Morisson *et al.,* 1999, Aguilar-Pérez *et al.,* 2009). Since reproduction is a major component of production efficiency in a beef cattle production system, numerous studies have been performed to understand the factors that influence reproduction in post-partum beef cows.

Body condition score has been reported to be a good indicator of body energy reserves and re-breeding performance (Houghton *et al.,* 1990, Morrison *et al.,* 1999; Ayres *al.,* 2009). It has been shown that cows with a greater BCS at parturition return early to oestrus and experience high conception rates (Laflamme and Connor, 1992; Osoro and Wright, 1992; DeRouen *et al.,* 1994; Morrison *et al.,* 1999; Ezanno *et al.,* 2005). The restriction of dietary energy intake during the peri-partum period demonstrates that luteinizing hormone (LH) pulse frequency and follicular growth in post-partum cows were influenced by negative energy balance (Beal *et al.,* 1978; Grimard *et al.,* 1995; Roche *et al.,* 2009). However, BCS has a low correlation with BW (Ayres *et al.,* 2009), indicating that the energy balance is probably a better predictor of reproduction in the post-partum period.

Research on the effects of energy balance on re-conception rates in extensive beef cows is limited. Leptin, a hormone secreted by adipose tissue, identified by Zhang *et al.* (1994), has been proposed to signal nutritional status in ruminants (Delavaud *et al.,* 2000; Tokuda *et al.,* 2000; Block *et al.,* 2001; Ludwik *et al.,* 2007). Whether leptin plays a central role in regulating reproduction in cattle has not been determined. However, plasma leptin concentration is related to adipose cell size and positively related to feeding level (Delavaud *et al.,* 2000). The decrease in plasma leptin concentration is associated with a reduction in the secretion of LH, Immunoglobulin F-I (IgF-I) and insulin in cattle (Amstalden *et al.,* 2000) and in sheep and goat (Morrison *et al.,* 1999; Azraqi, 2007). There is no information available on the effects of post-partum BCS, BW and their changes on leptin, follicle stimulating hormone (FSH), LH, ovarian steroids, their interactions and the related conception rates in extensive beef cattle.

Suckling is another important factor that extends post-partum anoestrus, affecting consequently the rebreeding performance of beef cows (Radford *et al.,* 1978; Williams, 1990; Stewart *et al.,* 1993; Lamb *et al.,* 1999; Marongiu *et al.,* 2002; Perea *et al.,* 2008; Pinheiro *et al.,* 2009). The mechanism by which this external stimulus impairs oestrus during the post-partum period is uncertain. Nevertheless, despite the fact that the LH concentrations in the anterior pituitary (Nett *et al.,* 1988) and in the plasma (Radford *et al.,* 1978) are similar after 30 days post-partum in suckling and non-suckling beef cows, the pulsatile LH pattern is low in suckling cows. Moreover, Murphy *et al.* (1990) and Crowe *et al.* (1998) report that the development of dominant follicles occurs in suckling and non-suckling cows, but no ovulation was observed in suckling cows.

Efforts to reduce the effects of suckling on the post-partum reproduction of beef cows have taken various forms, from understanding of suckling behaviour (Stewart *et al.,* 1993a; Paranhos da Costa *et al.,* 2006) to the effects of manipulation of different suckling times on the onset of post-partum oestrous (Reeves and Gaskins, 1981; Bell *et al.,* 1998; Lamb *et al.,* 1997; Lamb *et al.,* 1999; Alverez-Rodriguez *et al.,* 2010) or on conceptions rates (Stewart *et al.,* 1993b; Gazal *et al.,* 1999; Escrivão *et al.,* 2009). The manipulations of suckling times and calf withdrawal have yielded different results – either on the interval to first oestrus, subsequent conception rates or on calf weaning weights. However, referring to the effects of calf withdrawal on post-partum rebreeding, the existing reports fail to consider the nutritional status, metabolic rate, stress and gonadotropic hormones, since it is believed that the yielded results are the combinations of these factors rather than calf withdrawal itself.

1.2 Motivation

In view of defining a strategy to improve reproductive efficiency in post-partum suckling beef cows an understanding of the complex nutrition-suckling-reproductive hormone interactions and the manipulation of related factors have to be considered. However, studies regarding this matter have been mostly performed under intensive management conditions, with breed types and climatic conditions that differ significantly from extensive beef cattle production in Mozambique. Therefore, a study on the effects of post-partum BW, BCS and calf removal on reproductive and productive characteristics of extensive beef cattle could make an important contribution to the productivity of extensive beef cattle and also the development of the beef cattle industry in Mozambique.

Mozambique has a total area of 778,000 km^2 of which it is estimated that 440,000 km^2 is potentially rich in pastures (FAO, 2005). The available natural pastures are of a relatively good quality, allowing a carrying capacity of approximately 8 ha per Large Stock Unit (LSU) (adopted from Timberlake and Reddy, 1986, 2006). If 50% of the estimated area could be used for cattle production, Mozambique would support a total of 3,142,857 LSU in a sustainable manner. The cattle population in Mozambique is 1.4 million (DNSV, 2010), that is approximately 935,200 LSU, which means that the country´s potential for cattle production is used at 29.7%.

Approximately 80% of beef meat consumed in Maputo City (the major beef market in the country) is imported (DNSV, 2010). Considering the production characteristics of existing extensive beef cattle in the country (commercial and subsistence sectors), although the subsistence sector represents 70% of the cattle population, only the development of commercial farms could compete over the medium – or long term with imported beef. However, success will be attained if the management of commercial farms improves and emphasis is placed on reproduction and production management.

The calving rates in the commercial sector are still very low at <60% (Mandlate, 1985; Escrivão *et al.*, 1998; Escrivão *et al.*, 2009). A similar situation is evident in the family livestock production sector where calving rates are below 50% (Rocha *et al*., 1988). This poor calving rate represents one of the major constraints for the development of cattle production in Mozambique.

Previous studies in Mozambique have identified calving rate as the premium productivity indicator, but re-conception rates after calving lengthen the inter-calving period (Mandlate, 1985; Schwalback *et al.,* 1997; Escrivão *et al.,* 1999). None of the previous studies have focused on the factors that influence post-partum conception rates of suckling beef cattle under extensive production conditions in Mozambique. Small increases in productivity could be reasonably attained with lower cost through improvements in reproduction management and, more specifically, by increasing calving rates rather than by improvements in feeding management for slaughter animals (Burns *et al.,* 2010). In addition, financial resources are scarce for the majority of extensive beef cattle farmers in Mozambique, so reproduction management remains the most important approach to improving productivity.

For this reason, the present study consisted of a number of trials on the effects of BW, BCS and calf removal on reproductive and productive characteristics of extensive beef cattle in Mozambique. In order to ensure that these specific goals were achieved, the trials were conducted under extensive conditions. First, animals incorporated in the experiments were maintained in the herd without extra management but removed only for measurements or sample collections and returned afterwards. Second, the trials were designed in such a way that the results could be used to compile management strategies for similar extensive beef cattle production systems in Mozambique, including the communal farming sector.

1.3 General objectives

- To study the factors that influence post-partum reproduction of suckling beef cows in extensive production systems in Mozambique; and

- To develop new management strategies to improve reproductive efficiency of suckling *Bos indicus* beef cows in extensive production systems in Mozambique.

1.4 Specific objectives

To test the hypothesis in experiments 1, 2 and 3.

EXPERIMENT 1

Effects of post-partum body condition score, body weight, age and parity number and their interactions on ovarian steroids, cortisol, creatinine, urea and the related conception rates in *Bos indicus* cows in extensive production systems

Hypothesis 1

Ho. Post-partum BCS, BW, age and parity number do not affect ovarian steroids, cortisol, creatinine, urea and related conception rates of *Bos indicus* cows in extensive production system.

H1. Post-partum BCS, BW, age and parity number affect ovarian steroids, cortisol, creatinine, urea and related conception rates of *Bos indicus* cows in extensive production system.

EXPERIMENT 2

Effects of 12- hour calf removal on conception rates and calf weaning weights of *Bos indicus* cows in extensive production systems

Hypothesis 2

Ho. Restricting suckling at night from 45 days post-partum does not increase conception rates of *Bos indicus* cows in the subsequent breeding season in extensive production systems.

H1. Restricting suckling at night from 45 days post-partum increases conception rates of *Bos indicus* cows in the subsequent breeding season in extensive production systems.

Hypothesis 3

Ho. Calf weights at weaning are not affected by restricted suckling at night from 45 days post-partum in extensive production systems.

H1. Calf weights at weaning are affected by restricted suckling at night from 45 days post-partum in extensive production systems.

EXPERIMENT 3

Effects of 48-hour calf removal on conception rates and calf weaning weights of *Bos indicus* cows in extensive production systems

Hypothesis 4

Ho. Forty-eight-hour calf removal prior to breeding does not improve the conception rates of *Bos indicus* beef cows in extensive production system.

H1. Forty-eight-hour calf removal prior to breeding improves the conception rates of *Bos indicus* beef cows in extensive production system.

Hypothesis 5

Ho. Calf weights at weaning are not affected by 48-hour calf removal in extensive production systems.

H1. Calf weights at weaning are affected by 48-hour calf removal in extensive production systems.

In order to address all these aspects appropriately, the present thesis is divided into six chapters. A general introduction is provided in Chapter one, while the second chapter provides a detailed literature review on the relevant aspects related to extensive beef cattle production systems. Chapter two also incorporates the factors that influence post-partum reproduction of *Bos indicus* cows reared in extensive production systems. The effect of BCS, BW and suckling and the related effects on conception rates, as well as the influence of calf withdrawal on weaning weights are reviewed. The results of experiment one, two and three are presented and discussed in Chapters three, four and five, respectively. General discussion and conclusions are presented in chapter six, followed by a comprehensive bibliography.

2. Literature Review

Post-partum reproduction in suckling beef cows

2.1 Introduction

Post-partum rebreeding in suckling beef cows has been discussed (Hammond, 1927 cited by Short *et al.*, 1990; Wiltbank, 1991) and continues to be a vital subject for animal scientists (Goyache *et al.*, 2005; Satrapa *et al.*, 2010). Poor rebreeding is characterised by the non-appearance of oestrus (anoestrus) early in the post-partum period and extended interval to first service, which lengthens the calving interval above 365 days, reduces calf crop, and causes an economic loss to beef cattle producers (Short *et al.*, 1990; Yavas and Walton, 2000; Quintans *et al.* 2010).

After parturition, uterine involution is generally completed within four to six weeks (Gier and Marion, 1968; Dobson *et al.*, 2001; Zhang *et al.*, 2010). Thereafter, the duration of post-partum anoestrus is governed by the recommencement of the hypothalamic pituitary ovarian (HPO) axis activity. Due to this fact, much attention has been given to the nature of the signal that controls pituitary secretion of LH and FSH, the response of the ovary to LH and FSH (Bryner *et al.*, 1990; Martinez *et al.*, 2005) and to the other ovarian effects that are independent of gonadotropins (Paranhos da Costa *et al.*, 2006).

Several factors are implicated in the activation of the HPO axis activity during the post-partum period of suckling beef cows. These factors include suckling and nutrition (body energy reserves) as the major factors and many minor factors such as age, parity number, breed, individual genetic variation, presence of bull, diseases, twin births, dystocia, and retained placenta (Deutscher 1991; Grimard *et al.*, 1995; Marongiu *et al.*, 2002; Martinez *et al.*, 2005; Álvarez-Rodrigez *et al.*, 2010). The effects of body energy reserves, suckling and the related mechanisms that appear to influence post-partum reproduction of extensive beef cows are reviewed in sections 2.2 to 2.7.

2.2 Body condition score

Maintenance of body energy reserves in bovines has been considered to be the basis of any reproductive management strategy (Houghton *et al.,* 1990; Morrison *et al.,* 1999; Flores *et al.,* 2008). Body energy reserves were measured through weight loss, when energy reserves (fat) and protein reserves (muscle) are being depleted or, otherwise, through weight gain. Usually, weight gain or loss has been held as a good measure of well-being and productivity (Bishop *et al.,* 1994; Butler, 2003). However, weighing cattle is laborious and rarely done by veterinarians, animal nutritionists or farmers. A more useful method in assessing the energy reserves is based on assessing individual BCS, based on visual observation and more accurately by palpation of back, ribs and rear quarters. There are two main scoring systems: (1) the American scoring system on a nine-point scale (Herd and Spratt, 1986) and (2) the Scottish scoring system on a five-point scale (Edmundson *et al.,* 1989; Wiltbank, 1991). The Scottish body condition scoring system seems to have been adopted quite widely in the Southern African region, including Mozambique. Equivalence between these two scoring systems is presented in Table 2.1.

2.2.1 Other methods of assessing energy reserves in cattle

It is obvious that looking at the body weight of a cow does not give us an idea of existing energy reserves. Since an estimation of energy reserves represents an important tool for reproduction and production management, other techniques rather than BCS have been developed for cattle.

Subcutaneous fat thickness at the *longissimus dorsi* (UFAT; 12^{th} rib fat thickness) and rump fat thickness (RFAT) (Schroder and Staufembiel, 2006; Yokoo *et al.,* 2008) have been mostly used in dairy cattle to estimate the body energy reserves. Although the measurement of RFAT requires the use of ultrasonographic examinations that are non-invasive practices, skills are, however, needed for manipulation. Its use in beef cattle is limited and apparently not studied under extensive conditions. Nonetheless, a recent publication regarding RFAT showed its high correlation with BCS (Ayres *et al.,* 2009). These findings value the use of simple methods like BCS to estimate the body energy reserves. Due to this reason there is a probability that widespread use of RFAT in dairy and beef cattle may not take place.

Correlations between BCS and other direct methods of measuring energy reserves like energy balance, or indirect methods like circulating levels of hormones, leptin, were reported (Wathers *et al.,* 2007; Murrieta *et al.,* 2010). Recent results indicate that BCS remains the best indicator of nutritional status because of its high correlations with the related energy reserve indicators.

Table 2.1 Description of the Scottish Body condition score system and its American equivalent

Group	Score system Scottish (*American*)	Description
Thin condition	1 *(1 and 2)*	**Extremely thin** with severe muscle wasting may appear humped in the back with feet close together, usually weak; extremely prominent backbone, hooks, pins and ribs. *Similar to BCS 1 and 2 in 9-point system*
	2 *(3)*	**Thin** with little or no wasting of muscle structure; vigorous, little or no fat in ramp, rib or brisket; prominent backbone, hooks, pin and ribs normal appearing muscle structure. *Similar to BCS 3 in 9-point system.*
Moderate condition	3 *(5)*	**Ideal Condition**. Thrifty with normal muscle structure; some evidence of fat deposit in fore-rib, brisket and crops but limited around the tail-head. Some smoothness over the shoulder, ribs, backbone, hooks and pins. *Similar to BCS 5 in 9-point system.*
Fat condition	4 *(7)*	**Fat** but still firm; vigorous; considerable fat deposit over fore-rib; brisket protruding; tail-head full (bulging); very smooth over backbone with no skeleton visible except at hooks. *Similar to BCS 7 in 9-point system.*
	5 *(8 and 9)*	**Very fat** with considerable softness; very fat over the fore-rib and shoulder; large prominent brisket; broad flat top-line; large patchy fat deposit around the tail-head; body curvature become squares in appearance. *Similar to BCS 8 and 9 in 9-point system.*

Scores in italics ~ American equivalent

Source:

Edmundson *et al.*, 1989; Wiltbank, 1991 (Scottish BCS system)

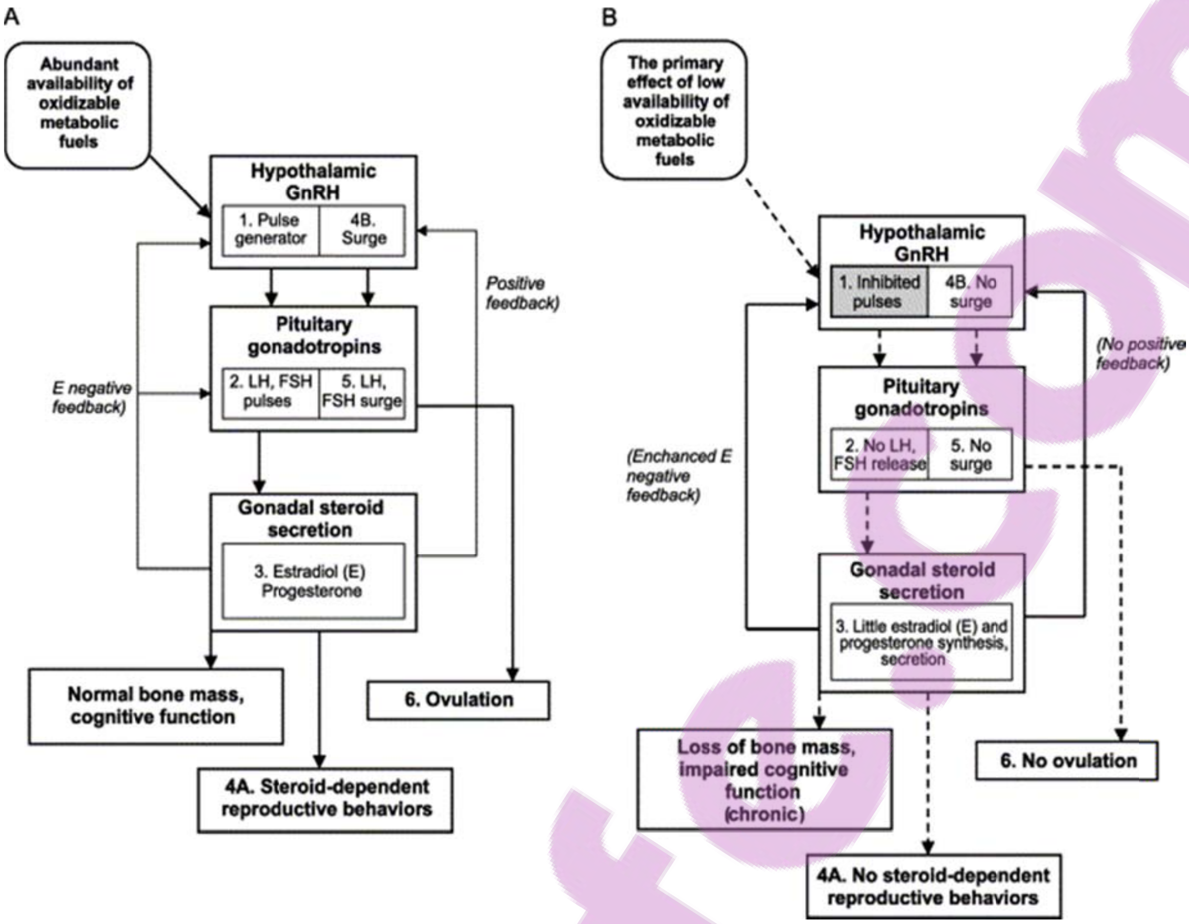
Herd and Spratt, 1986 (American BCS System)

2.3 Effect of changes in body condition score on post-partum reproduction

The effects of BCS on postpartum rebreeding of beef cows (Bishop *et al.,* 1994; Spitzer *et al.,* 1995; Renquist *et al.,* 2006; Flores *et al.,* 2008) and dairy cows (Roche *et al.,* 2009; Allbrahim *et al.,* 2010) have been discussed. Variation in BCS of beef cows in the post-partum period has a number of practical implications for bovine reproduction, such as the association with the length of the post-partum interval to oestrus and ovarian activity (Bishop and Pfeiffer, 2008), and conception rates (Renquist *et al.,* 2006; Roche *et al.,* 2009).

It is well known that the Gonodatropin releasing hormone (GnRH) pulse generator system and the secretion of GnRH are inhibited by under nutrition (Randel, 1990; Wade and Schneider, 1996; Nqeno *et al.,* 2010). Nevertheless, the physiology of nutrition illustrates that the oxidisable metabolic fuels are used for all physiological functions in the body and the excess is stored to be retrieved when a deficit occurs and, thus, to maintain production. Moreover, energy is partitioned by a priority to first maintain the life of the cow and then to propagate the species (Short *et al.,* 1990). The approximate order of priority has being indicated to be (1) basal metabolism; (2) activity; (3) growth; (4) basic energy reserves; (5) pregnancy; (6) lactation; (7) additional energy reserves; (8) oestrus cycle and initiation of pregnancy; and (9) excess reserves (Short *et al.,* 1990). Thus, reproduction takes place when basic physiological functions are satisfied in terms of energy.

The functioning of the hypothalamus-pituitary-ovarian axis when not energetically challenged is presented in Figure 2.1 (A), while when energetically challenged in Figure 2.1 (B). Figures 2.1 (A and B) illustrate that the prerequisite for the resumption of ovarian activity in the post-partum cows is an increased pulse frequency of episodic release of LH, which may occur in cows with moderate to good BCS (Bishop *et al.,* 1994).



Source: Schneider (2004)

Figure 2.1 Functioning of the hypothalamus-pituitary-ovarian axis when not energetically challenged in (A), while energetically challenged in (B).

2.4 Effect of suckling on post-partum reproduction

Suckling is important for calf survival and forms the basis of high and sustainable income in beef cattle production systems. However, there is a concern that this exteroceptive stimulus prolongs the post-partum anoestrus, probably through a neural-mediated inhibition of LHRH or an inhibitory effect of RH on gonodatrophins or action at the ovary (Acosta *et al.,* 1983; Convey *et al.,* 1983; Pérez-Hernández *et al.,* 2002). Notwithstanding the evidence that suckling might act in a chronic fashion to inhibit LH secretion through the post-partum period (Convey *et al.,* 1983; Garcia-Winder *et al.,* 1986; Crowe *et al.,* 1998; Pérez-Hernández *et al.,* 2002; Quintans *et al.,* 2009), the true mechanism by which suckling extends the post-partum anoestrus is uncertain.

Suckling may occur six to nine times a day, with young calves suckling more frequently than older calves (Shimada *et al.,* 1989; Stewart *et al.,* 1993; Gazal *et al.,* 1999; Das *et al.,* 2000; Paranhos da Costa *et al.,* 2006). Available reports indicate also that suckling frequency is high in first parity cows along with the short duration of the suckling meal (Stewart *et al.,* 1993; Paranhos da Costa *et al.,* 2006).

There is a breed-related difference in daily suckling frequency. *Bos indicus* cattle have a higher daily suckling frequency than *Bos taurus* and cross-breed cow-calf pairs (Das *et al.,* 2000).

Although the precise mechanism by which suckling extends the post-partum anoestrus is uncertain, evidence exists that suckling frequency is the characteristic that correlates best with the anticipated onset of oestrus in the post-partum period (Radford *et al.*, 1978; Shimada *et al.,* 1989; Williams, 1990; Stewart *et al.,* 1993; Lamb *et al.,* 1999; Gazal *et al.,* 1999; Marongiu *et al.,* 2002; Álvarez-Rodriguez *et al.,* 2010). In rodents, the inhibitory effect has been shown to be proportional to suckling intensity (Ford and Melamphy, 1973; Hammons *et al.,* 1973) with comparable suggestion in ruminants (Quintans *et al.,* 2009).

2.5 Effects of suckling on post-partum LH and FSH concentrations

Around parturition the hypothalamus-pituitary axis responds to a negative feedback effect of the placental and ovarian steroids by suppressing FSH release and accumulating this hormone in the anterior pituitary and depleting LH stores (Yavas and Walton 2000). Severe increase in FSH is observed after parturition followed by the emergence of first follicular waves of which the dominant follicles do not ovulate, leading to the development of second follicular waves in both suckling- and non-suckling beef cows (Murphy *et al.,* 1990; Breuel *et al.,* 1993; Crowe *et al.,* 1998). There are similarities between suckling- and non-suckling beef cows in LH concentration in the anterior pituitary (Nett *et al.,* 1988) and in the plasma (Radford *et al.,* 1978, Walters *et al.,* 1982) after 30 days post-partum. For Garcia-Winder *et al.* (1986), the plasma LH concentration in suckling beef cows is low irrespective of post-partum period. On the other hand, there is an indication of low LH pulse frequency in suckling cows after 30 days post-partum (Walters *et al.,* 1982; Garcia-Winder, 1986; Quintans *et al.;* 2004) which is implicated in un-ovulation of dominant follicles emerged from second follicular waves (Williams *et al.,* 1983; Acosta *et al.,* 1983; Breuel *et al.,* 1993; Crowe *et al.,* 1998). Since reports suggest that the pattern of LH pulse frequency is crucial for oestrus to occur, the given data have reinforced the hypothesis that the suckling stimulus increases time to first ovulation by increasing the sensitivity of the hypothalamus to the negative feedback of estrogens during the post-partum period, resulting in decreased LH release.

The elimination of the suckling stimulus for 48 hr in *Bos taurus* beef cattle in intensive production systems increases serum LH concentration as well as pulse frequency and amplitude by 24 hr after calf removal, peaking by 48 hr, and then causes a decrease in LH concentration after the calf returns (Walters *et al.,* 1982; Whisnant *et al.,* 1985; Edwards; 1985). There is, however, a lack of information on the effects of temporary calf removal (12 hr or 48 hr) on *Bos indicus* cows in extensive production systems.

2.6 Effects of suckling on cortisol concentrations

Adrenocortical activity has been widely used as an indicator of social stress and, therefore, as an indicator of animal welfare (Milleder *et al.,* 2003). Koolhaas *et al.,* (1999) reportd that the difference in adrenocortical activity might express basic differences in physiology rather than in stress level. In addition, measuring glucocorticoid concentration in plasma has two methodological problems: (1) blood sampling itself causes an increase in glucocorticoid concentration; and (2) frequent sampling is necessary due to considerable fluctuations. Because reproductive performance is altered in cattle subjected to physiological stress (Dobson and Smith 2000), circulating cortisol levels have been measured together with plasma gonadotropins to analyse their relationship and to understand to which extent stress could impair reproduction (Echternkamp, 1984; Lyimo *et al.,* 1999). Despite the fact that several studies on the correlation between cortisol and reproduction in cattle have been performed in dairy cattle and in *Bos taurus* beef cattle in intensive production systems, the influence of stress on gonadotrophin secretion and subsequent reproductive responses is dependent on the magnitude of the adrenal steroidogenic response and the animal's adaptability to the stress.

During the post-partum period of suckling beef cows, cortisol correlates negatively with LH (Dunlap *et al.,* 1981; Echternkamp, 1984). The increase in systemic cortisol of about 20-fold subsequent to intensive stress suppresses pulsatile LH release (Echternkamp, 1984). Unfortunately, very limited information is available on the effect of calf removal or restricted suckling on cortisol concentrations. Whisnant *et al.,* (1985) studying hormonal changes during 48-hour calf removal reported that serum cortisol concentration did not differ between cows with removed calves as opposed to cows with suckling calves, but a transient elevation was notable in the calf-removed group from 9 to 12 hours after calf removal. Because LH concentration was greater in cows that have weaned than in suckling cows and cortisol pattern followed the above described trend, it was concluded that cortisol may not be a physiological inhibitor of LH secretion in the post-partum period of suckled beef cows.

2.7 Strategies to reduce the suppressive effect of suckling on LH

The existing knowledge of mechanisms by which suckling seems to interfere in the post-partum rebreeding of beef cows was used to shorten the interval to first oestrus and to increase subsequent conception rates. The effects of restricted suckling, calf removal or complete weaning at different days post-partum ranging from 1 to 30 days (Convey *et al.,* 1983; Edwards, 1985) or after 30 days (Dunlap *et al.,* 1981; Walters *et al.,* 1982; Whisnant *et al.,* 1985; Dunn *et al.,* 1985) were reported, but the research was generally restricted to *Bos taurus* breeds and their crosses. Restricted suckling and partial calf removal make up the majority of the studies done in this field, probably because complete weaning would imply early weaning of calves, with subsequent negative effects on growth rate as well as the removal of the effects of suckling on re-conception rates.

The manipulation of suckling times was performed either to test the effect of suckling on development of dominant follicles or to analyse the responsiveness of the anterior pituitary on calf withdrawal. Salfen *et al.* (2001) reported that the development of dominant follicles was independent of suckling following a 48-hr calf removal on days two, four and eight during the post-partum period. The same authors observed an increase in oestrus rate of cows synchronised at 25 days post-partum following a 48-hr calf withdrawal. This observation supports the results reported by Walters *et al.* (1982) and Odde *et al.* (1986), in which 48-hr calf removal preceded the start of the breeding season.

There is no defined post-partum day on which calf withdrawal has to be implemented in order to achieve a desirable result from the anterior pituitary. The day of withdrawal may depend; however, on farmer's reproductive management and the cow's body condition score without neglecting the puerperal period.

At approximately 30 days post-partum, in cows with a moderate to good BCS, a 48-hr calf withdrawal may induce oestrus but the reestablishment of the oestrus cycle is uncertain (Salfen *et al.,* 2001).

Reeves and Gaskins (1981); Hoffman *et al.* (1996) and Lamb *et al.* (1999) found that when restricted suckling began before 30 days post-partum once or twice-daily-suckling did not shorten the interval to first oestrus. But, when the same management strategy was carried out after 30 days post-partum or from about 30 days prior to the breeding season, the interval to first oestrus was shortened, with increased conception rates in the following breeding season (Reeves and Gaskins, 1981; Odde *et al.,* 1986; Bell *et al.,* 1998; Gazal *et al.,* 1999; Escrivão *et al.* 2009).

Bell *et al.* (1998) working with *Bos taurus* primiparous cows, reported that once daily suckling and early weaning 30 days before the onset of the breeding season decreased the post-partum interval to first oestrus. But early weaning had a negative effect on weaning weights at 205-days. Conversely, Randel (1981); Odde *et al.* (1986) report that once or twice daily suckling had no adverse effect on weaning weights.

Successful results for the interval to first oestrus and conception rates were related to restricted suckling combined with 48-hr calf removal. Similar results were also observed when 48-hr calf removal was implemented prior to the breeding season (Walters *et al.,* 1982a; Walters *et al.,* 1982b). A summary of existing reports on the effect of suckling management on the postpartum rebreeding performance of cattle is presented in Tables 2.2, 2.3 and 2.4.

Table 2.2 Effects of once-daily suckling on post-partum interval to oestrus and conceptions rates

Author (Year)	Breed	Days post Partum	PPI** to oestrus (days) (ad libidum suckling)	Conception rates (%) (ad libidum suckling)
Reeves and Gaskins (1981)	*Bos taurus*	21 days	41 ± 2.9 (61 ± 4)	Not recorded
Reeves and Gaskins (1981)	*Bos taurus*	> 30 days	38 ± 3 (47 ± 3)	Not recorded
Odde *et al.,* (1981)	*Bos taurus*	> 30 days	52.2 ± 2.6 (55.2 ± 2.7)	93 % (82.1%)
Randel (1981)*	Crossbred (Bt X Bi)	> 30 days	68.9 ± 6.2 (168.2 ± 13.8)	Not recorded
Bell *et al.* (1998)*	*Bos taurus*	> 30 days	Decreased 12 d compared to *ad libidum suckling*	Nor recorded

* First parity cows
** PPI – post-partum interval
Bt – *Bos Taurus*
Bi – *Bos indicus*

Table 2.3 Effects of 12-hour calf removal on post-partum interval to oestrus and conception rates

Author (Year)	Breed	Days post partum	PPI** to oestrus (days) (ad libidum succkling)	Conception rates (%) (ad libidum suckling)
Stewart *et al.* (1993)	*Bos taurus*	> 30 days*	Not recorded	86% (66%)
Gazal *et al.* (1999)	Crossbreed	From day 9*	36 ± 11 (40 ± 3.9)	100% (93%)
Escrivão *et al.* (2009)	*Bos indicus*	From 45* days to breeding	Not recorded	80% (59%)

* Day suckling cows

**PPI – post-partum interval

Table 2.4 Effects of 48-hr calf removal on post-partum days to oestrus and conception rates

Author (Year)	Breed	Days post partum	PPI* to oestrous (days) (ad libidum suckling)	Conception rates (%) (ad libidum suckling)
Odde et al., (1981)	*Bos taurus*	> 30 days	54.9 ± 2.6 (55.2 ± 2.7)	90.9 % (82.1%)
Meirelles et al (1994)	*Bos indicus*	Prior to breeding season	Not recorded	55% (20%)
Escrivão *et al.* (2011)	*Bos indicus*	Prior to breeding season	Not recorded	76% (55%)

* PPI – post-partum interval

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 *et 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.

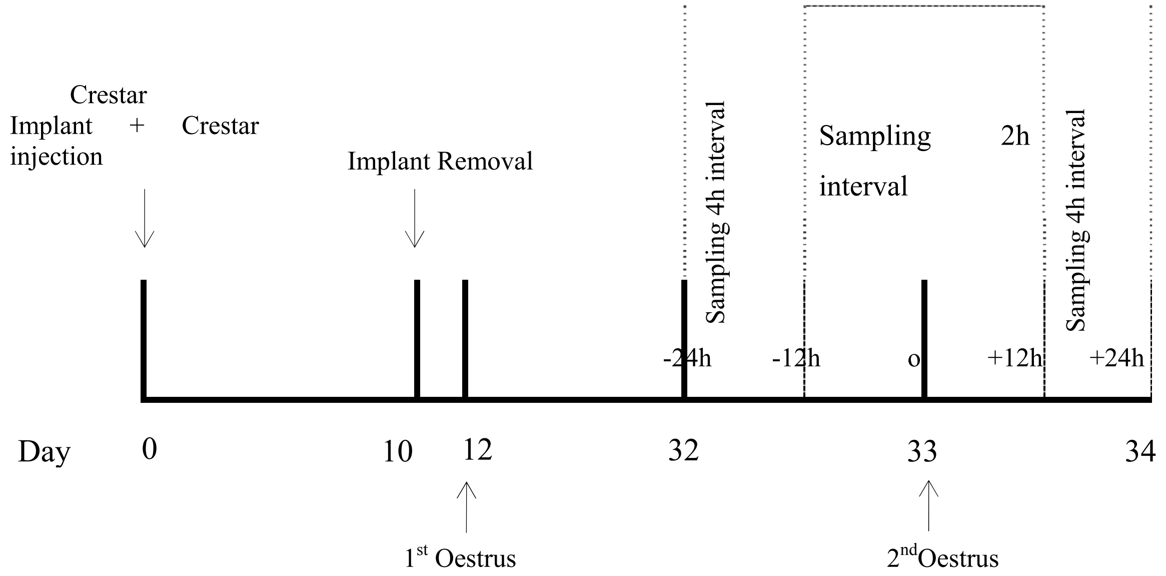


Figure 3.1.Diagram of oestrus synchronisation and blood sampling of *Bos indicus* cows for hormonal assay around oestrus

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^{0}C;
- Dispenses 200 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.

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:

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

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).

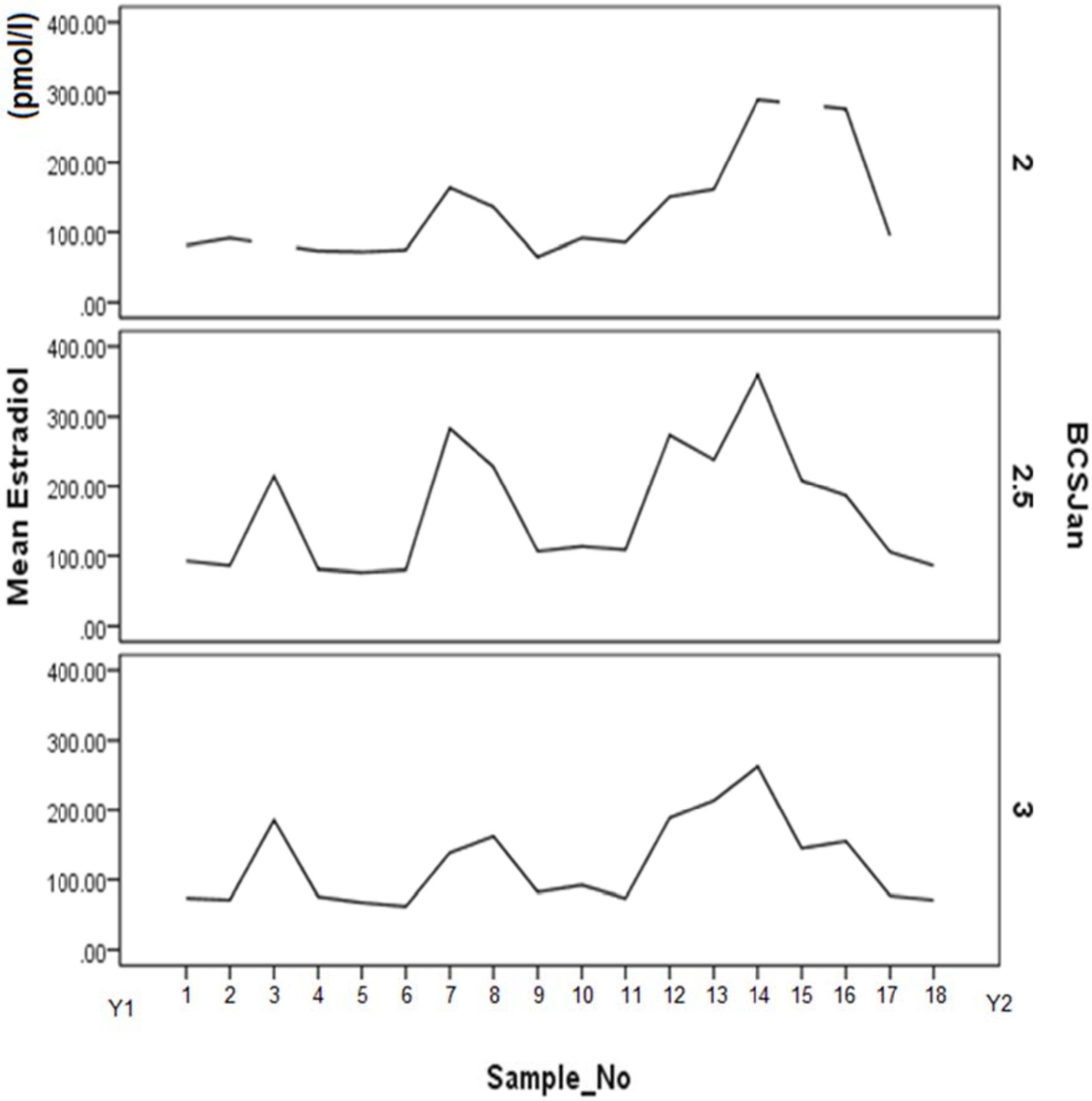


Figure 3.2 Effects of BCS on estradiol concentrations around oestrus

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

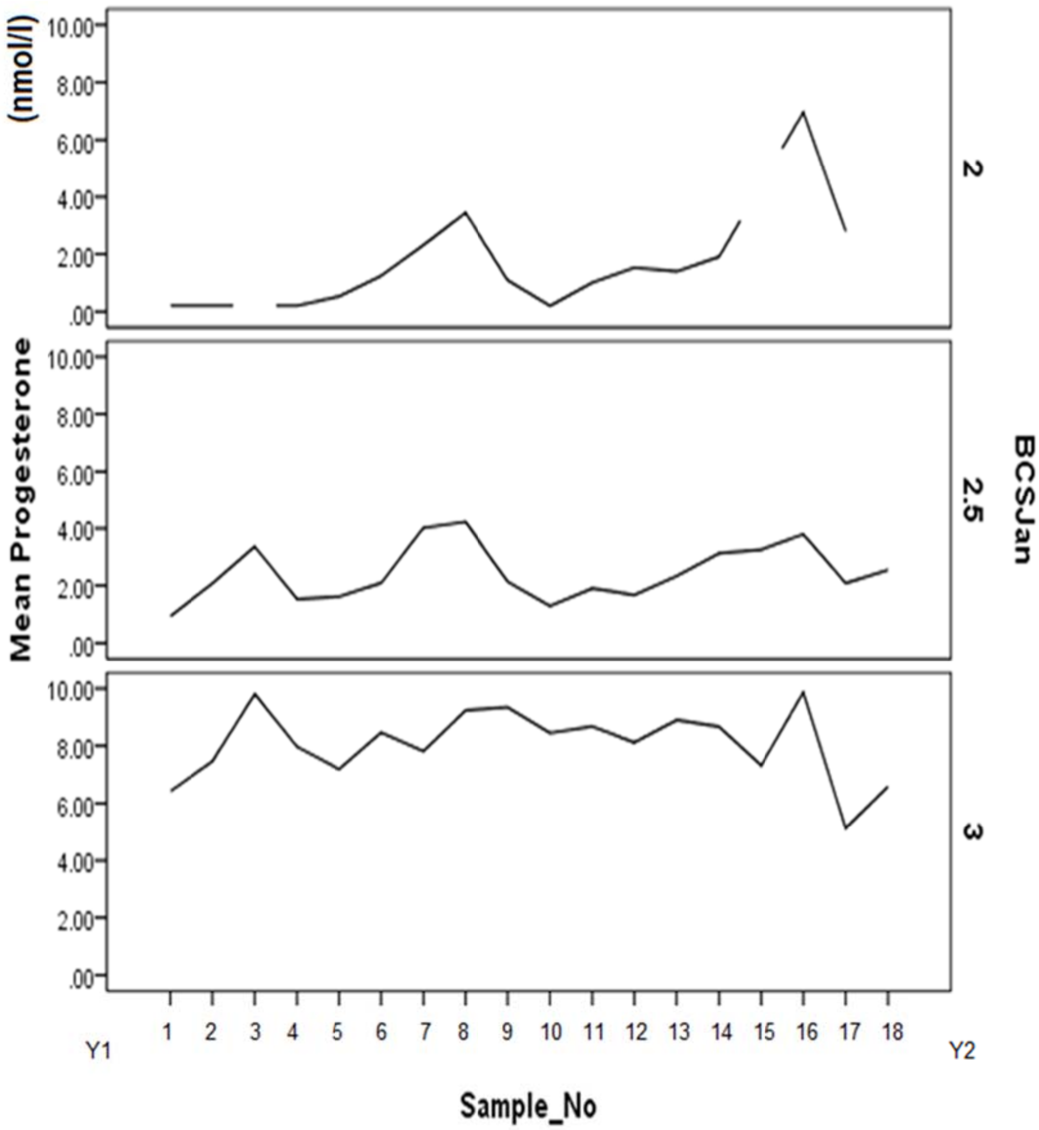


Figure 3.3 Effect of BCS on progesterone concentrations around oestrus
Progesterone at BCS 2; 2.5 and 3 (illustrated on axis –Y_2) differed significantly ($p<0.05$)

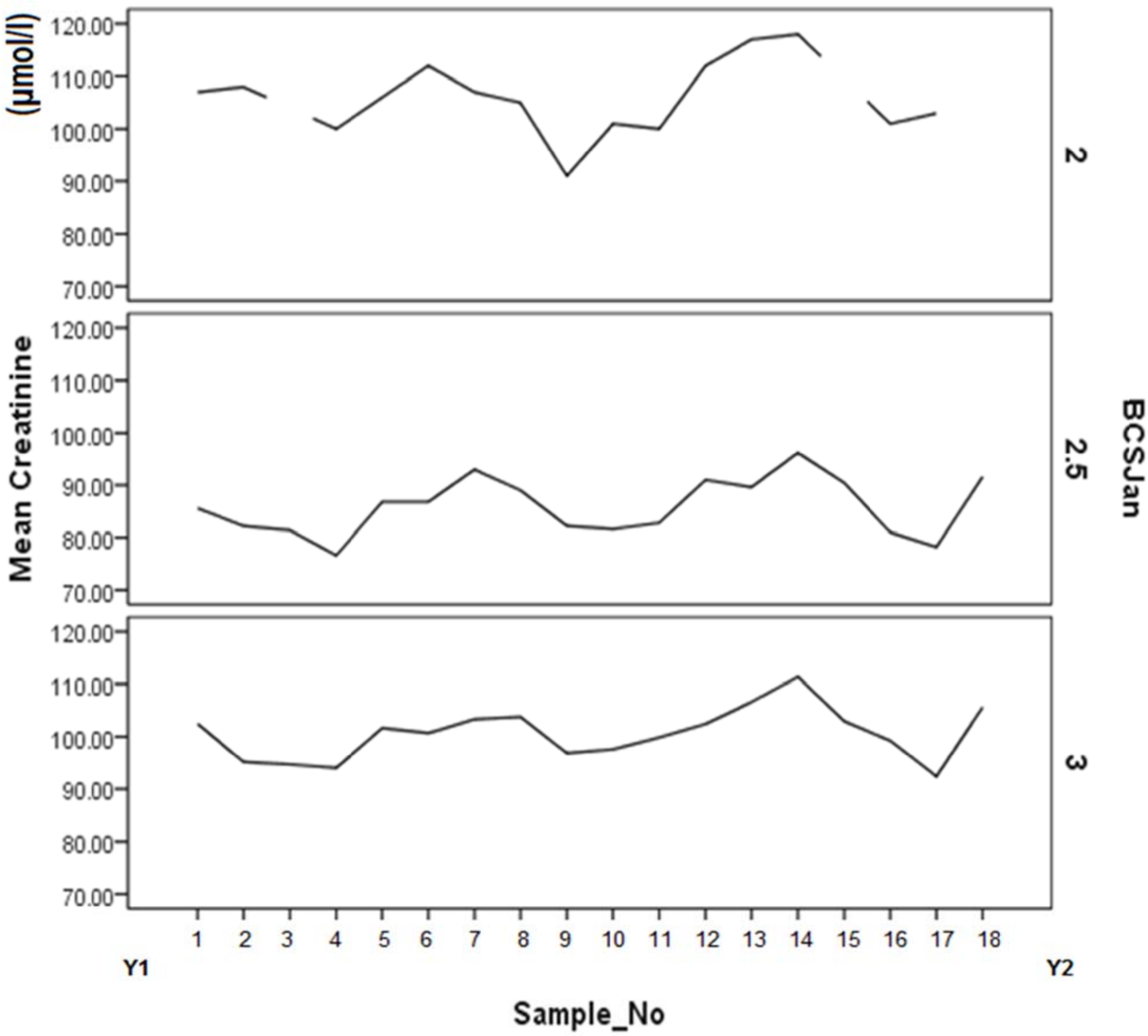


Figure 3.4 Effect of BCS on creatinine concentrations around oestrus

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

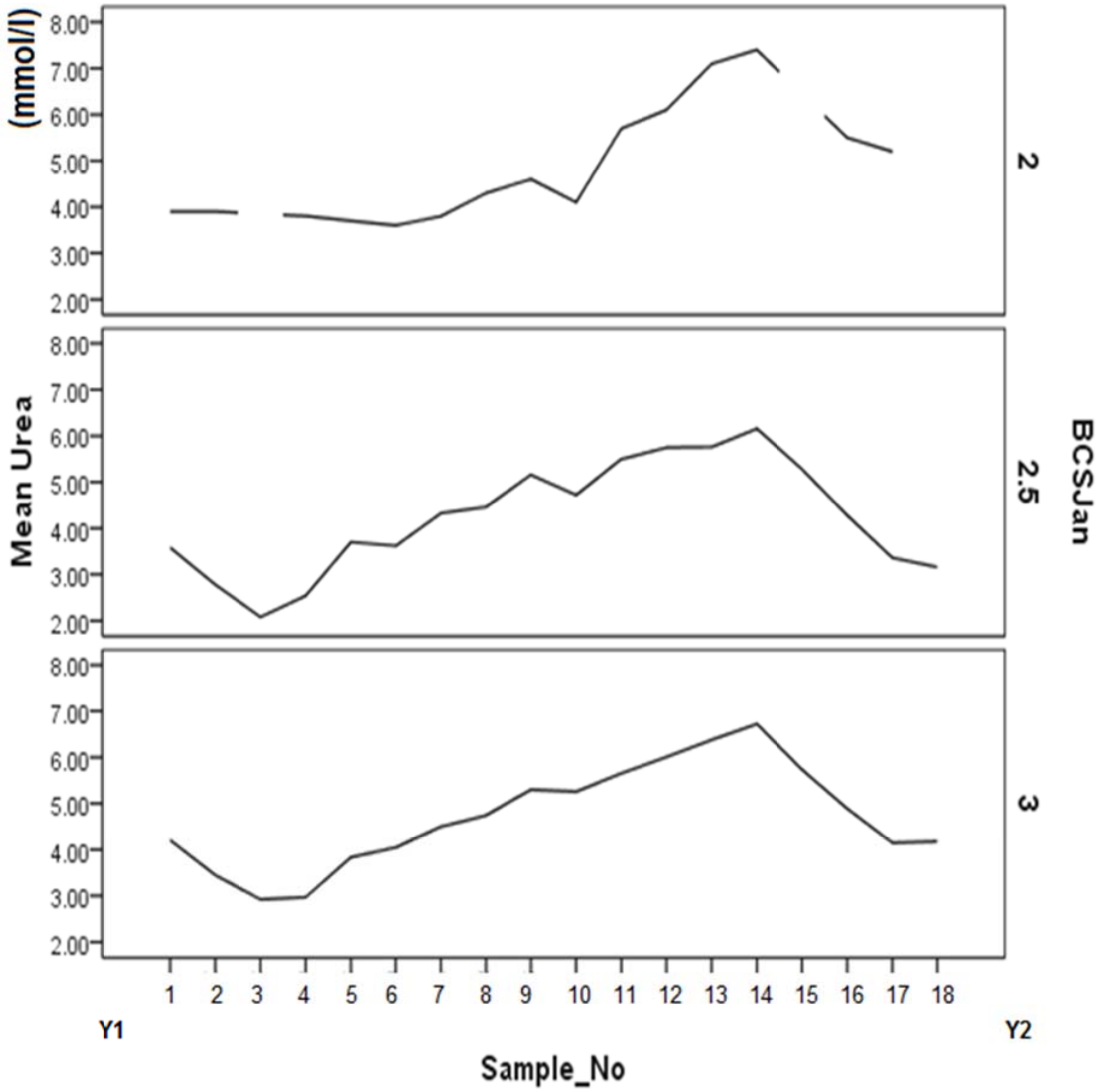


Figure 3.5 Relationship of BCS and urea concentrations around oestrus

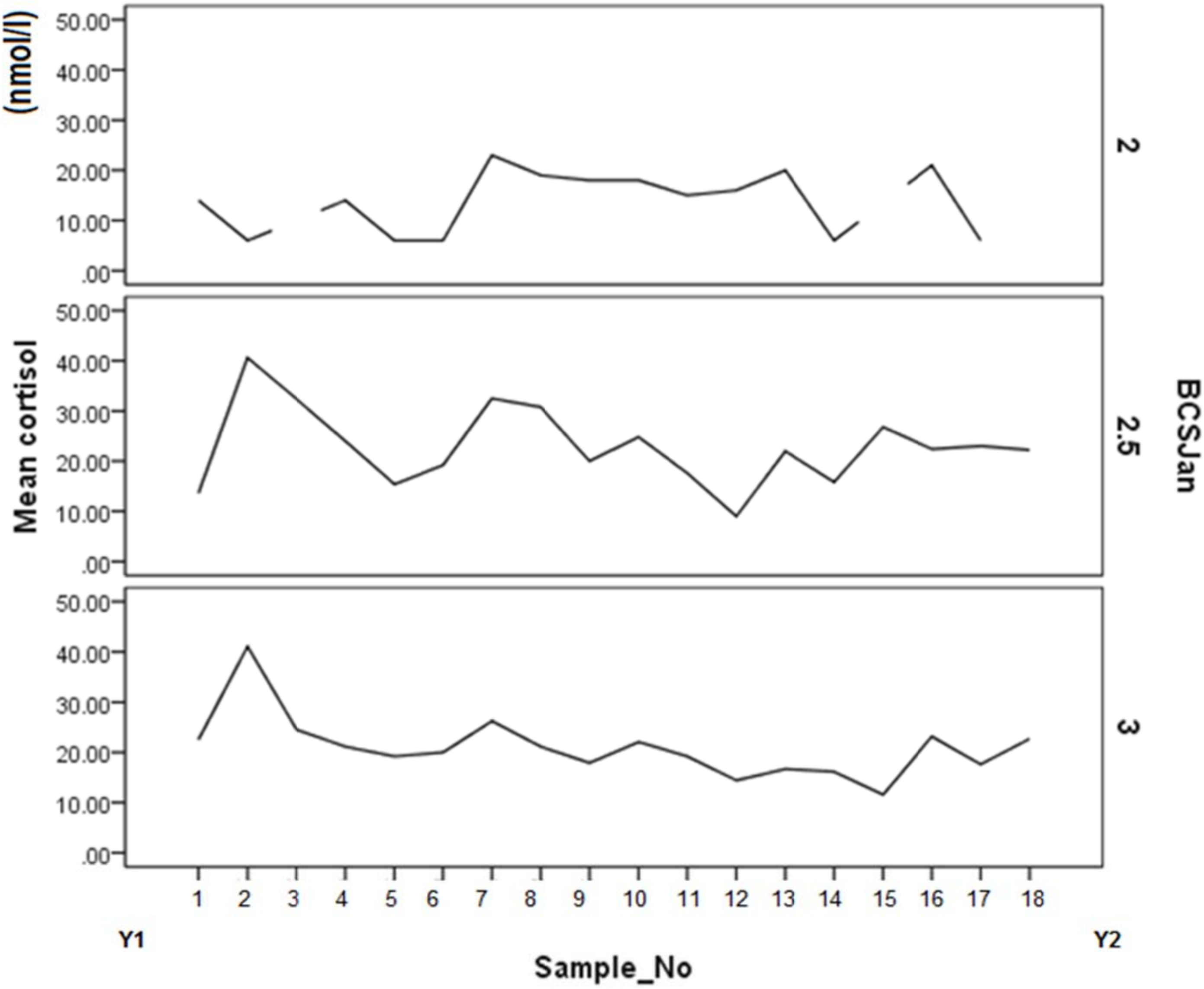


Figure 3.6 Relationship BCS and cortisol concentrations around oestrus

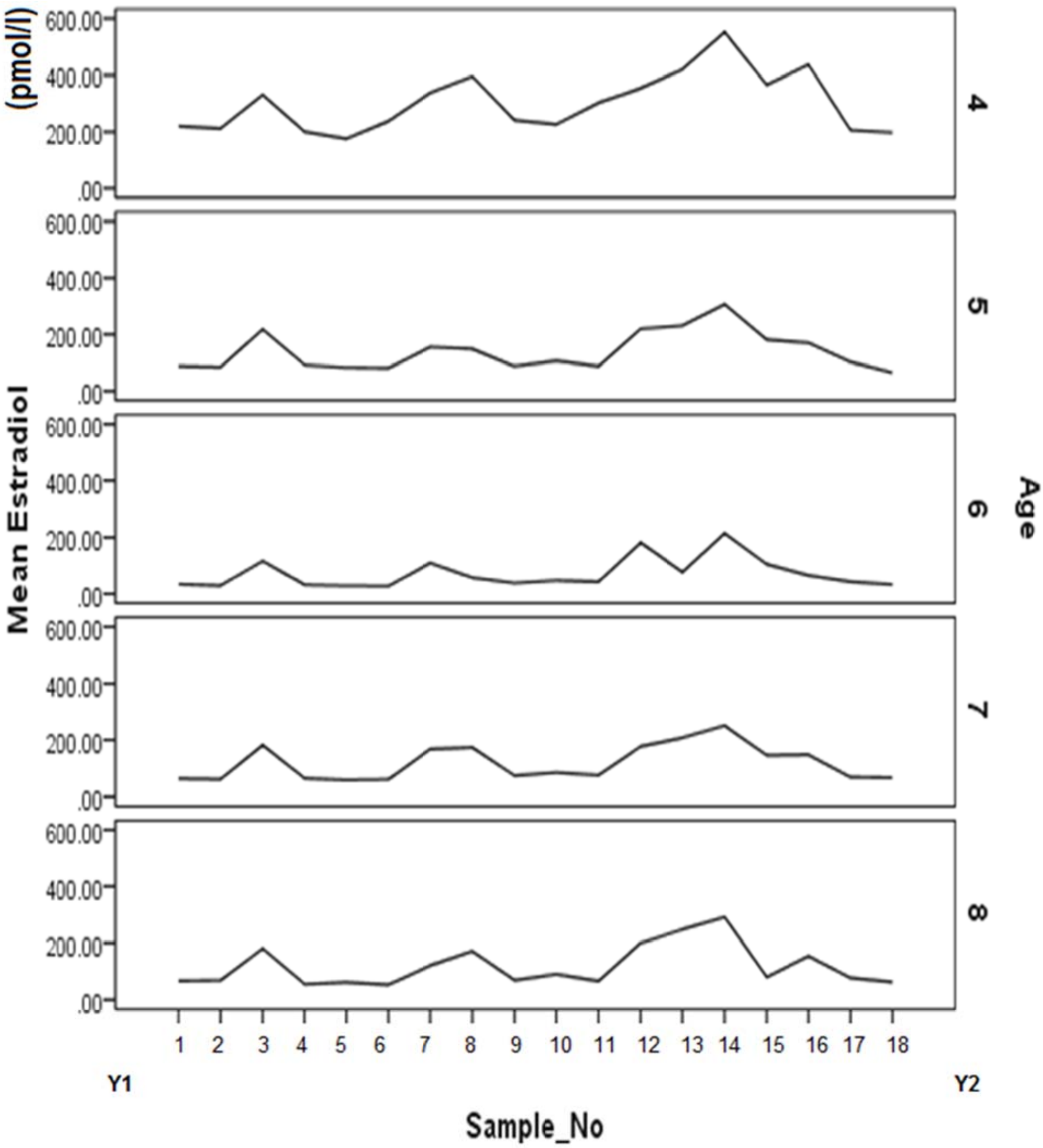


Figure 3.7 Effect of age of cows on estradiol concentrations around oestrus

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

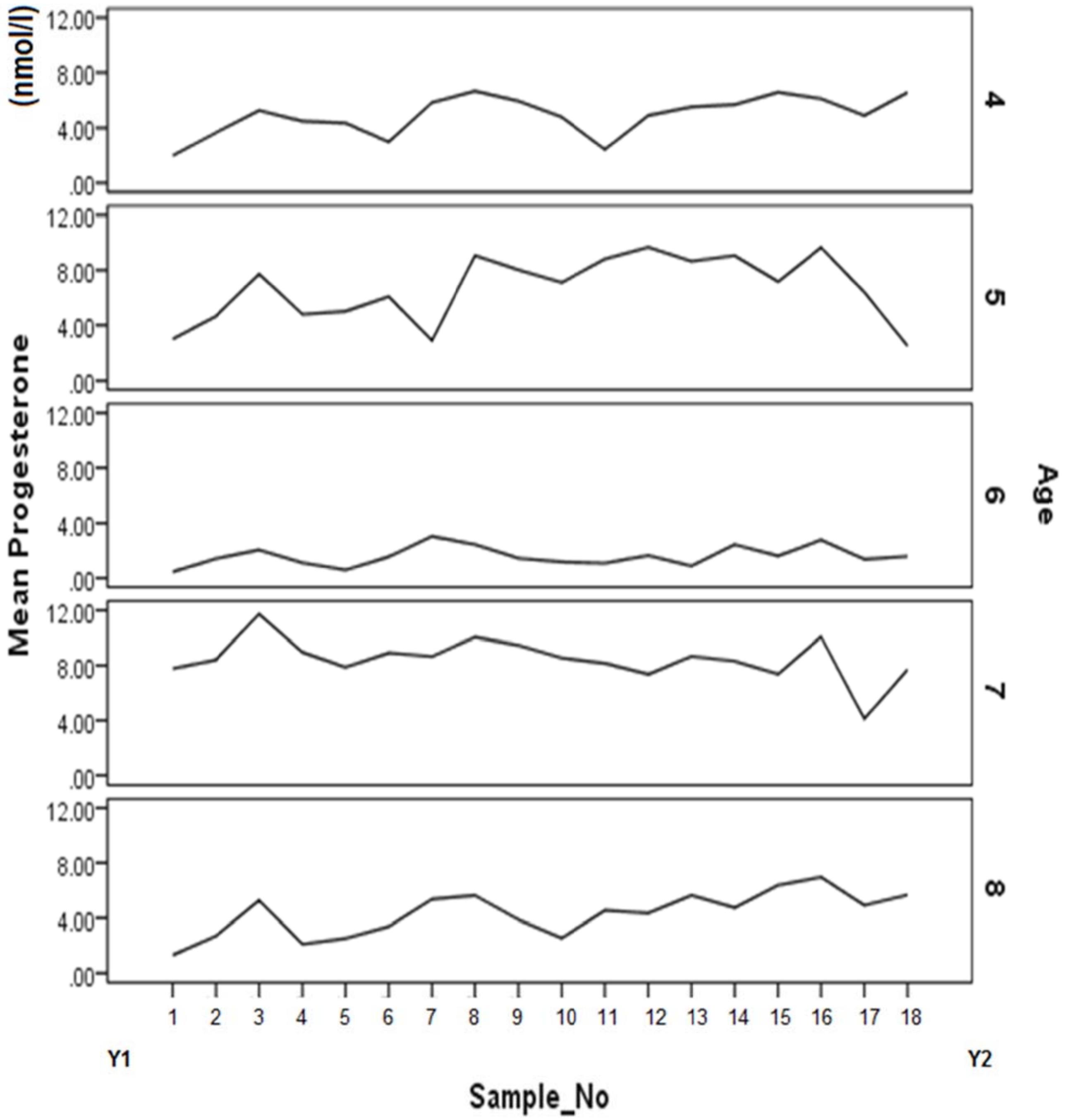


Figure 3.8 Effect of age of cows on progesterone concentrations around oestrus

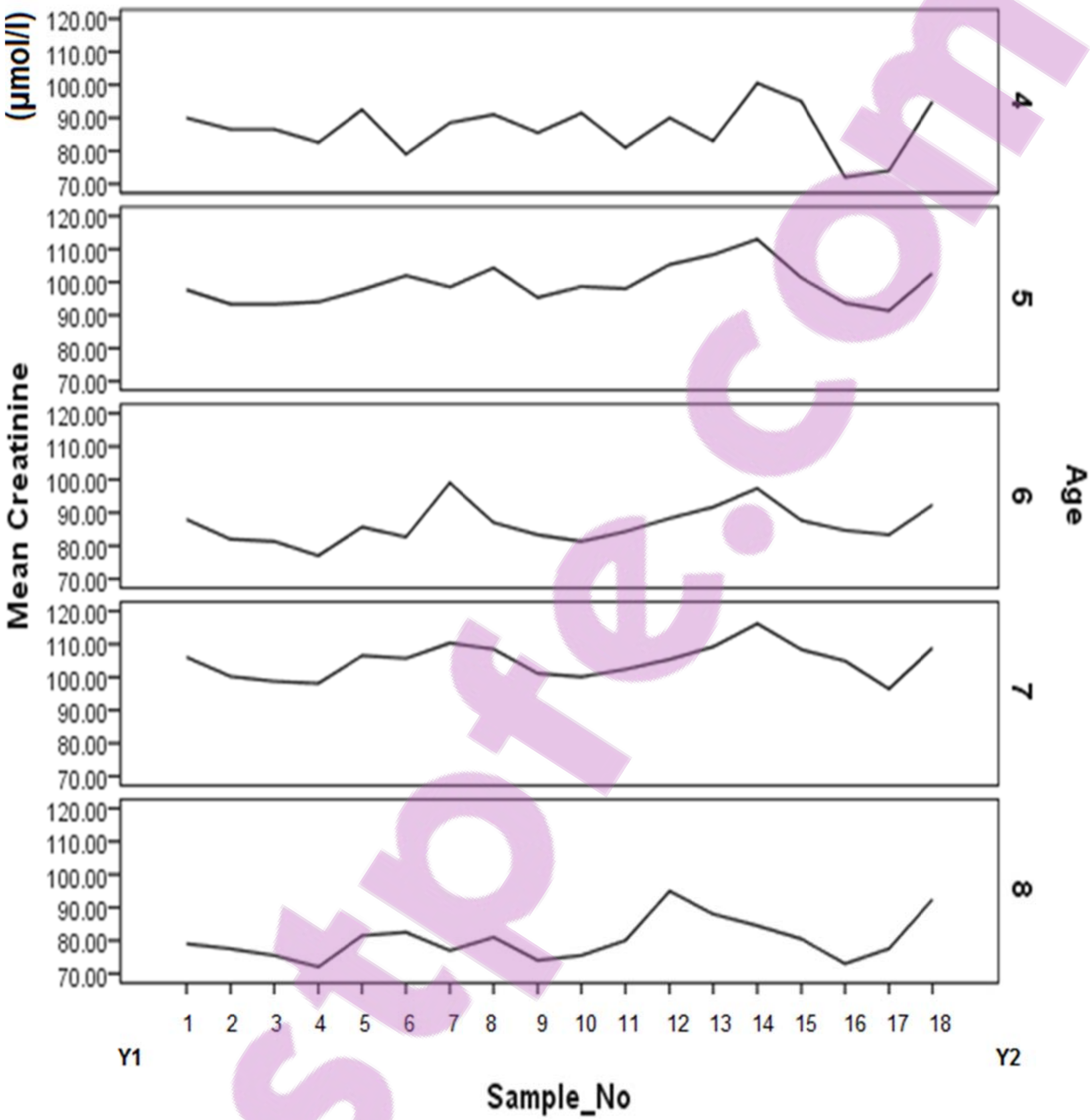


Figure 3.9 Effect of age of cows on creatinine concentrations around oestrus

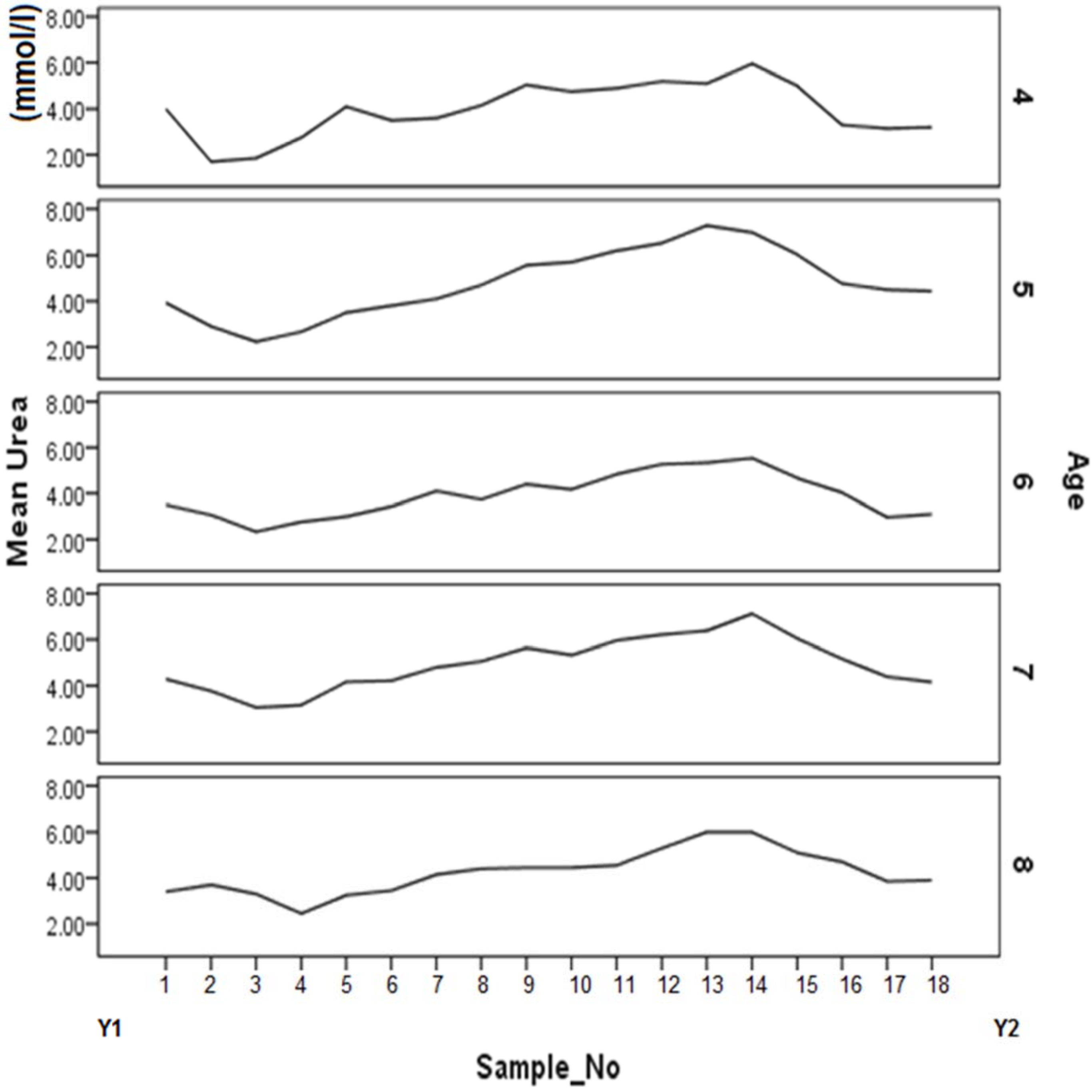


Figure 3.10 Relationship age of cows and urea concentrations around oestrus

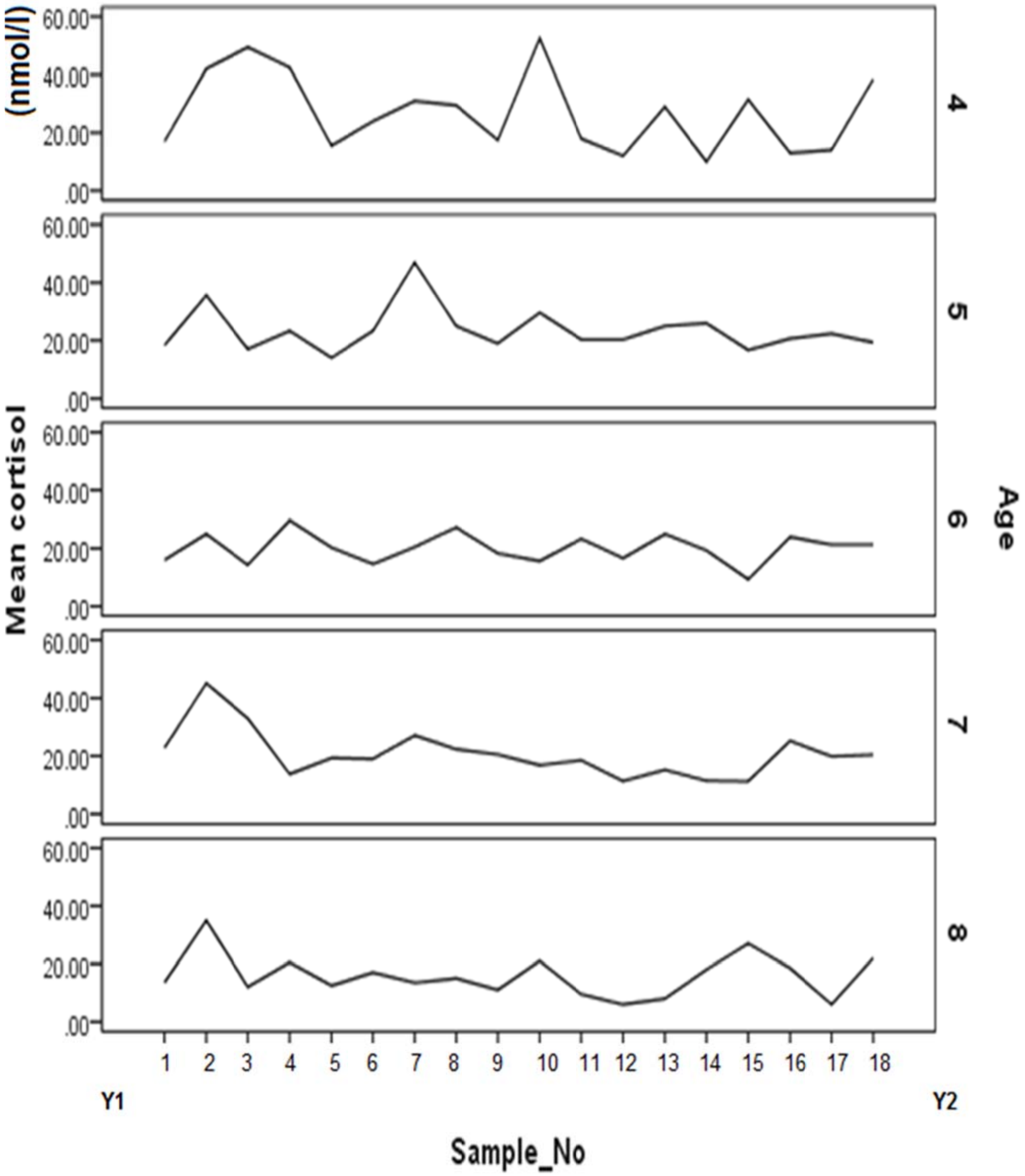


Figure 3.11 Relationship age of cows and cortisol concentrations around oestrus Cortisol at age 4; 5; 6; 7 and 8 (illustrated on axis –Y_2) differed significantly ($p<0.05$)

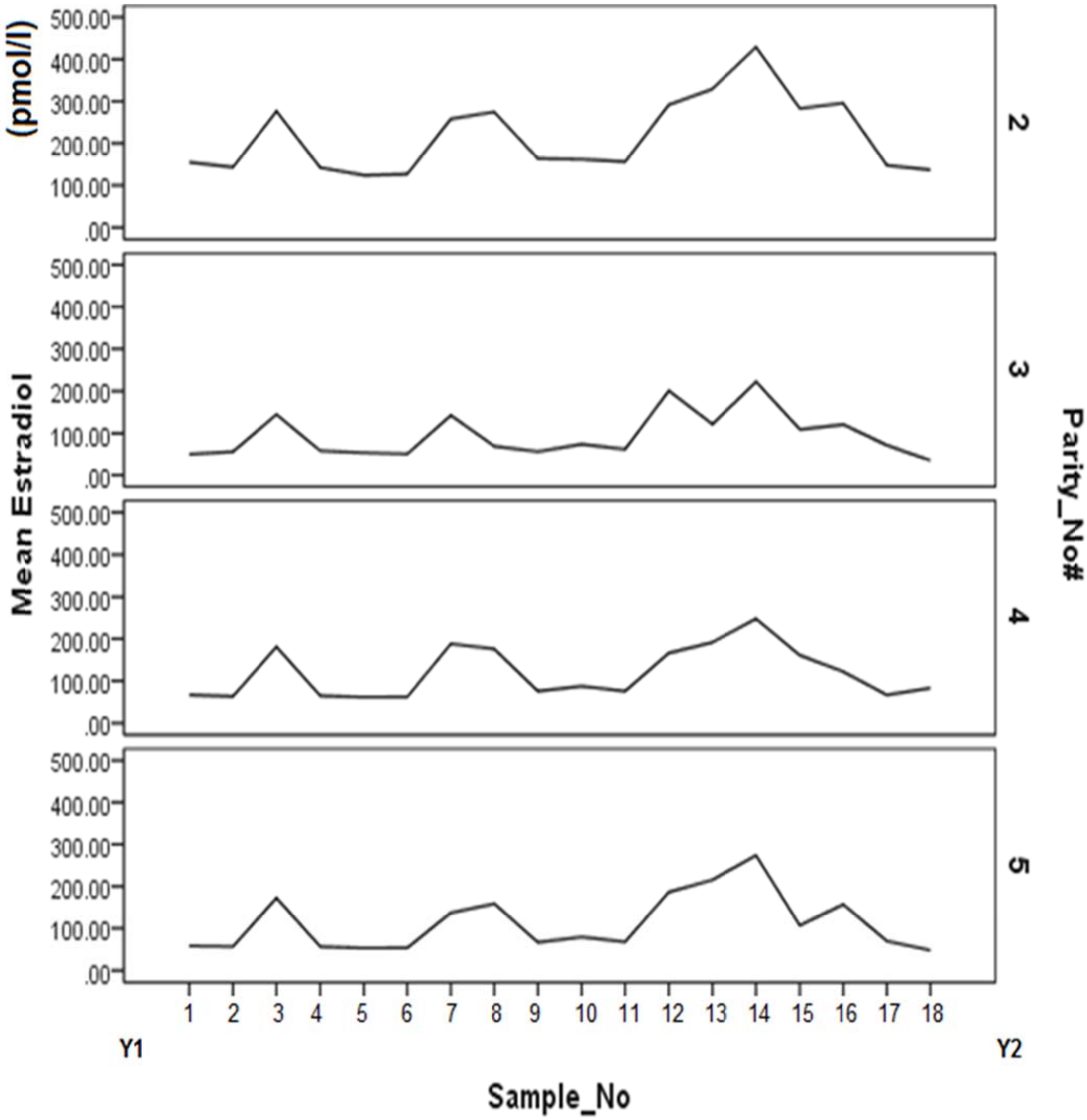


Figure 3.12 Effect of parity number on estradiol concentrations around oestrus
Estradiol at parity number 3; 4; 5 and 6 (illustrated on axis $-Y_2$) differed significantly ($p<0.05$)

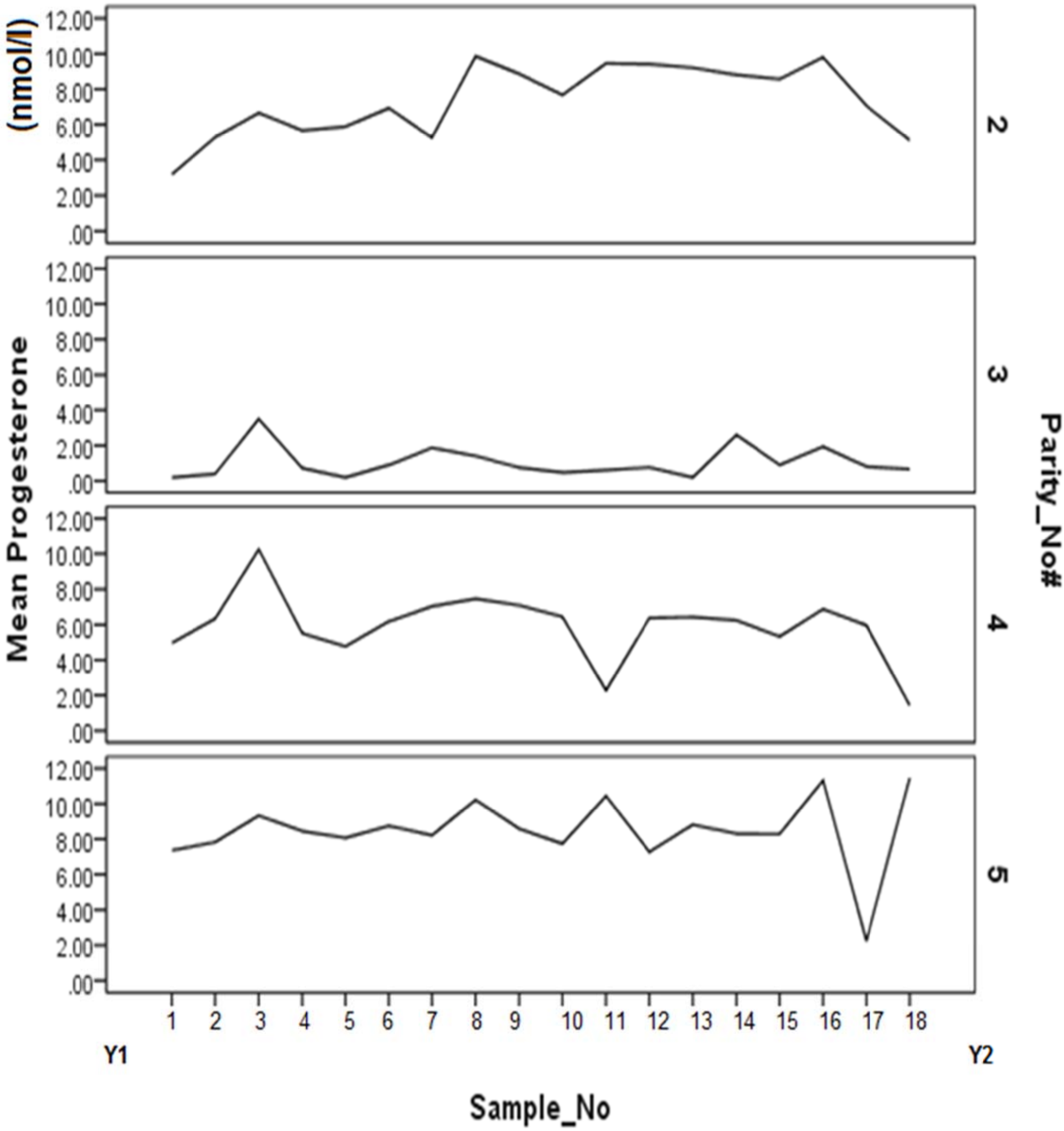


Figure 3.13 Effect of parity number on progesterone concentrations around oestrus

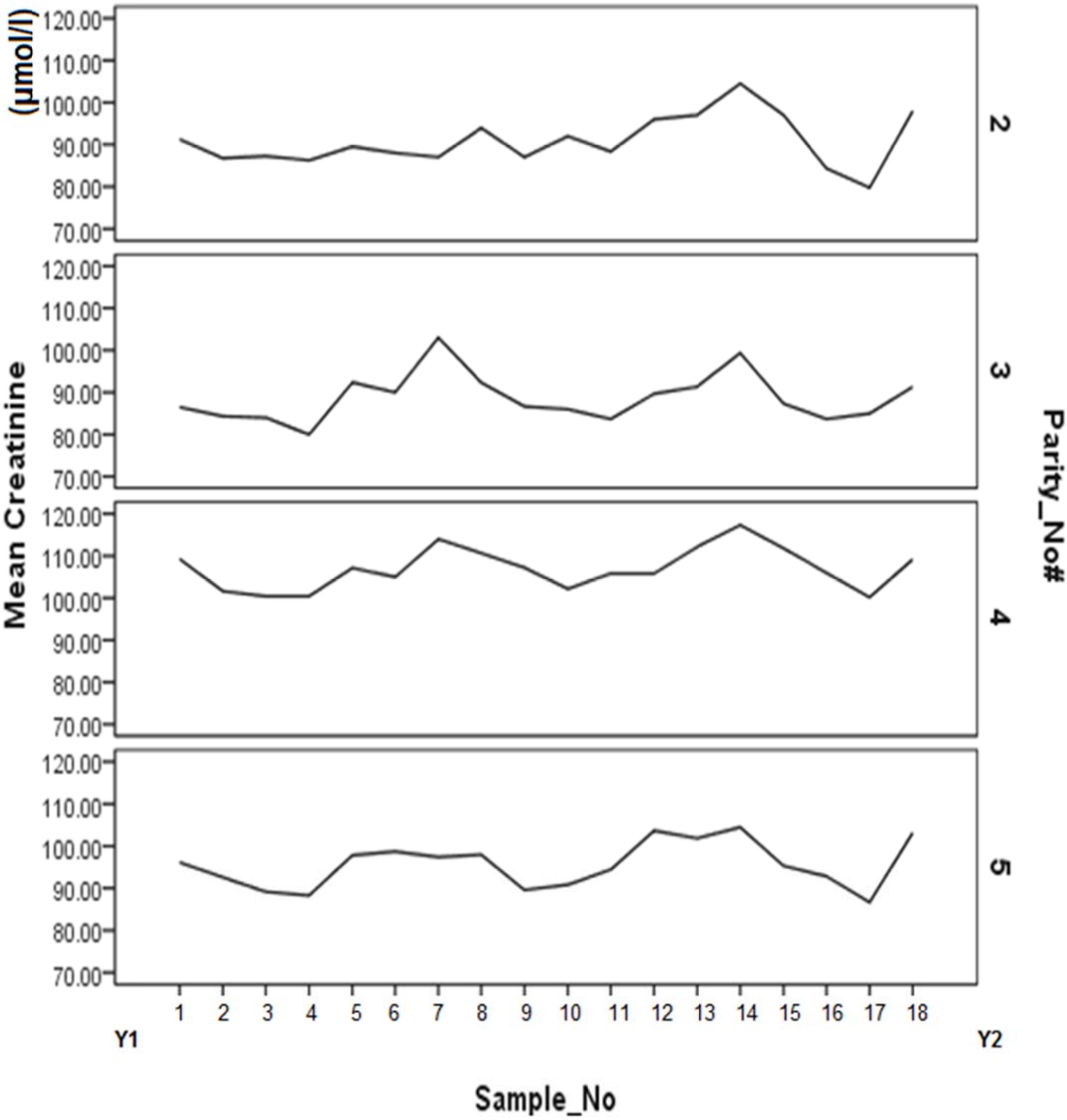


Figure 3.14 Relationship parity number on creatinine concentrations around oestrus

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

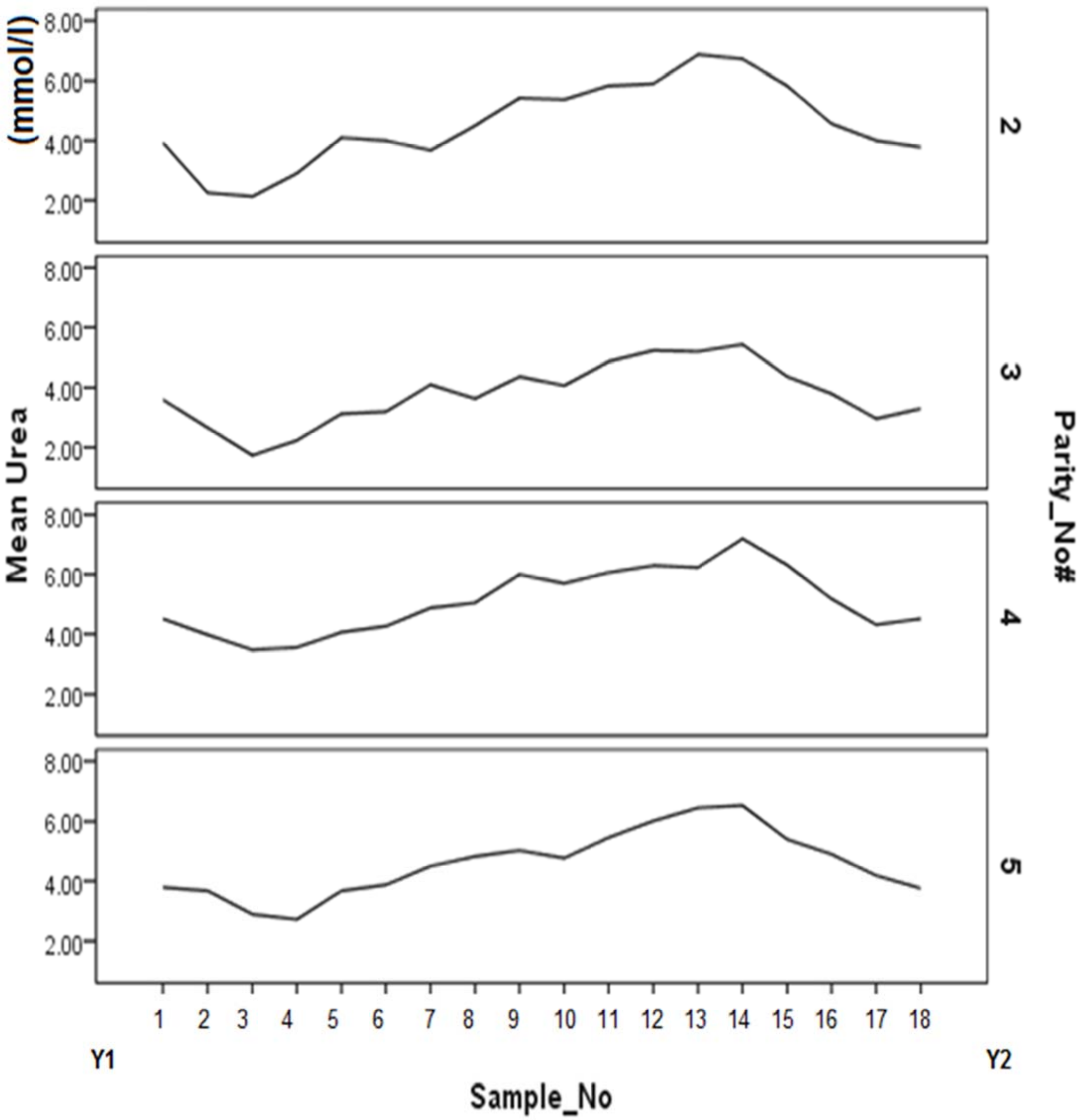


Figure 3.15 Relationship parity number and urea concentrations around oestrus

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

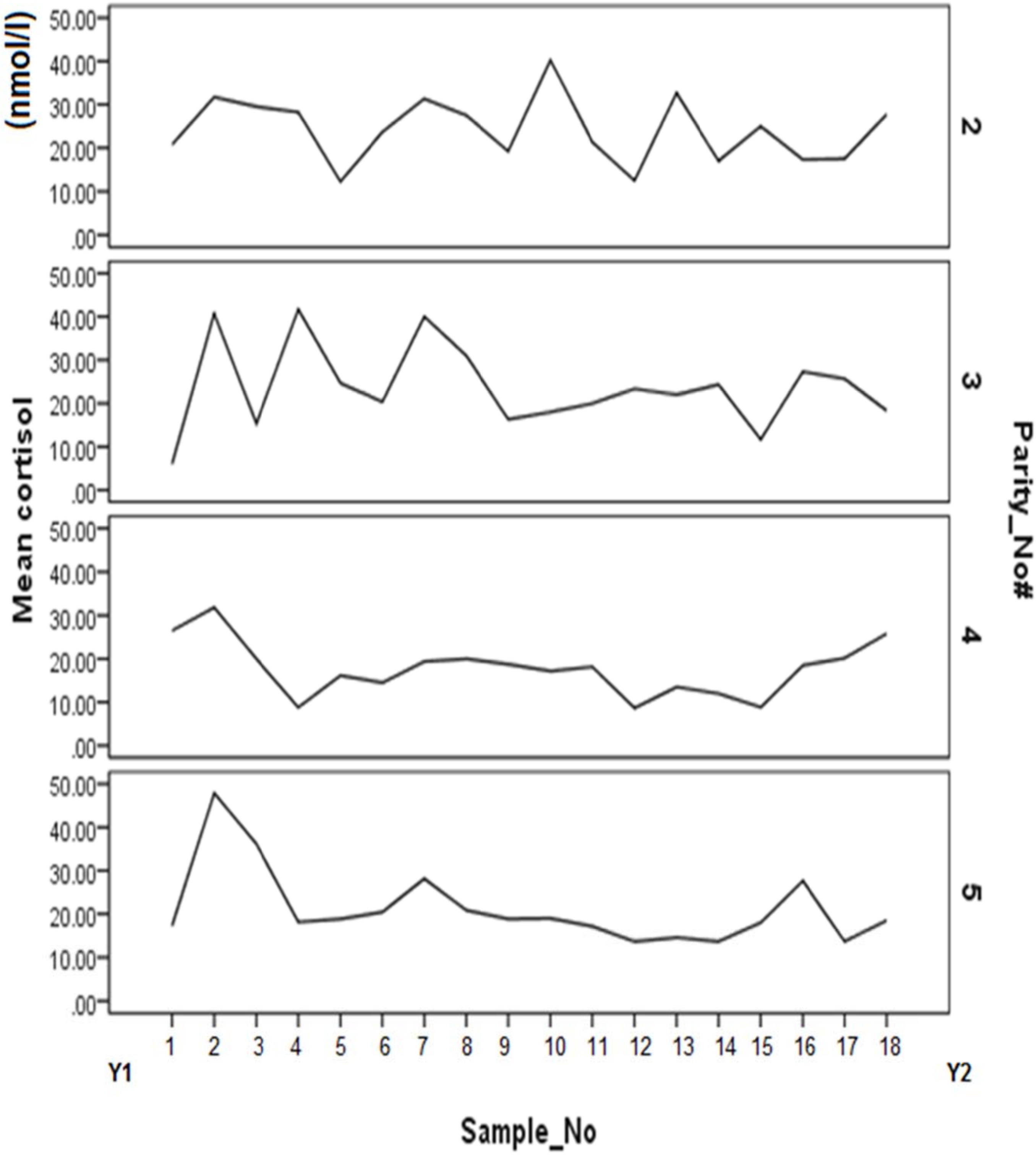


Figure 3.16 Relationship parity number and cortisol concentrations around oestrus

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

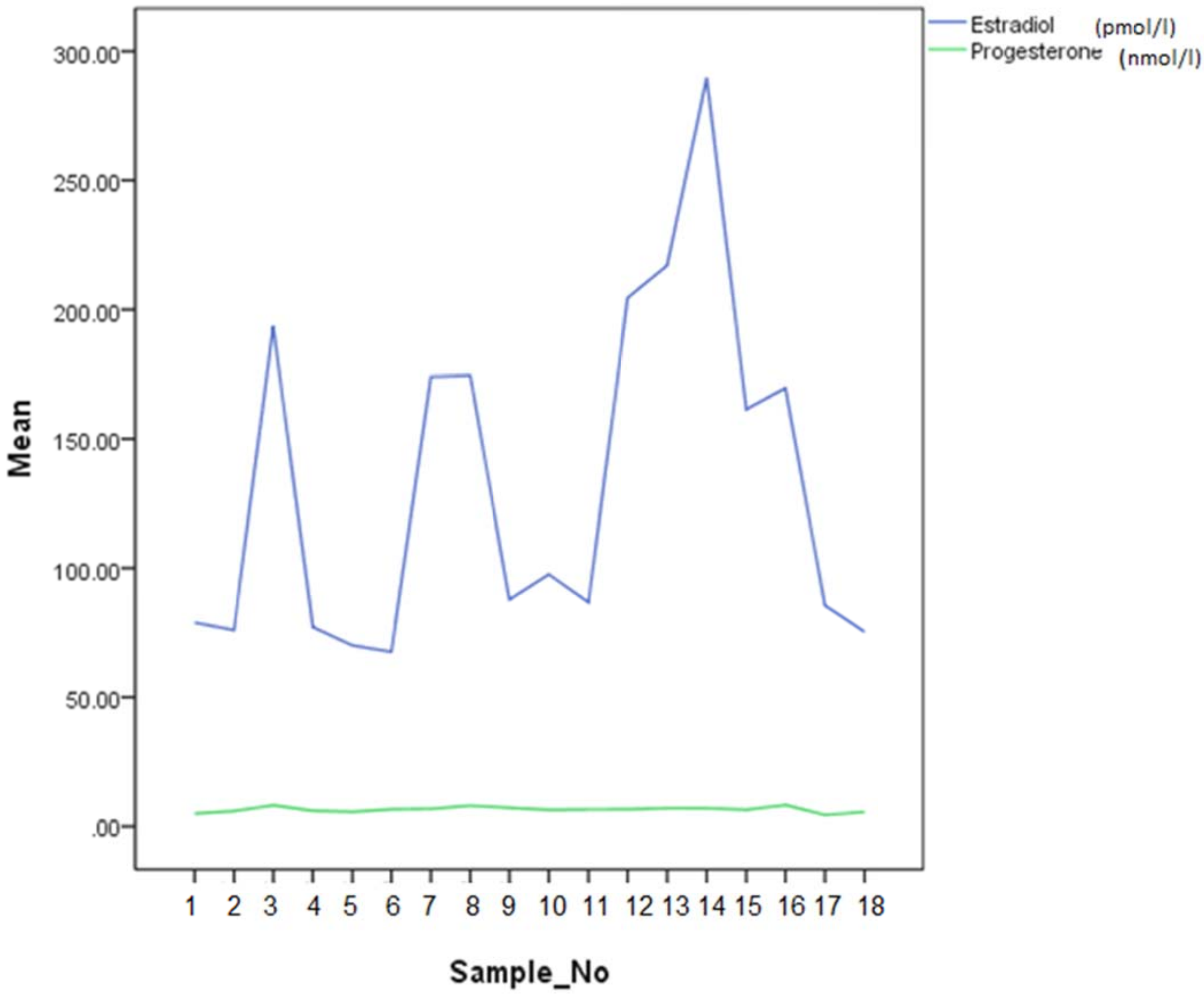


Figure 3.17 Pattern of plasma concentrations of estradiol and progesterone around oestrus in *Bos indicus* cows under extensive conditions

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

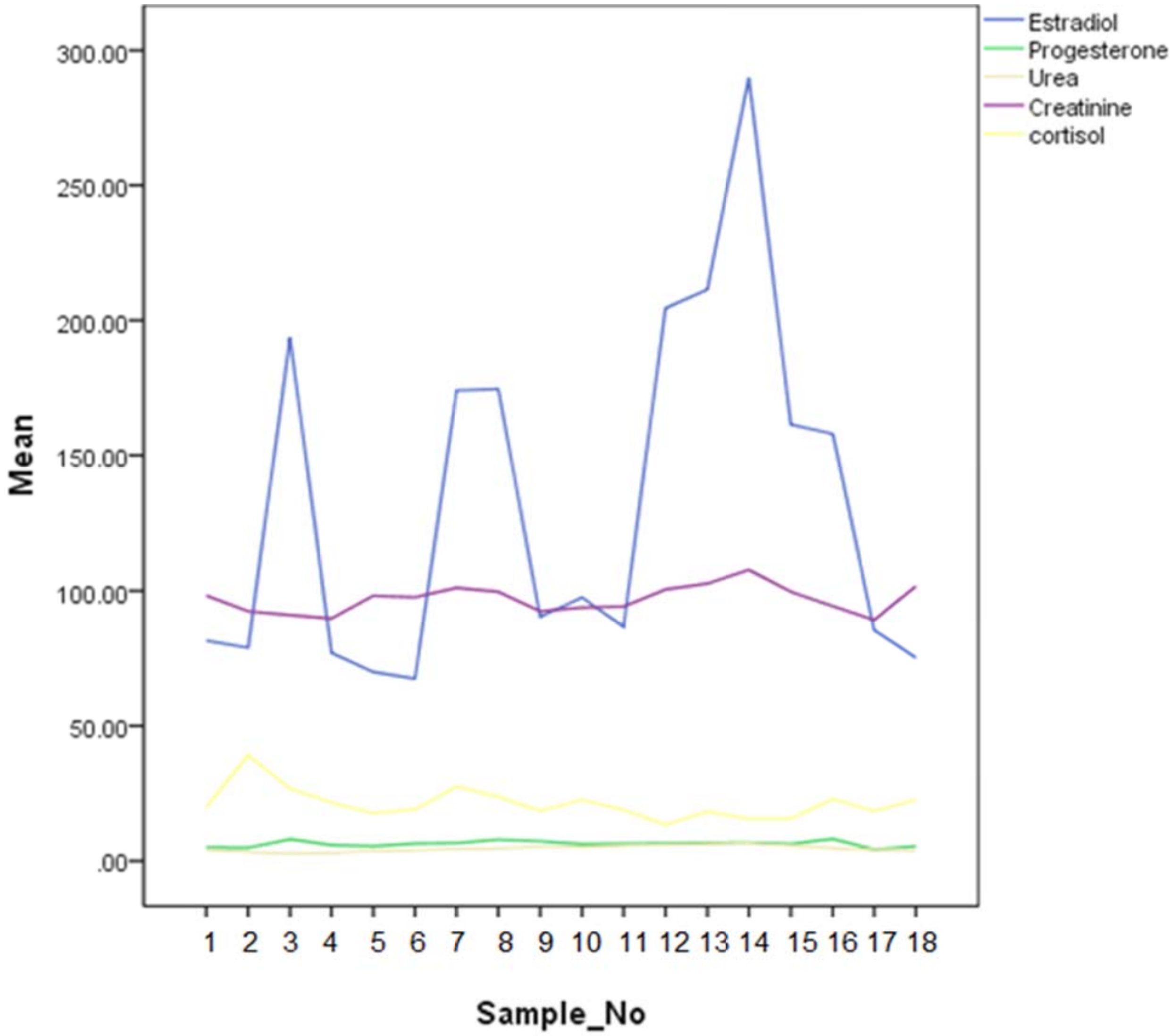


Figure 3.18 Pattern of steroids and other metabolites during oestrus in *Bos indicus* cows under extensive conditions

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.

4. Effects of 12-hour calf withdrawal on conception rates and calf performance of *Bos indicus* beef cattle in extensive production systems

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Summary

This study was conducted to evaluate if restricted suckling at night from 45 days post-partum increases the conception rates of *Bos indicus* beef cows in extensive production systems in sub-tropical conditions and to quantify the related effects on calf-weaning weights. Fifty-two multiparous Brahman type cows with reproductive tract scoring (RTS) ≥ 4 at 45 days post-partum were randomly assigned to two groups of 26 cows each separated into an *ad libitum* suckling group or calf non-removal group (NRG) and treatment group or calf removal group (RG). Calves in the treatment group were separated for 12 hr during the night from 45 days post-partum to the onset of the breeding season. Satisfactory classified bulls were used at the ratio of 1:20 cows for a breeding season of 90 days.
BCS and BW of cows were recorded 45 days post-partum, at the start of the breeding season, and at pregnancy diagnosis that took place 60 days after the end of the breeding season. Pregnant cows were monitored throughout the gestation period. Calves were weighed at calving and weaning. Weaning weights were corrected to 205 days. BW and BCS at the onset of the breeding season was 395.8 ± 50 kg and 2.5 ± 0.3 for the RG and 410.5 ± 40 kg and 2.6 ± 0.3 for the NRG. Calving to breeding intervals were 93 ± 17.5 days for RG and 99 ± 22.1 days for NRG, respectively. Calving to conception intervals differed significantly between the experimental groups (110.9 ± 10 days for RG and 132.8 ± 19 days for NRG) and a similar result was obtained for the commencement of the breeding season to conception intervals (17.8 ± 15 days for RG and 31.1 ± 18.9 days for NRG). Conception rates were 80% for the RG and 59% for the NRG, which correlated better with BW than BCS at the

onset of the breeding season. Weaning weights differed significantly between control and treatment groups (149.3 ± 18 kg for RG and 134.5 ± 20 kg for NRG). From 45 days post-partum to the onset of the breeding season, cows in the RG experienced a positive energy balance (3%) while those in the NRG had a negative energy balance (-0.1%). It was concluded that 12-hr calf separation at night increases the conception rates and improves the calf-weaning weights of *Bos indicus* beef cattle in extensive production systems under sub-tropical conditions.

4.1 Introduction

Twelve-hour calf separation shortens the interval to first oestrus post-partum and increases the conception rate of *Bos taurus* beef cows in intensive production systems (Stewart *et al*., 1993b; Gazal *et al.,* 1999). There is, however, no information on 12-hr calf separation in *Bos indicus* cows in extensive production systems under sub-tropical conditions as well as the influence of calf separation on the weaning weights of calves. The present study was thus conducted to determine if restricted suckling at night from 45 days post-partum increases the conception rates in *Bos indicus* beef cows in extensive production systems and to quantify the related effects on calf weaning weights.

4.2 Materials and Methods

4.2.1 Study location

The experiment was carried out at the Inácio de Sousa extensive beef cattle farm located in Manhiça district, about 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 is concentrated during the rainy season that last for six months (October to March), with 50% of the rain distributed in December and January.

4.2.2 Experimental design

Fifty-two multiparous Brahman-type cows were randomly allotted from a group of post-partum cows that had all calved during the calving season (October to December), normal parturition, irrespective of the sex of their calves. The cows were randomly assigned to two groups of 26 cows each: treatment group (RG) and control group (NRG). Reproductive tract scoring (RTS) was performed at 45 days post-partum (calculated based on the actual calving dates) via rectal palpation using a 5-point cows reproductive tract scoring method as described by Schwalback *et al.* (2000). Only cows with a RTS of 4 or above were incorporated in the experiment.

Cows were maintained in the herd under the same management as the whole farm, taken to grazing areas between 7:00 hours and 17:00 hours, without supplementary feeding and calves from the treatment group were removed at 18:00 hours after the herd returned to the coral.

Separation of calves for a period of 12 hr (12-hr night-calf-separation; 18:00 hours to 6:00 hours) began 45 days post-partum until the beginning of the breeding season and then calves were allowed to suckle *ad libitum*. Calves from the control group remained with their dams for the entire period. Breeding soundness examinations were performed using the system of Hopkins and Spitzer (1997) and only satisfactory classified bulls were used at a ratio of 1:20 cows for a breeding season of 90 days (January to March).

The post-partum interval to the start of the breeding season (CBI) and the duration of treatment were calculated. Body weight was recorded using a standard scale for cattle (Richter scale Company® - Livestock &Animal Scale, Model CS-001 Series, Cattle Scale Weigh Beams) 45 days post-partum and at the onset of the breeding season and during pregnancy diagnosis along with the BCS determinations. BCS determinations were done using a 5-point Scottish scoring method (Wiltbank, 1991). The energy balance of the cows (weight loss or weight gain) at the onset of the breeding season was calculated and expressed as a percentage. Pregnancy diagnosis took place 60 days after the end of the breeding season.

Pregnant cows were monitored to the end of the gestation period, and calving dates were recorded. Calving intervals (CI) were calculated and then used to calculate the calving to conception intervals (CCI) and breeding to conception intervals (BCI), as follows:

CCI = CI – 280 days
BCI = CCI – CBI

280 days is the mode gestation length for Brahman type cows used in the present study (Escrivão, 1998).

The weights of the calves were recorded at calving, within 48 hr of parturition and at weaning.

As calving dates differed marginally, but all calves were weaned on the same day, a correction factor was included to correct for these differences in weaning weights. Weaning weight was corrected to 205 days using the following formula:

$$C_{ww} = B_w + [\ (W_w - B_w) / W_a)^* 205)]$$

Where:
C_{ww} – Corrected weaning weight
B_w – Birth weight
W_w – Weaning weight
W_a – Weaning age

4.2.3 Statistical analysis

Data was analysed using the analysis of variance (ANOVA) procedure in SPSS version 14.0 for Windows, by including the treatment group as fixed factor in the model and the variables BW, BCS, energy balance, pregnancy status, post-partum interval to breeding, birth weights, corrected weaning weight and the corresponding interactions. Pearson product moment correlation coefficients were calculated between variables as well as the significance levels. Differences between factors were assessed at a significance 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 of treated and control cows was compared by Chi-square analyse (SPSS, 2005).

4.3 Results

Cows incorporated in the present study showed at 45 days post-partum the following reproductive tract characteristics: vulva and vagina were normal with moist pink mucosa, intra-pelvic location of the cervix, involuted uterus and approximately symmetric and thin wall without content, good tone and active ovaries with either follicle or corpus luteum.

Body weight and BCS of cows in the experimental groups were typical for Brahman-type cows in an extensive production system in Mozambique (Schwalback *et al*., 1997), taking into consideration the physiological status of the cows and season of the year.

From calving to breeding, the available forage increased in both quantity and quality as a result of the seasonal variation on forage production associated with the sub-tropical climate. Nevertheless cows in the control group experienced a negative energy balance, though the BCS were similar (Table 4.1).

Under the true extensive production conditions with minimal management on which the experiment took place the BW, BCS and energy balance of cows at the start of the breeding season and, birth weights of calves, corrected weaning weights, calving to breeding interval, breeding to conception interval and conception rates are summarised in Table 4.1, while correlations between variables are presented in Table 4.2.

Table 4.1 Effects of calf removal on live weight, BCS, energy balance, weaning weights of the calves and re-conceptions characteristics of *Bos indicus* beef cows in extensive productions systems (LS means ± SD)

	Group	
Trait	RG	NRG
Live weight at calving (kg)	384 ± 43	410.9 ± 32
Live weight (kg)*	395.8 ± 50	410.5 ± 40
Body condition score*	2.5 ± 0.3	2.6 ± 0.3
Energy balance (kg)*	11.8 ± 10[a] (3%)	–0.4 ± 12[b] (-0.1%)
Birth weigth (kg)	28.3 ± 0.9	28.5 ± 0.9
Corrected weaning weight (kg)	149.3 ± 18[a]	134.5 ± 20[b]
Calving to breeding interval (days)	93 ± 18	99 ± 22
Calving to conception interval (days)	111 ± 10[a]	133 ± 19[b]
Breeding to conception interval (days)	18 ± 15[a]	31 ± 17.3[b]
Conception rate (%)	80	59

* At the start of the breeding season

[a,b] Means with different superscripts in the same row differ significantly ($p < 0.05$)

Table 4.2 Pearson product moment correlations coefficients between BW, BCS and conceptions and re-conceptions data of *Bos indicus* beef cows under extensive conditions

Control Variables			C_{WW}	*CBI*	*BwOBS*	*BCSOBS*	*BWMBS*	*BCSMBS*	*BWPD*	*BCSPD*	*PD*
Group	C_{WW}	Correlation	1.000								
		Significance (2-tailed)	.								
		Df	0								
	CBI	Correlation	-.212	1.000							
		Significance (2-tailed)	.158	.							
		Df	44	0							
	BwOBS	Correlation	.046	-.062	1.000						
		Significance (2-tailed)	.761	.683	.						
		Df	44	44	0						
	BCSOBS	Correlation	.160	-.003	.254	1.000					
		Significance (2-tailed)	.289	.986	.088	.					
		Df	44	44	44	0					
	BWMBS	Correlation	.038	-.060	.957	.250	1.000				
		Significance (2-tailed)	.802	.693	.000	.093	.				
		Df	44	44	44	44	0				
	BCSMBS	Correlation	.074	-.173	.008	.477	.109	1.000			
		Significance (2-tailed)	.626	.251	.955	.001	.471	.			
		Df	44	44	44	44	44	0			
	BWPD	Correlation	.087	.012	.759	.292	.793	.166	1.000		
		Significance (2-tailed)	.567	.936	.000	.049	.000	.271	.		
		Df	44	44	44	44	44	44	0		

BCSPD	Correlation	-.035	.064	.153	.399	.244	.585	.484	1.000	
	Significance (2-tailed)	.817	.671	.311	.006	.102	.000	.001	.	
	Df	44	44	44	44	44	44	44	0	
PD	Correlation	-.086	.313	.356	.348	-.007	.241	.008	.419	1.000
	Significance (2-tailed)	.570	.034	.015	.018	.964	.107	.960	.004	.
	Df	44	44	44	44	44	44	44	44	0

Cww – Corrected weaning weight; CBI – calving to breeding interval; BWOBS – Body weight at the onset of breeding season; BCSOBS – Body condition score at the onset of the breeding season; BWMBS – Body weight at the mid of breeding season: BCSMBS – Body conditions score mid breeding season; BWPD – Body weight at pregnancy diagnosis; BCSPD – Body condition score at pregnancy diagnosis; PD – Pregnancy data. **In Bold - (p<0.05)**

4.4 Discussion

The present study was conducted on *Bos indicus* beef cattle with the 12 hr night-calf removal for 48 ± 18 days prior to the breeding season in extensive production system in sub-tropical region. Previous studies have been performed on *Bos taurus* beef cattle within weeks prior to the onset of the breeding season (Stewart *et al*., 1993b) and in crossbreed *Bos taurus x Bos indicus* beef cattle from 9 to 12 days post-partum to first luteal phase or 100 days post-partum in intensive production systems (Gazal *et al*., 1999).

The conception rates were 80% for the RG and 59% for the NRG. Although the conception rates did not differ (p>0.05) between groups, the numerical difference of almost 20% was considerable (Table 1). The observed numerical increase in conception rates for cows in the RG agrees with the results of Stewart *et al*. (1993b) but contrast with those from Gazal *et al*. (1999) who reported that 12-hr (night or day) calf separation did not affect the duration of post-partum anestrus. The agreement with Stewart's results is probable due to the fact that both experiments were performed in sub-tropical conditions and prior to breeding season, although the breed type differed significantly. In addition, results of the present study indicated that by performing a 12-hr calf withdrawal at night there is an increase on BW of cows with consequent positive energy balance (3%), which, if combined with the reduced teat stimulation frequency, triggered the hypothamuls-ovarian function (BCI of 18 ± 15 days). The *ad libitum* suckling group had a conception rate of about 59%, which concurs with the previous results of Escrivão (2005) (unpublished report) for extensive beef cattle in the south of Mozambique.

Like BW and BCS at the onset of the breeding season, CBI is an important predictor of pregnancy rate. In the present study, conception rates were affected (p <0.05) by CBI, which concurs with the findings in literature (Requist *et al.*, 2006), but the treatment effects were stronger because the conception rates were higher for cows in the RG and they had a shorter CBI. The BCI of cows in the RG was only 18 ± 15 days, which represents a meaningful improvement in reproductive efficiency of extensive beef cattle, where the norm is in the order of 31 ± 17 days (NRG). This improvement is probably due to the fact that cows in the RG calved earlier in the calving season, weaned a heavier calf, and had enough time to recover for the following breeding season. These results agree with those of Stewart *et al*. (1993b) where 56.8% of the day-suckled cows showed estrus activity during the first 30 days of the breeding season, compared to 22% of the night-suckled cows.

In the current study, weaning weights increased ($p < 0.05$) with 12-hr calf removal at night contrary to the common belief. A number of reports in the literature suggest that temporarily calf removal does not affect the weaning weights of calves (Odde *et al*., 1986; Bell *et al*., 1998). The finding in the present study that 12-hr calf withdrawal has a positive effect on weaning weights of calves is probably because of the period of calf removal coinciding with the availability of good green pastures and calves making better use of it and compensatory growth seems to play a role.

The correlations observed between BCS, BW, energy balance at the start of the breeding season and conceptions rates demonstrated the importance of these variables in terms of reproduction. Results of the present study agree with those of other studies (Osoro and Wright, 1992; DeRouen *et al.,* 1994; Morrison *et al.,* 1999). Although animal scientists and veterinarians use BCS more often than BW in their routine work, this study indicated that BW when assessed in a consecutive manner is the most appropriate indicator of body energy reserves and re-breeding performance of *Bos indicus* beef cows in extensive production systems under sub-tropical conditions.

4.5 Conclusions

Twelve-hour calf removal at night for about 48 days prior to the breeding season increases the percentage of cows that exhibit estrus within the first 21 days of the breeding season, enhance conception rates and has a beneficial effect on calf weaning weights of *Bos indicus* beef cattle in extensive production systems.

5. Effects of 48-hour calf removal on conception rates of *Bos indicus* cows and calf-weaning weights in extensive production systems

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Summary

The current study aimed to determine if 48-hr calf removal prior to the breeding season affects (1) ovarian steroids, cortisol, urea and creatinine; (2) improves the conception rates; and (3) influence the calf-weaning weights of *Bos indicus* cattle in extensive production systems. Sixty multiparous Brahman-type cows were randomly selected in the early post-partum period and equally allocated into a calf removal group (RG) and a non-removal group (NRG). Calves from cows in the RG were removed for 48 hr prior to the breeding season and returned afterwards, whereas in the NRG the calves remained with their dams until weaning. BW and BCS of cows were recorded at the beginning of the breeding season, mid-breeding season and just after pregnancy diagnosis.

Pregnant cows were monitored throughout the gestation period and calving dates were accurately recorded. The calving season was divided into early, mid and late, corresponding conceptions occurred in the early, mid and late part of the breeding season, respectively. Calves were weighed at birth and at weaning. Weaning weights were corrected to 205 days. BW and BCS were similar throughout the experimental period. Conception rates (CR) were 76% for RG and 55% for NRG but did not differ significantly between the groups. However, differences ($p<0.05$) between the groups were observed for conception rates in the early and late part of the breeding season. CR was correlated with CBI and BCS at the onset of the breeding season. Product-limit survival curves Vs CCI differed significantly ($p<0.05$) between treatment groups. It was estimated with 95% certainty that 50% of the cows in the RG would conceive within the

first 19 days of the breeding season while for the NRG within the first 38 days of the breeding season. Weaning weights were 135.2 ± 22 kg for the RG and 135.5 ± 19 kg for the NRG. In the RG estradiol concentrations increased with sampling time, contrary to progesterone. Cortisol decreased with sampling time for both groups but with higher concentration in the RG. It was concluded that 48-hr calf removal prior to breeding enhances the conception rates with the majority of cows conceiving in the early part of the breeding season. It was also concluded that 48-hr calf removal increases plasma concentration of cortisol without adversely affecting reproduction and does not affect calf weaning weights of *Bos indicus* beef cattle in extensive production systems.

5.1 Introduction

In a beef cow-calf operation the optimum calving percentage can be attained if the first post-partum oestrus and the reestablishment of oestrous cycle occur prior to the breeding season. Walters *et al*. (1982) and Odde *et al*. (1986) found that 48-hr calf removal preceding the onset of the breeding season increases the number of cows in oestrus in the early breeding season. Salfen *et al*. (2001) observed an increase in oestrus rate of cows synchronised at 25 days post-partum following a 48-hr calf removal. Better results in interval to first oestrus and conception rates were related to restricted suckling combined with 48-hr calf removal or only the latter, when these were implemented prior to the breeding season (Odde *et al*., 1981; Walters *et al*., 1982a, Walters *et al*., 1982b; Meirelles *et al*., 1994) or in synchronisation protocols using GnRH and $PGF_{2\alpha}$ with 48-hr calf removal (Yelich *et al.,* 1995; Gear *et al*., 2001; Vasconcelos *et al.,* 2009). There is, however, a lack of information on the effects of 48-hr calf removal on the rebreeding performance of *Bos indicus* cows in extensive production systems.

In Mozambique, the breeding season is used to better manage reproduction in a few extensive beef cattle farms but conception rates are yet relatively low (<60%). The current study aimed to determine if 48-hr calf removal prior to the breeding season: (1) affects ovarian steroids, cortisol, urea and creatinine in *Bos indicus* cows in extensive production systems; (2) improves the current conception rates of *Bos indicus* cows in extensive production systems and (3) affects the calf-weaning weights.

5. 2 Materials and Methods

The study was carried out at the same location as described in 4.2.1.

Sixty multiparous Brahman-type cows were randomly selected in the early post-partum period and assigned to two groups of 30 cows: a calf removal group (RG) and a non-removal group (NRG). Calves from cows in the RG were removed for 48-hr prior to the breeding season and returned afterwards, whereas calves from cows in the NRG remained with their dams until weaning. BW and BCS of cows were recorded at the beginning of the breeding season, mid-breeding season and just after pregnancy diagnosis using the methods as described by Escrivão *et al.* (2009). 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).

Cows from both groups were submitted to the breeding season at the same time. Pregnancy diagnoses were done by rectal palpation 60 days after the end of the breeding season. Calves from both groups were weighed approximately 48-hr after parturition and again at weaning. Weaning weights were corrected to 205 days (Escrivão *et al.*, 2009). The interval from calving to the onset of the breeding season was calculated and defined as calving to breeding interval (CBI). Pregnant cows were monitored throughout the gestation period and calving dates were accurately recorded, while non-pregnant cows were re-bred in the follow-up breeding programme. The calving season was divided into early-calving season (ECS) (first 21days of the calving season), mid-calving season

(MCS) (second 21 days of the calving season) and late-calving season (LCS) (third 21 days of the calving season and above) and corresponding conceptions in the early, mid and late parts of the breeding season, respectively.

5.2.1 Blood sampling

Blood samples were collected by caudal venepuncture at 24 and 36 hours after calf removal (24 and 12 hours prior to breeding season) from all cows in the RG and NRG using sterile vacuum tubes containing EDTA and centrifuged immediately after collection. Plasma was harvested and stored at - 20ºC until hormone analysis. Collection of blood samples at time zero (t_0 = time of calf removal) was not considered due to the fact that cow-calf management from both groups was similar by the time of calf withdrawal. In addition, reports on *Bos taurus* beef cows in intensive production systems indicate that when 48-hr calf removal is performed the first LH peak is observed approximately 24 hr after calf removal (Walters *et al*., 1982; Edwards, 1985; Whisnant *et al*., 1985).

5.2.2 Hormonal assay

Estradiol, progesterone, creatinine, cortisol and urea were assayed using ADVIA Centaur Assay and SYNCRON LX Systems, while creatinine was assayed by Cobas Modular P, as described in 3.2.4.

5.2.3 Statistical analysis

Data were analysed by means of the analysis of variance (ANOVA) in the GLM procedure of SAS by including treatment group, sampling time as fixed factors, as well as the corresponding interactions in the model. The variables measured included BW and BCS at the onset of the breeding season, pregnancy data, CBI, CCI, and corrected weaning weight, progesterone, estradiol, cortisol, creatinine and urea. When appropriate the BW of the cows at the start of the breeding season and CBI were included as covariates. 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 of treated and control cows were compared by Chi-square analysis (SAS, 1996). To compare the survival distributions (in the time before conception) the Log-rank test (Nathan*, 1966*) was applied to the two experimental groups. Cows that did not conceive were included in the analysis as censored data with a weighting factor to compare conception rates between treatment groups. The Kaplan-Meier estimator was used to estimate and graphically display the survival functions (Kaplan and Meier, 1958).

5.3 Results

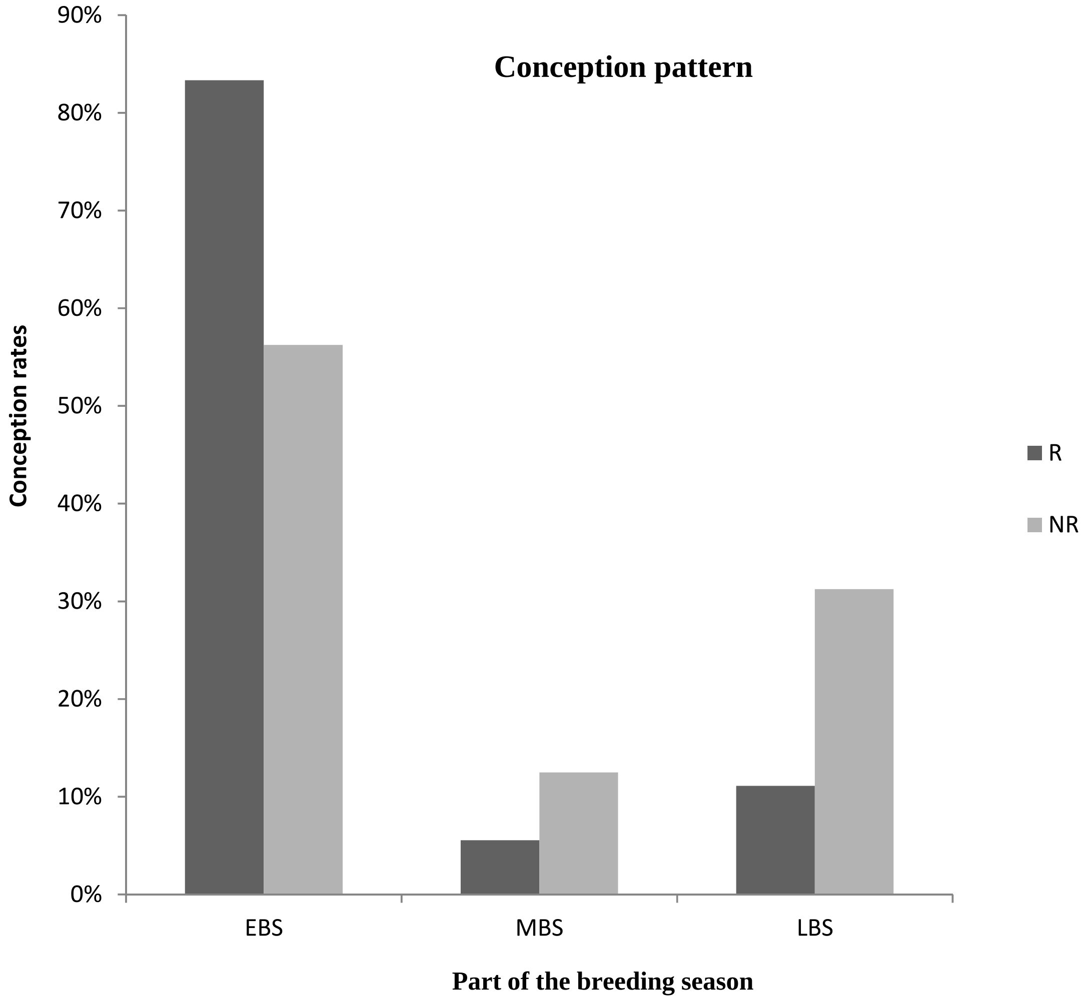
Calves of two cows incorporated in the experiment died before the onset of the breeding season and were thus excluded from the data set. Body weight and BCS of cows were similar ($p>0.05$) between and within the groups throughout the experimental period- namely at the onset of the breeding season, mid-breeding season and around pregnancy diagnosis, as illustrated in the Table 5.1.

Table 5.1 BW and BCS of cows at the onset (OBS), mid breeding season (MBS) and around pregnancy diagnosis (PD) for the RG and NRG

	Group	
Trait	RG	NRG
BW OBS (kg)	409 ± 38[a]	421 ± 48[a]
BW MBS (kg)	408 ± 41[a]	420 ± 44[a]
BW PD (kg)	415 ± 42[a]	431 ± 49[a]
BCS OBS	2.8 ± 0.5[a]	2.8 ± 0.4[a]
BCS MBS	2.4 ± 0.3[a]	2.4 ± 0.3[a]
BCS PD	2.8 ± 0.4[a]	2.8 ± 0.3[a]

[a,a] Means with same superscripts in the same row do not differ ($p>0.05$)

The conception rates were 76% for the RG and 55% for the NRG. Although a numerical difference was evident, conception rates did not differ ($p>0.05$) between treatment groups. However, when conception rates were allocated into early, mid and late parts of the breeding season, differences ($p<0.05$) between RG and NRG were observed. Conception rates differed between the RG and NRG during the early and late part of the breeding season but not in mid-breeding season. The conception pattern is presented in Figure 5.1.



(↔) indicates significant differences ($p<0.05$)

Figure 5.1 Conception rates in early (EBS), mid (MBS) and late part of the breeding season (LBS) for RG and NRG

Survival analysis indicated that product-limit survival curves Vs CCI differed significantly ($p<0.05$) between the removal and non-removal groups (Figure 5.2). In addition, survival analysis showed that CBI and BCS at the beginning of the breeding season were significant ($p<0.001$) predictors of conception.

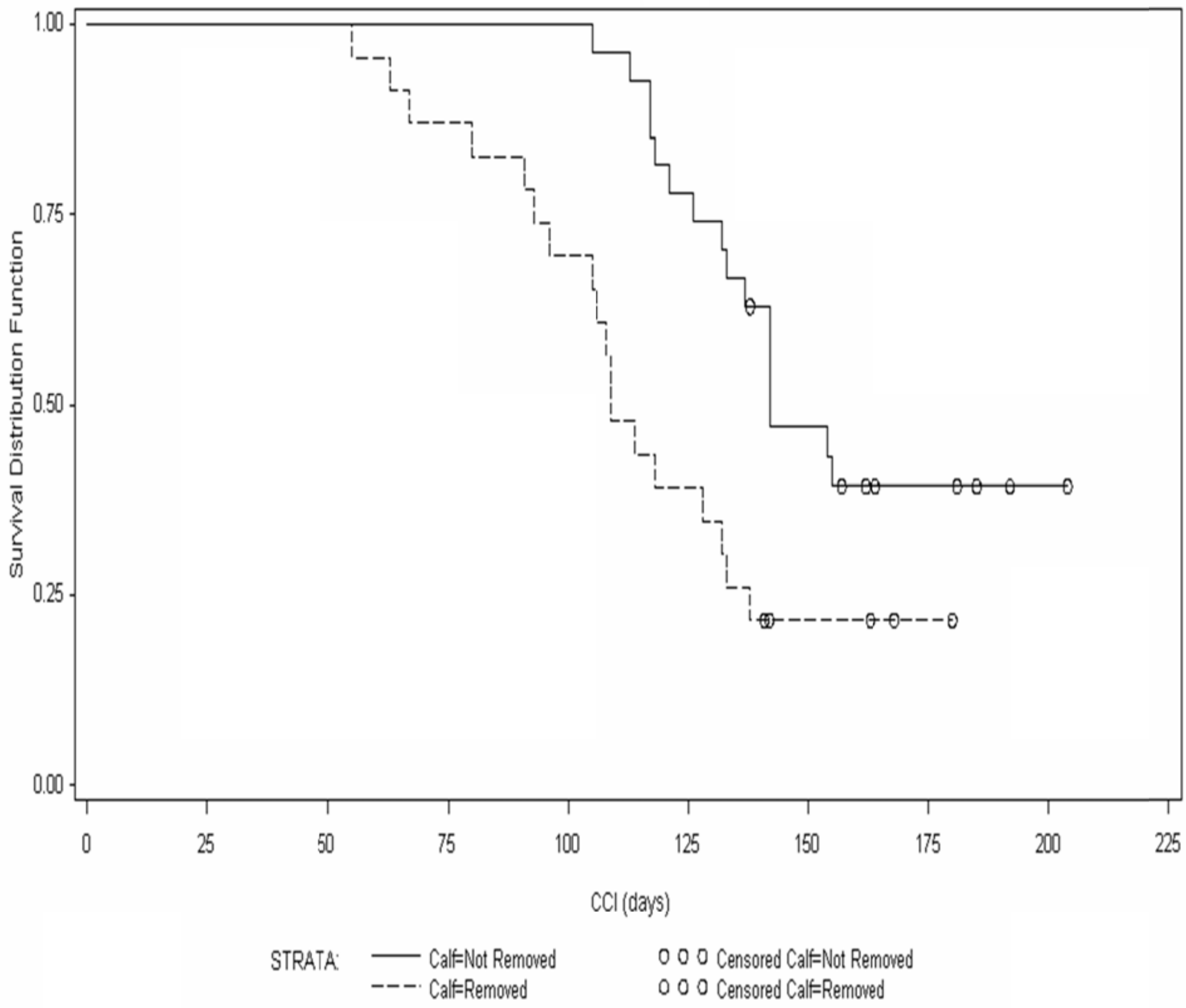


Figure 5.2 Product-Limit survival curves Vs calving to conception intervals of cows with calves removed 48 hours compared to cows without calves removed

Survival analysis estimates that 50% of cows in the RG will conceive an average within the first 19 days of the breeding season while for NRG in the first 38 days of the breeding season. The approximate speed of conception for cows in the RG and NRG is presented in Table 5.2.

Table 5.2 Speed of conception for Removal and Non removal groups

	Day of Conception			
	Removal Group		Non Removal Group	
% of Cows Conceiving	CCI[1]	BCI[2]	CCI[1]	BCI[2]
25%	93 [55 - 108]	3	126 [113- 142]	22
50%	109 [96 - 132]	19	142 [≥ 132]	38
75%	138 [≥ 114]	38	[≥ 154]	

[1]Calving to conception interval: Average (95% confidence interval)

[2] Breeding to conception interval

The weights of calves were similar at birth ($p>0.05$) and again at weaning (C_{ww} was 135.2 ± 22 kg for R group and 135.5 ± 19 kg for NR group) but gender differences ($p<0.05$) in terms of weaning weights of calves were observed as bull calves were 9% heavier than heifer calves.

The effect of 48-hr calf withdrawal on ovarian steroids, metabolites and their variation with sampling time is presented in Table 5.3.

Table 5.3 Effect of 48-hr calf withdrawal on circulating estradiol, progesterone, cortisol, urea and creatinine concentrations in beef cows under extensive conditions (Mean ± SD)

		Group	
Characterists	SP[1]	R group	NR group
Estradiol (pmol/l)	24 hr	118.8 ± 100.1[a]	84.7 ± 58.3[a]
	36 hr	151.9 ± 110.1[b]	141.3±86.3[b]
Progesterone (nmol/l)	24 hr	11.7 ± 26.2	4.2 ± 4.6
	36 hr	7.4 ± 6	4.7 ± 3.2
Cortisol (nmol/l)	24 hr	26.2 ± 18.7	19.1 ± 18.4
	36 hr	19.4±14	17.6±17
Urea (mmol/l)	24 hr	3.3 ± 2[c]	2.9 ± 1.4[d]
	36 hr	3.9 ± 1.5[c]	2.5 ± 0.9[d]
Creatinine (µmol/l)	24 hr	92.1 ± 17.9[a]	89.5 ± 17[a]
	36 hr	82.6 ± 17.9[b]	75.4 ± 14.6[b]

SP[1] = sampling time

[a,b] Means with different superscripts in the same column differ significantly ($p < 0.05$)

[cd] Means with different superscripts in the same row differ significantly ($p < 0.05$)

Cows with longer calving to breeding interval showed an increased ($r=0.393$ and $p=0.001$) estradiol concentration compared to cows with shorter CBI. There was an association between the concentration of plasma cortisol and urea ($r=0.344$ and $p=0.01$), as well as concentration of cortisol and creatinine ($r=0.278$ and $p=0.05$).

Table 5.4 Correlation between variables – Effects of 48-hr calf removal on rebreeding performance of *Bos indicus* cows and calf-weaning weights in extensive production systems

Control Variables			EBS	MBS	LBS	BwDec	BCSDec	PD	Estrad.	Prog	Urea	Creat	Cort	Dayspp	Cww
Group and Samp_time	EBS	Correlation	1.000												
		Significance (2-tailed)	.												
		Df	0												
	MBS	Correlation	-.508	1.000											
		Significance (2-tailed)	.000	.											
		Df	54	0											
	LBS	Correlation	-.680	-.285	1.000										
		Significance (2-tailed)	.000	.033	.										
		Df	54	54	0										
	BwDec	Correlation	-.145	-.045	.200	1.000									
		Significance (2-tailed)	.285	.742	.139	.									
		Df	54	54	54	0									
	BCSDec	Correlation	-.089	.132	-.013	.551	1.000								
		Significance (2-tailed)	.515	.333	.922	.000	.								
		Df	54	54	54	54	0								
	PD	Correlation	-.032	.022	.017	.082	.340	1.000							
		Significance (2-tailed)	.815	.874	.901	.546	.010	.							
		Df	54	54	54	54	54	0							

Control Variables			EBS	MBS	LBS	BwDec	BCSDec	PD	Estrad.	Prog	Urea	Creat	Cort	Dayspp	Cww
	Estradiolpmoll	Correlation	.092	-.030	-.076	-.054	.100	.112	1.000						
		Significance (2-tailed)	.502	.827	.575	.690	.463	.409	.						
		Df	54	54	54	54	54	54	0						
	Progst_nmoll	Correlation	.022	.000	-.024	-.123	-.274	-.628	.017	1.000					
		Significance (2-tailed)	.874	.999	.859	.365	.041	.000	.900	.					
		Df	54	54	54	54	54	54	54	0					
	Urea_mmoll	Correlation	.165	-.097	-.101	.145	.088	.025	-.066	.019	1.000				
		Significance (2-tailed)	.225	.479	.459	.287	.521	.855	.628	.892	.				
		Df	54	54	54	54	54	54	54	54	0				
	Creat_umoll	Correlation	.066	.089	-.148	-.015	.303	.074	.169	.001	-.034	1.000			
		Significance (2-tailed)	.631	.516	.275	.915	.023	.589	.212	.992	.802	.			
		Df	54	54	54	54	54	54	54	54	54	0			
	Cortisol_nmoll	Correlation	-.072	.130	-.030	-.023	.084	-.285	-.173	.146	.344	.278	1.000		
		Significance (2-tailed)	.597	.341	.826	.864	.537	.033	.203	.282	.009	.038	.		
		Df	54	54	54	54	54	54	54	54	54	54	0		
	Dayspp	Correlation	.471	-.057	-.475	-.428	-.089	-.210	.393	.179	.018	.160	.158	1.000	
		Significance (2-tailed)	.000	.676	.000	.001	.516	.120	.003	.188	.895	.240	.243	.	
		Df	54	54	54	54	54	54	54	54	54	54	54	0	
	Cww	Correlation	-.082	-.183	.248	.159	.094	.160	-.085	-.074	-.104	-.176	-.362	-.471	1.000
		Significance (2-tailed)	.546	.177	.066	.242	.490	.240	.535	.588	.447	.195	.006	.000	.
		Df	54	54	54	54	54	54	54	54	54	54	54	54	0

5. 5 Discussion

Results of the present study indicate that BW and BCS were similar between RG and NRG over the experimental period, which was likely to occur because cows were randomly allocated to the two experimental groups and were in the same extensive management system. In addition, the observed results were congruent with those of other studies on 48-hr or 96-hr calf removal (Quintans *et al.*, 2004).

As cows in both groups had a good body condition score at the onset of the breeding season (BCS ≥ 2.7) and since this variable has been reported to be strongly correlated with conception rates (Osoro and Wright, 1992; DeRouen *et al*., 1994; Morrison *et al*., 1999; Renquist *et al*., 2006), similar conception rates between the groups tested in this study were expected. However, conception rates were numerically greater for the RG (76%) than for the NRG (55%), which finding agrees with the results of several others studies (Walters *et al*., 1982; Fanning *et al.*, 1995; Geary *et al*., 2001; Roche *et al.,* 2009).

Despite the shorter CBI in RG and similar BCS and BW between the groups at the onset of the breeding season, the higher conception rates observed in RG highlights the positive effect of 48-hr calf removal on conception rates of *Bos indicus* cows in an extensive production system in sub-tropical conditions. Furthermore, the beneficial effect of 48-hr calf removal on herd productivity was evident through increasing the number of cows that conceived early in the breeding season (Figure 5.1 and Figure 5.2). Similar results have been previously reported (Walters *et al*., 1982; McCartney *et al.,* 1990; Belloso *et al.* 2002).

Cows in the NRG had lower conception rates and conceived later in the breeding season. These cows weaned lighter calves (calves were younger at weaning and weighed less) and according to Marshall *et al.* (1990) they had an increased likelihood of conceiving later in the subsequent breeding season or even not conceiving. Non-pregnant cows are often culled due to reproductive inefficiency and represent an economic loss to the farmer (Grossi *et al,* 2008).

It was found in the present study that weaning weights of calves were not affected by 48-hr calf removal and these findings agree with those from other studies (Odde *et al.*, 1986; Fanning *et al.*, 1995; Bell *et al.,* 1998).

In the RG estradiol concentrations increased and progesterone concentration decreased with sampling time, which is in concurrence with previous studies (Walters *et al*., 1982; Whisnant *et al*., 1985). The observed trend for the concentration of estradiol and progesterone in the RG and the fact that the majority of cows in the same group conceived in the early part of the breeding season illustrates that the withdrawal of suckling may have provoked estrus in RG by removing the negative effect of suckling.

It was expected that cortisol concentration would decrease with sampling time due to the adaptation of cows to the stress of blood sampling (Koolhaas *et al.,* 1999). Despite the lack of any statistical difference between the groups, cortisol concentrations were numerically higher in the RG than in NRG, probably due to the combined stress of blood sampling and calf removal. Previous data on 48-hr calf removal in *Bos taurus* cows in an intensive production system (Whisnant *et al.,* 1985) indicated a temporary increase in cortisol concentrations, 9 to 12 hr after calf removal. Our findings in *Bos indicus* cows in an extensive production system in sub-tropical conditions show a similar increase in cortisol concentrations, although these were higher at 24 and 36 hr after calf removal compared to the NRG.

High plasma urea and creatinine concentrations have been reported to be associated with catabolism of protein due to negative energy balance or stress (Wikhund *et al*., 1996; Butter, 2000). In the current study the concentrations of urea and createnine were within the reference ranges (Ndlovu *et al.,* 2007) but urea differed significantly between the groups, suggesting that the catabolism of protein was higher in the RG than in NRG. In addition, urea concentration in the NRG and creatinine in both groups followed the cortisol trends with sampling time and these variables were correlated with cortisol. Therefore, stress may explain part of the variations observed in the concentration of creatinine and urea and cortisol was more pronounced in the RG than in the NRG. However, the results suggest that stress due to calf removal did not interfere with reproduction hormones or conception rates which agree with the finding from other studies (Wikhund *et al*., 1996; Butter *et al.,* 2000).

5.6 Conclusions

Forty-eight-hour calf removal prior to the breeding season seems to block the negative effect of suckling and enhance conception rates, so that the majority of cows conceive in the early part of the breeding season. Calf removal marginally increased plasma concentrations of cortisol, creatinine and urea without adversely affecting reproduction or calf-weaning weights of *Bos indicus* beef cattle in extensive production systems.

6. General Discussion and Conclusions

The major factors that influence post-partum re-conception rates in *Bos indicus* cows under extensive management conditions were reviewed (Chapter 2). There is a need for a better understanding of the reproductive hormones and metabolite profile during the post-partum period in extensively managed *Bos indicus* cows. These aspects have only been studied in detail in *Bos taurus* cows under intensive management conditions (Alverez-Rodiguez *et al.,* 2010; Forde *et al.,* 2011). The primary focus of the present study was thus on the effects of post-partum BCS, BW, age and parity number on ovarian steroids, metabolites and the related conception rates in *Bos indicus* cows under extensive management conditions.

The difficulties of obtaining the hormonal profile of *Bos indicus* cows in true extensive management conditions over the entire oestrous cycle was taken into consideration and this is probably the reason for the limited number of reports in this field. In order to overcome these problems the present study was performed based on: (1) synchronisation of oestrus to avoid low oestrus detection in *Bos indicus* cows; (2) analysis of concentrations and changes in concentrations of plasma hormones and metabolites from 24 hours before oestrus to 24 hours after oestrus; and (3) maintenance of experimental animals under extensive management conditions throughout the study period (Chapter 3).

The results of the present study on post-partum plasma concentrations of estradiol and progesterone (Figure 3.17) around oestrus in *Bos indicus* cows under extensive conditions were similar to that reported in *Bos taurus* cows in intensive management systems (Forde *et al.,* 2011). In addition, the present experiments enclose an extended analysis by including the metabolic status (energy balance) of the cows to the start of the breeding season. The concomitant analysis of metabolic status of the cows (BCS and energy balance) observed in the present study provide a better interpretation of the effects and interactions of BCS on plasma concentrations of steroids, metabolites and subsequent conception rates. The results obtained on the correlation between BCS at the start of the breeding season with estradiol, indicate with confidence the necessity of maintaining at least a BCS of 2.5 at the beginning of breeding season in order to maximise the post-partum re-conception rates. These new findings differ from those of previous reports (Shwalback *et al.*1996; Renquist *et al.,* 2006) in which a BCS of at least 2 is recommended in extensive and semi-extensive *Bos indicus* beef cows and their crosses.

Despite the fact that the effects of BCS at the start of the breeding season or at calving were related to the subsequent conception rates (Ezanno *et al.,* 2005; Renquist *et al.,* 2006; AlIbrahim *et al.,* 2010) the present study provides more data to stress the importance of BCS in post-partum reproduction, based on the positive correlation complex between BCS-estradiol-conception rates. The practical implications of the present findings are more important for the management of *Bos indicus* cattle in extensive conditions, with emphasis on Mozambican farmers where the study took place and where the conception rates are generally rather low (< 60%).

Results of the metabolites that indicate the catabolism of protein (urea and criatenine) show that at a BCS 2, 2.5 and 3, in cows experiencing a positive energy balance the requirements for energy seems to be satisfied as the breakdown of protein did not exceed the upper level concentrations of creatinine and urea in the circulating blood.

The hypothesis that stress could impair the post-partum re-conception rates in beef cows under extensive conditions was the topic of a number of studies but the data were inconclusive (Dieleman *et al.,* 1986; Dobson and Smith, 2000; Walker *et al.,* 2008). In the present study, similar results were obtained for plasma cortisol concentrations, compared to previous studies on *Bos taurus* beef cows and dairy cows but with a transient elevation at the beginning of the blood sampling and decreasing thereafter. Since cortisol did not correlate with estradiol or progesterone, the observed results support the thesis that stress probably did not interfere with post-partum re-conception rates of extensive beef cows.

The average conception rate (90.5%) obtained for cows in the experimental group in the present study (Chapter 3) was high. This data represents a tremendous increase in cattle productivity in Mozambique compared to the conception rates that are generally below 60%. Furthermore, the fact that for 60% of the cows, conceptions occurred in the first 21 days after the start of the breeding season indicates that the reproductive management based on BSC of at least 2.5 to the breeding season contributed positively to the weaning weight of the calves and, therefore, to the herd productivity.

The mechanisms involved in the effect of suckling on post-partum re-conception rates and on calf-weaning weights were considered in the present study by testing two hypotheses: (1) If restricting suckling at night from 45 days post-partum increases conceptions rates in the subsequent breeding season; and (2) If 48-hr calf removal prior to the breeding season improves the conceptions rates of *Bos indicus* cows under extensive conditions. For productive characteristics calf-weaning weights were compared between treatment and control groups.

Results from both hypotheses demonstrate a considerable increase in post-partum re-conception rates: 80% for 12 hr calf removal and 76% for 48-hr calf removal compared to their control groups 59% and 55%, respectively. Although a numerical difference was evident, the conception rates did not differ significantly between treatment and control groups. Similar results were observed in terms of period of occurrence of conception during the breeding season in both suckling-management strategies in which conceptions were concentrated in the first 21 days after the start of the breeding season.

The analysis of conception data by means of Chi-square analysis showed no statistical difference between treatment and control groups. But when the evaluation of conception data was performed by means of survival analysis (conception over time of the breeding season) it was found that the product-limit survival curves Versus CCI differed significantly ($p< 0.05$).

This new way of analysing conception data (by means of survival curves) provides a better understanding of the factors that influence reproduction in post-partum *Bos indicus* beef cows under extensive conditions by simultaneous inclusion of time and binary variables and predict the conception rates over the breeding season (Table 5.2). Results from the present study indicate that the use of Chi-square analysis to compare conception rates of extensive beef cows under natural breeding over a defined period of breeding season do not take into account the events over time. For this reason, the analysis might not express the reality. In addition, conception rates are true rates (Transfield *et al.,* 1996) and these rates measures the speed at which a cow conceives over a defined period of time. It follows that survival analysis provides a more accurate method for comparing conception rates between groups of animals.

The 12-hr-calf-removal strategy used in the present study is similar to that used in subsistence production systems but the purpose of this practice in subsistence systems is to increase milk yield for the following morning. The later practice does not result in an increase in subsequent conception rates or reduce inter-calving periods because the milk is used for household purposes followed by excessive suckling by the calf after milking.

In terms of conception rates and calf weaning weights the reproductive management strategy based on 12-hr calf removal from 45 days post-partum to the start of the breeding season is more advantageous under extensive conditions since it improves conceptions rates and increases the calf-weaning weights compared to 48 hr-calf removal.

Results from the experiments performed in the present study emphasise the benefits of calf removal on post-partum re-conception rates of *Bos indicus* cows under extensive management. In addition, the findings stress the correlation complex of BCS-estradiol-conception rates. Furthermore, maximum post-partum re-conception rates can be achieved if the BCS at the beginning of the breeding season is at least 2.5, regardless of the calf-removal strategy.

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